

Original articles

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ANTI-MICROINFLAMMATORY LIPID SIGNALS GENERATED FROM DIETARY N-3 FATTY ACIDS *VIA* CYCLOOXYGENASE-2 AND TRANSCELLULAR PROCESSING: A NOVEL MECHANISM FOR NSAID AND N-3 PUFA THERAPEUTIC ACTIONS

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Aspirin therapy inhibits prostaglandin biosynthesis; yet *via* acetylation of cyclooxygenase 2 (COX-2) it leads to bioactive lipoxins epimeric at carbon 15 (15-*epi*-LX, also termed aspirin-triggered lipoxin or ATL). Here, we review our findings indicating that inflammatory exudates from mice treated with ω -3 PUFA and aspirin (ASA) generate a novel array of bioactive lipid signals. Also, human endothelial cells, both HUVEC and microvascular, with upregulated COX-2 and treated with ASA converted C20:5 ω -3 to 18*R*-hydroxyeicosapentaenoic acid (HEPE) and 15*R*-HEPE. Human PMN activated with serum treated zymosan (STZ) utilized each of these *R*-HEPEs to generate novel classes of trihydroxy-containing mediators including 5-series 15*R*-LX and 5,12,18*R*-triHEPE. The novel products were potent inhibitors of human PMN transendothelial migration and infiltration of PMN in dorsal air pouches *in vivo*. In addition to ASA, both acetaminophen and indomethacin also permitted 18*R*-HEPE and 15*R*-HEPE generation with recombinant human COX-2 as well as ω -5 and ω -9 oxygenations of other fatty acids that act on leukocytes, platelets and endothelial cells. These findings establish new transcellular routes for producing arrays of lipid mediators *via* COX-2-NSAIDs and cell-cell interactions that impact microinflammation. Moreover, they provide novel mechanism(s) that could underlie the many reported therapeutic benefits of ω -3 dietary supplementation of interest in inflammation, cancer, and vascular disorders.

Key words: dietary PUFA, eicosanoids, leukocytes.

INTRODUCTION

Many reports of the past 25 years suggest that supplementation of dietary omega-3 polyunsaturated fatty acids (ω -3 PUFA) has beneficial effects in human diseases and laboratory animals (1, 2). These include antithrombotic,

immunoregulatory and anti-inflammatory responses relevant in arteriosclerosis, arthritis and asthma (1) as well as antitumor and antimetastatic effects (3). Their potential for preventative actions in cardiovascular diseases were recently bolstered with the finding that major dietary ω -3 PUFA, eicosapentaenoic acid (C20:5 ω -3, EPA) and docosahexaenoic acid (C22:6 ω -3; DHA) have a dramatic effect on ischemia-induced ventricular fibrillation and can protect against sudden cardiac death (4). Emergence of such preventative and/or therapeutic actions of ω -3 PUFA supplementation in infant nutrition, cardiovascular diseases and mental health have recently called for recommended dietary intakes by an international workshop (5). However, the molecular mechanism(s) for dietary ω -3 protective actions remain largely unexplained.

It is believed that the actions of the major lipid of fish oil, C20:5, are caused by i) preventing conversion of arachidonic acid (C20:4 ω -6, AA) to proinflammatory eicosanoids (i. e., PG and leukotrienes¹ (LT)); ii) serving as an alternate substrate producing 5-series LT that are less potent; and/or iii) conversion by cyclooxygenase (COX) to 3-series prostanoids (i. e., PGI₃) with potencies equivalent to their 4-series PG counterparts to maintain antithrombotic actions (1, 3, 4). These and other explanations offered (1, 3—5) are not embraced because of the lack of molecular evidence *in vivo* and the high concentrations of ω -3 PUFA required to evoke putative "beneficial actions" *in vitro*. Although the proinflammatory roles of LT and PG are well appreciated (6, 7), there is new evidence that other eicosanoids derived from arachidonate, namely lipoxins (LXs) and their endogenous analogs, the aspirin-triggered 15 epimer lipoxins (ATL), potent counterregulators of PMN-mediated injury and acute inflammation (8—10). Acetylation of COX-2 by aspirin (ASA) prevents the formation of prostanoids (11), but the acetylated enzyme remains active *in situ*, generating 15-*R*-hydroeicosatetraenoic acid (15-*R*-HETE) from C20:4 (12, 13), which is converted by inflammatory cells to 15-epimeric lipoxins (ATL). Synthetic analogs of these natural local mediators with prolonged bio-half-life display potent anti-inflammatory properties (10, 14, 15), providing evidence that cell-cell "cross-talk" can convert arachidonic acid to mediators with anti-inflammatory properties (16).

¹Abbreviations

COX-2, cyclooxygenase 2

EPA, eicosapentaenoic acid

LC/MS/MS, liquid chromatography tandem mass spectrometry

LT, leukotrienes

LX, lipoxin

LXA₄, 5S, 6R,15S-trihydroxy-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid

15-epi-LXA₄, 5S,6R,15R-trihydroxy-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid

NSAID, nonsteroidal anti-inflammatory drug

Along these lines, oxidation of C20:4 in endothelial cells (EC) may lead to 11,12-epoxyicosatetraenoic acid via P450, which blocks EC activation (17). Since PMN-vessel interactions are pivotal to recruitment and PMN-dependent tissue injury (18), the local signals involved in their "cross-talk dialog" are of interest. Our finding that aspirin-acetylated COX-2 remains active *in vivo* (12) to generate specific ATL that can be effectors of well-established anti-inflammatory therapy offers a mechanism for ASA beneficial impact that cannot be attributed to prostanoids (7, 11). Since new therapeutic uses for ASA and related NSAIDs continue to be uncovered that require molecular definition, including prophylaxis against colorectal cancer and lower risk of myocardial infarction (reviewed in 19), and in view of the qualitatively overlapping beneficial profiles assigned to dietary ω -3 PUFA in human disease (1, 3–5), we sought evidence for novel mechanisms involved in the generation of lipid-derived signals that would provide a basis as well to explain some of the beneficial actions of ω -3 PUFA. Our recent results (Serhan *et al.*, submitted, 2000) are reviewed in this contribution.

MATERIALS AND METHODS

Materials used in LC/MS/MS analyses were from vendors given in (20) and in Serhan *et al.* (submitted, 2000).

Human cell types

Human PMN were freshly isolated from venous blood of healthy volunteers, that declined taking medication for 2 weeks before donation, by Ficoll gradient and enumerated, and human umbilical vein (HUVEC) or microvascular (HMVEC) endothelial cells were cultured for transendothelial migration and performed as in (9). HMVEC monolayers (1, 2, or 3 passages) were seeded ($\sim 2 \times 10^5$ cells/cm²) on polycarbonate permeable supports pre-coated with 0.1% gelatin for incubations with NSAIDs and PUFA.

Murine air pouches

Inflammatory exudates were initiated with intrapouch injection of TNF α (R&D Systems) into 6 d dorsal air pouches (15) with 6–8 wk male FVB mice followed by ASA (500 μ g) at 3.5 h and 300 μ g C20:5/pouch at 4 h. At 6 h pouches were lavaged (3 ml saline), exudate cells enumerated and activated (4 μ M A₂₃₁₈₇, 37°C, 20 min). Inhibition of TNF α stimulated (100 ng/pouch FVB) PMN infiltration with *i.v.* tail injection of either 18R-HEPE, 5,12,18R-HEPE or 15-epi-LXA₄ analog was determined (15) with pouch lavages taken at 4 h.

Recombinant molecules and analytical procedures

Specific [³H] LTB₄ (NEN) binding was performed with HEK293 cells stably transfected with human BLTR (10). Human recombinant COX-2 (a gift from Dr. Copeland, DuPont Merck, DE) was overexpressed in Sf9 insect cells (ATCC) and microsomal fractions ($\sim 8 \mu$ l) suspended in Tris

(100 mM, pH 8.0) as in (21), and NSAIDs were incubated (i.e., ASA, ~1 mM) at 37°C 30 min before addition of PUFA (20 μ M). *B. megaterium* was grown in Bacto Nutrient Broth (Fisher Scientific) at 30°C with shaking. To produce biogenic standards for 18R-HEPE, sonicates were incubated in 2 M Tris buffer (pH 8.1) with NADPH (2 mM) and C20:5 (330 μ M). Similar conditions were used to convert LTB₅ (15 μ M) to novel products; see results. All incubations were extracted (8) with internal standard and subject to LC/MS/MS analysis (12, 15) using a Finnigan LCQ (San Jose, CA) equipped with a LUNA C18-2 (150 \times 2 mm; 5 μ M) column and a rapid spectra scanning UV/Vis detector. Chiral cell OB-H column (J. T. Baker) was used to determine *R* and *S* alcohol configurations of monohydroxy-PUFA using isocratic (hexane:isopropanol; 96:4 vol:vol). Detailed procedures for isolation, quantitation and structural determination of lipid-derived mediators were reported (20) and used essentially as described for the elucidation of the novel products (Serhan *et al.*, submitted, 2000). The design of experiments was approved by a local Ethical Committee.

RESULTS

Since ASA triggered the formation of previously undescribed bioactive eicosanoids (8), we tested the concept that NSAIDs might promote the formation of novel mediators from ω -3 PUFAs. Inflammatory exudates formed in murine air pouches via intrapouch injections of TNF α with ω -3 and ASA on board (2 h) generated several novel products. LC/MS/MS analyses of these exudate-derived materials demonstrated monohydroxy acids, depicted as selected ion chromatograms from acquired results recalled at *m/z* 317, i.e., 18-hydroxy-eicosapentaenoic acid (18-HEPE) and 5-HEPE which coeluted with synthetic 5*S*-HEPE as well as novel trihydroxy-containing products derived from C20:5. LC retention times and MS/MS spectra gave product ions consistent with structures shown in the respective insets, namely *m/z* 317 = [M-H]⁻, 299 = [M-H]⁻-H₂O, 273 = [M-H]⁻-CO₂, 255 = [M-H]⁻-H₂O-CO₂. Diagnostic ions consistent with 18-HEPE identification were present at *m/z* 259 (Panel B) and 5-HEPE at *m/z* 115. These criteria were used throughout for identification. The chirality at carbon 18 was established for the exudate-derived 18-HEPE using a chiral column and a reference 18*R*-HEPE prepared via biogenic synthesis with *B. megaterium* (see Methods), which monooxygenates fatty acids and for example converts arachidonic acid to 18*R*-HETE (22, 23). The alcohol chirality at position 18 proved to be >98% *R*. These findings indicated that murine inflammatory exudates exposed *in vivo* to ω -3 20:5 and ASA produced 5-lipoxygenase pathway 5-series 5*S*-HEPE, a product also identified with human PMN (24), as well as the novel 18*R*-HEPE, whose route of formation was determined (*vide infra*). Air pouch inflammatory exudate cells from these ASA- and EPA-treated mice contained predominantly PMN, which were 25–60% lower in number than in exudates formed with TNF α alone (*n*=3). When activated with ionophore A₂₃₁₈₇ (4 μ M),

these exudates generated essentially equivalent amounts of 18R-HEPE (10.2 ± 4.3 ng/ 10^6 cells) and 5S-HEPE (10.9 ± 2.9 ng/ 10^6 cells).

Evidence for novel trihydroxy-containing products was also obtained in these inflammatory exudates (Serhan *et al.*, submitted, 2000). Ions present within MS/MS were consistent with a trihydroxy-containing product from C20:5 with a parent ion m/z 349 = $[M-H]^-$ and product ions of structural significance present at m/z 291 and 195 that are consistent with fragmentations denoted in the inset. Also, a 270 nm UV absorbance maximum was evident, indicative of a conjugated triene, and the presence of the m/z = 291 (cleavage C17-C18 positions) as well as the twenty-carbon structure implicated that 18R-HEPE and triHEPE were biosynthetically related.

It was of interest to determine whether these new products were also generated by human cells and if they possess bioactivities. To this end, human endothelial cells known to induce COX-2 with IL-1 β (8) or hypoxia were pulsed with EPA, treated with ASA, and extracted materials subject to LC/MS/MS analysis. Selected ion monitoring at m/z 259 revealed that HUVEC treated with ASA converted EPA to 18R-HEPE. Also, HMVEC treated with ASA and EPA generated 18-HEPE (10.6 ng/ 10^6 cells) and 15-HEPE (6.0 ng/ 10^6 cells) ($n = 2$, $d = 4$; not shown). These observations implicated the involvement of COX-2 in the generation of these products, which proved to be the case with recombinant human COX-2 exposure to ASA and ω -6 or ω -3 PUFA (Table 1).

Table 1. Products from Polyunsaturated Fatty Acids with Recombinant Human COX-2*

PUFA	Treatment	Monohydroxy Product ng		
		ω -2	ω -5	ω -9
C18:2	+ ASA	--	<u>13-HODE</u> 2.9 \pm 0.6	<u>9-HODE</u> 25.2 \pm 17.8
		--	55.0 \pm 18.7	243 \pm 68.6
C20:4	+ ASA	--	<u>15R-HETE</u> 234.0 \pm 112.5	<u>11R-HETE</u> 1.4 \pm 1.6
		--	11.5 \pm 8.2	1.8 \pm 1.0
C20:5	+ ASA	<u>18R-HEPE</u> 16.2 \pm 3.3	<u>15R-HEPE</u> 16.8 \pm 5.8	<u>11R-HEPE</u> 5.4 \pm 3.3
		7.0 \pm 3.3	17.9 \pm 5.2	40.7 \pm 10.3

* $n = 3$ mean \pm SEM. Products were identified and quantitated using LC/MS/MS as reported in Serhan *et al.* (submitted, 2000).

Results in *Table 1* show that linoleic acid (C18:2) was converted to both 13-HODE (ω -5 oxygenation) and 9-HODE (ω -9), which were greatly diminished by ASA but not completely abolished. Arachidonic acid was converted to 15*R*-HETE (ω -5) as well as 11*R*-HETE (ω -9), consistent with earlier findings (8) and an ASA-triggered appearance of a lipoxygenase activity that switched to 15*R*-HETE production by acetylated COX-2 (13), which did not appear to influence 11*R*-HETE formation (*Table 1*). 11*R*-HEPE was the major product with EPA and COX-2, with lesser amounts of 15*R*-HEPE (ω -5) and 18*R*-HEPE (ω -2). ASA acetylation of COX-2 led to \sim 2-fold increase in 18*R*-HEPE (ω -2) with a $> 85\%$ reduction in 11*R*-HEPE (the ratio of positional oxygenations with C20:5 was 1:1:0.3 with 18*R* 15*R* $>$ 11*R*). Hence, together they suggested that acetylated COX-2 in endothelial cells was a dominant source of 18*R*-HEPE and 15*R*-HEPE. Of interest, yet unlike the isolated COX-2 product profiles, neither 11*R*-HEPE (from C20:5) nor 11*R*-HETE (from C20:4) were major products of the vascular endothelial cells. These results suggest that ASA treatment at local sites of inflammation along with ω -3 PUFA (i. e. EPA; 20:5) administration, as exemplified by cytokine-driven acute inflammation (*Fig. 1*), can convert EPA via induced COX-2 to 18*R*-HEPE and 15*R*-HEPE.

Human PMN convert ASA-triggered COX-2-derived 15*R*-HETE to 15-epi-LXA₄ and EPA is converted to 5-series LX by human leukocytes as well as trout macrophages (8, 25). Therefore, we evaluated whether activated human PMN engaged in phagocytosis handle acetylated COX-2-derived 20:5 products 18*R*-HEPE and 15*R*-HEPE. Serum-treated zymosan (STZ), the phagocytic stimulus, initiated the utilization and conversion of acetylated COX-2 C20:5-derived products to two classes of trihydroxy-containing EPE, evident again by selected ion monitoring at m/z 349.5 $[M-H]^-$, the expected base peak molecular ion for these products (Serhan *et al.*, submitted, 2000). Also, synthetic LTB₅ incubated with *B. megaterium* homogenates (pH 8.0 and NADPH (23) to facilitate hydroxylations) was transformed to a trihydroxy product with a m/z 291 ion characteristic for the presence of the 18*R* alcohol group as observed from human PMN. These independent lines of evidence indicated that PMN take up 18*R*-HEPE, which is converted by their 5-lipoxygenase and in subsequent steps to 5-hydroperoxy-18*R*-DiHEPE and 5(6) epoxide formation to 5,12,18*R*-triHEPE (an 18*R*-carrying "LTB₅-like" product) that is likely to possess the stereochemistry of LTB₅ (24), retaining the 18*R* chirality of the precursor.

In an analogous biosynthetic fashion, 15*R*-HEPE was converted by PMN via 5-lipoxygenation to a 5-series LXA₅ analogue (*Fig. 1*) that also retains their C15 configuration. Its MS/MS gave prominent ions here, m/z 305, 233, and 251, depicted in the MS/MS spectrum, namely 15-epi-LXA₅, consistent with LX₅ structure (5 series) observed from endogenous sources of EPA in trout

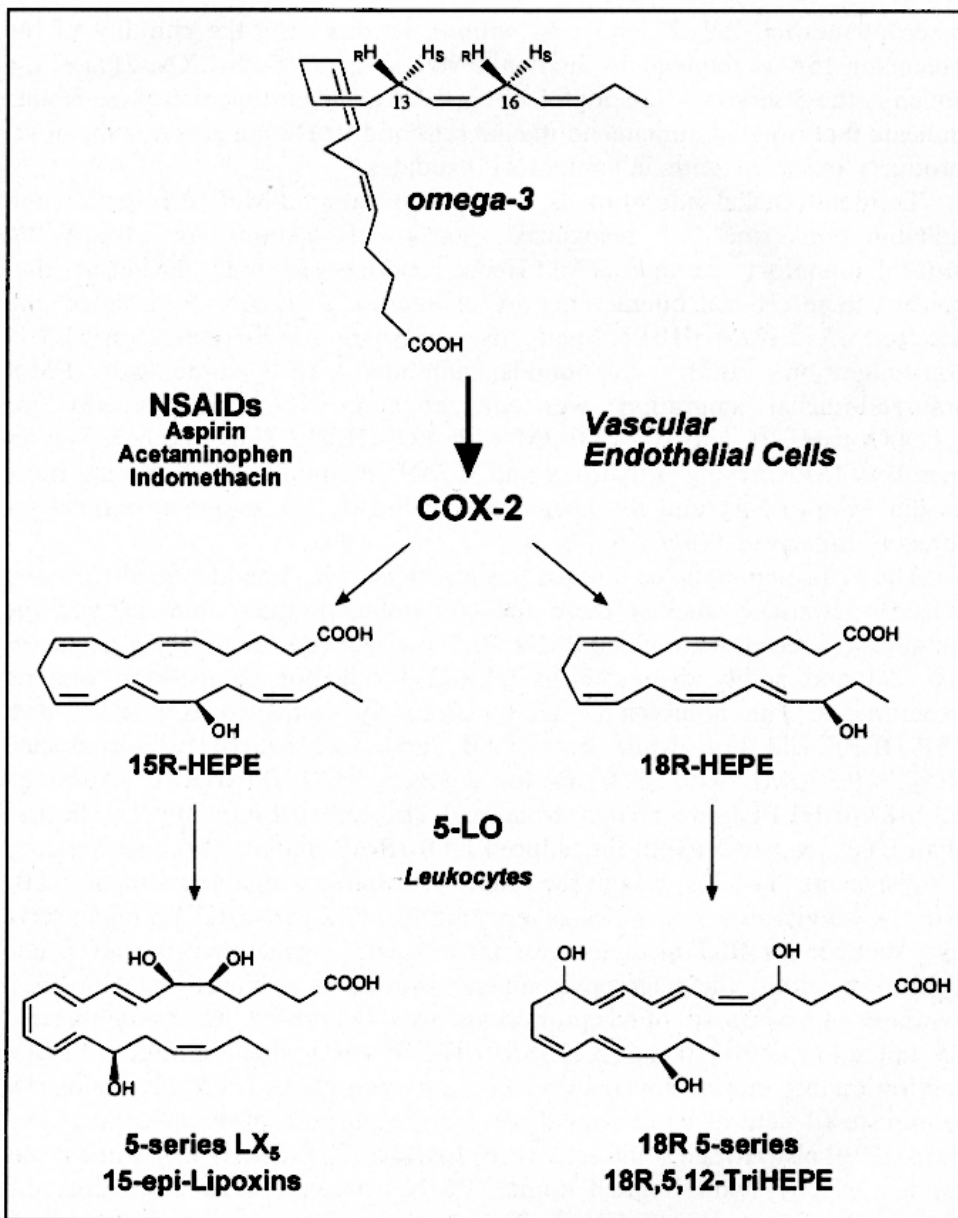


Fig. 1. Proposed Scheme for Generating Functional Arrays of Lipid Signals from ω -3 PUFA: An Example of Endogenous Inhibitors of Microinflammation.

In inflammatory cells with upregulated COX-2 treated with NSAIDs, PG formation from C20:4 is blocked and available ω -3 PUFA are converted via a COX-2-NSAID lipoxygenase-type mechanism with stereospecific hydrogen abstraction at C16 or C13 in C20:5 to give *R* insertions of molecular O₂ (e.g. 15*R*-HEPE or 18*R*-HEPE) products that signal with cells within the local microenvironment, inhibiting recruitment (see text for details). The complete stereochemistry of the novel trihydroxy products remains to be determined.

macrophages (cf. Ref. 25 and refs. within). In this case, the chirality of the precursor 15R is retained by human PMN to give 15-epi-LXA₅ (Panel D), which is the 5-series ω-3 analog of 15-epi-LXA₄. Taken together, these results indicate that isolated human endothelial cells and PMN can generate the novel products observed with inflammatory exudates.

Transendothelial migration is a pivotal event in PMN recruitment and inflammation and a recognized locus of action for traditional anti-inflammatory therapies (26). Hence, endogenous lipid mediators that control these cell-cell interactions are of interest. Therefore we isolated and assessed 5,12,18R-triHEPE and its precursor 18R-HEPE on PMN transmigration. Both compounds inhibited LTB₄-stimulated PMN transendothelial migration with an apparent IC₅₀ at 50 nM for 5,12,18R-triHEPE and IC₅₀ 1.0 μM with 18R-HEPE. Thus, the new 5-series members 18R-carrying trihydroxy and 18R-HEPE inhibited PMN migration, as did 15-epi-LXA₄ and its omega end analog (9, 15), tested in parallel for direct comparison (Table 1).

The G protein-coupled surface receptor for LTB₄ was identified (27), and thus to determine whether these 18R-containing products interact with the human BLT receptors to block PMN, BLT was cloned from reported sequences (10, 27) and stably expressed in HEK-293 cells for competition binding experiments. The homoligand LTB₄ effectively competed IC₅₀ ≈ 2.5 nM. 18R-HEPE did not, while both LTB₅ and 5,12,18R-triHEPE competed (IC₅₀ ~ 0.5 μM), with a trend for LTB₅ > 5,12,18R-triHEPE. Although 5,12,18R-triHEPE and a related structure, LTB₅, were substantially less effective than LTB₄, consistent with the reduced LTB₅ PMN activity (24), their potency for displacing ³H-LTB₄ was in the range of currently available synthetic LTB₄ receptor antagonists (not shown), suggesting that 5,12,18R-triHEPE might serve as a damper for BLT-mediated responses *in vivo* if generated in appropriate quantities within the microenvironment as well as a biotemplate for total synthesis of new classes of receptor antagonists. Of interest, when administered i. v. tail at low levels (100 ng), 5,12,18R-triHEPE was a potent inhibitor of PMN infiltration into murine dorsal air pouches, as was a 15-epi-LX stable analog (15) administered here at equivalent doses for the purpose of direct comparison. 18R-HEPE also carried some activity *in vivo* (< 5,12,18R-triHEPE), while it was far less effective with isolated human PMN transendothelial migration and apparently did not interact with BLT at these concentrations.

In addition to ASA, other widely used NSAIDs (i. e. acetaminophen and indomethacin) were tested with human recombinant COX-2 and 20:5 (10 μM, 37°C) as in Table 1 to determine whether they altered conversion to HEPE. Each inhibited 11-HEPE by > 95%. Of interest, 18R-HEPE and 15R-HEPE formation persisted (~ 1:1 ratio) in the presence of either acetaminophen or indomethacin at concentrations as high as 2 mM, albeit the levels of 15R- and

18R-HEPE were reduced by 3–8 times their levels in the absence of inhibitors ($n = 3$). These findings indicate that the oxygenation of ω -3 fatty acids to R-containing monohydroxy- and monohydroperoxy-containing products is not restricted to ASA treatment and arachidonate and that these commonly used NSAIDs may still permit PUFA oxygenation by endothelial cells (Fig. 1).

DISCUSSION

The discovery of COX-2 has opened a new appreciation of the prostanoids and related compounds in health and disease (11) and was successfully targeted for pharmacologic interventions in several human disorders (28). Increased awareness of this enzyme has raised the possibility that, in wound healing and resolution, COX-2 can participate in the biosynthesis of anti-inflammatory mediators (i. e., PGE₂, PGD₂, or lipoxins) (29, 30). In this context, the acetylation of COX-2 by ASA treatment in vascular endothelial cells, epithelial cells and at inflammatory sites *in vivo* initiates the biosynthesis of 15-epi-lipoxins during cell-cell interactions that can serve as endogenous mediators of some of ASA's therapeutic actions (14). This concept is further supported by results obtained with LX and 15-epi-LX analogs (Fig. 1 and Refs. 14, 15).

Despite the many reports of ω -3 PUFA's (i. e., C20:5) beneficial impact in humans (1, 3–5), oxygenation by COX-2 to generate monohydroxy products has not been addressed in humans or isolated cells. In fish, C20:5 as well as C20:4 are mobilized and utilized in macrophages and platelets to produce both 5-series-derived eicosanoids and 4-series including PG, LT, and LX with equal abundance (25). This is not the case in humans, where C20:4 appears to be preferentially mobilized by phospholipase A₂ for conversion to 4-series eicosanoids (6, 7). Attention to EPA conversion via COX-2 may have been underappreciated because when monitored by O₂ consumption alone COX-1 cells expressing COX-2 with C20:5 gives ~ 20% of C20:4 O₂ consumption (31). However, with fetal calf aorta Powell and Gravelle (32) showed that C20:5 has a higher affinity for cyclooxygenase C20:5 > C20:4. COX-2 is an integral membrane protein that resides with endoplasmic reticulum in fibroblasts (33). Yet, the relationship and/or role of membrane bilayer contributions to substrate channel presentation of ω -3 PUFA with COX-2 and their governance by NSAIDs or COX-2 selective inhibitors remain to be established *in situ*.

Our recent findings indicate that inflammatory exudates (Serhan *et al.*, submitted, 2000) from mice treated with ASA and EPA generate novel products (Fig. 1) that are also produced by human endothelial cells and PMN. Given the mg to g amounts of ω -3 PUFA taken as dietary supplements (1, 2) and the large area of the microvasculature in humans lined by endothelial cells, the conversion from EPA by these and neighboring cells as observed in our

experiments is likely to represent significant amounts at local microenvironments, especially in inflamed or diseased tissues where COX-2 will be focused and a determinant that impacts metabolism (12) when NSAIDs might be of therapeutic benefit, namely microinflammation. Analogous to 15-epi-LX generation, EPA COX-2-derived 15R-HEPE is converted by 5-lipoxygenation and 5(6)epoxide formation in leukocytes to 15-epi-LX₅ (Fig. 1). The stable analogs of 15-epi-LXA₄, modified at their C15 position through position 20 with bulky groups, resist inactivating enzymes and are more potent *in vivo*, inhibiting PMN traffic as well as formation and actions of key proinflammatory cytokines (9, 15, 34). Hence, the 5-series 15-epi-LX should act in a similar fashion since they possess a $\Delta 17-18$ double bond as the only modification from 15-epi-LXA₄ and thus can function as an ω -3-derived 15-epi-LX analog.

This novel 18R oxygenation led to bioactive products *in vivo* that blocked PMN infiltration and transendothelial migration, providing a basis for a novel mechanism of action for assessing the clinical utility of NSAIDs and dietary ω -3 supplementation, namely the endogenous generation of functional arrays of new lipid signals (Table 1, Fig. 1). These new lipids that carry information could, by inhibiting key events in microinflammation (e.g., adhesion and transmigration), mediate some of the beneficial actions noted earlier for ω -3 treatments in human trials (1, 3-5). In this context, 13-HODE, a lipoxygenase product that downregulates platelet-endothelial cell interactions (35), is also generated by COX-2 (Table 1), and joins the lipid signaling array. The identification of these novel ω -3 processing routes governed by cell-cell interactions and impact of NSAIDs opens new avenues for ω -3 supplementation in the generation of local endogenous mediators that control microinflammation, namely signal events at the postcapillary venules between leukocytes and vascular endothelial cells. In addition, these findings offer a basis to circumvent unwanted effects of current anti-inflammatory therapies as well as potential biochemical indices and/or markers of effective dietary n-3 supplementation.

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