Published in final edited form as:

Biochim Biophys Acta. 2011 November ; 1811(11): 737–747. doi:10.1016/j.bbali.2011.06.007.

Chiral Lipidomics of E-Series Resolvins: Aspirin and the Biosynthesis of Novel Mediators

Sungwhan F. Oh, Thad W. Vickery, and Charles N. Serhan

Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Harvard Medical School, Harvard Institutes of Medicine, 77 Avenue Louis Pasteur, Boston, MA 02115, USA

Sungwhan F. Oh: soh@zeus.bwh.harvard.edu; Thad W. Vickery: tvickery@zeus.bwh.harvard.edu

Abstract

Control of the inflammatory response is of wide interest given its important role in many diseases. In recent years we identified novel mechanisms and lipid mediators that play an active role in stimulating the resolution of self-limited acute inflammation. These novel pro-resolving mediators include the essential fatty acid-derived lipoxins, resolvins, protectins and maresins. Members of each possess a unique pro-resolving mechanism of action; each limits neutrophilic infiltration, regulates local mediators (chemokines, cytokines) as well as stimulates macrophage-enhanced clearance of apoptotic PMN, cellular debris and microbes. Given this unique mechanism of action, resolvins have already been shown to play pivotal roles in regulating key events in a wide range of experimental inflammatory diseases. These pro-resolving mediators also provide a molecular link between omega-3 essential fatty acids (e.g. EPA, DHA) and the resolution process of inflammation and tissue homeostasis. Here, we review recent evidence obtained using chiral LC-MS-MS-based lipidomics to identify a novel 18S-series of resolvins derived from EPA. Resolvin E1 possesses potent actions in vivo and in vitro demonstrated now in many laboratories, and herein we review comparisons in E-series resolvin biosynthesis and action of 18S-resolvin E1 and 18S-resolvin E2. The biosynthesis and formation of both 18S and 18R-series are enhanced with aspirin treatment and involve the utilization of dietary EPA as well as recombinant human 5-lipoxygenase and LTA₄ hydrolase in their stereospecific biosynthesis. Herein we also demonstrate the utility of LC-MS-MS-based lipidomics in identifying resolvins, protectins and related products in marine organisms such as *Engraulis* (Peruvian anchovy). These new findings emphasize the utility of chiral LC-MS-MS lipidomics and the potential for identifying new resolution circuits with chiral LC-MS-MS-based lipidomics and metabolomics.

Keywords

Resolution; inflammation; omega-3 fatty acids; leukocytes; nutrition

© 2011 Elsevier B.V. All rights reserved.

Corresponding Author: Prof. Charles N. Serhan, Director, Center for Experimental Therapeutics, Harvard Institutes of Medicine, 77 Avenue Louis Pasteur, HIM 829, Boston, MA 02115-5727, Tel.: 617-525-5001, Fax: 617-525-5017, cnsrhan@zeus.bwh.harvard.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

The acute inflammatory response is a protective self-limited response of the host activated by invading microbes and/or tissue injury. The host tightly regulates the orchestration of the cellular and biochemical events in a timely manner to restore homeostasis. Hence the ideal outcome, or inflammatory resolution, is a coordinated active process, that produces specialized pro-resolving mediators (SPM) identified in the resolution phase which act locally to stimulate the tissue reorganization and return to homeostasis [1]. Excessive and uncontrolled inflammation is now appreciated to be a key component in the pathophysiology of many widely occurring diseases such as cardiovascular diseases, metabolic disorders, as well as cancers [2].

Hence, the endogenous control mechanisms for the acute inflammatory response and the potential for novel anti-inflammatory and pro-resolving agents is of wide interest as new approaches are needed since the current pharmacopeia is not without unwanted side effects [1]. For example, aspirin (acetyl salicylic acid, ASA) is well known to dampen pro-inflammatory signals and more recently was demonstrated to jump-start resolution of acute inflammation [3]. The mechanism involves acetylation of cyclooxygenase-2 (COX-2) by aspirin. The aspirin-treated enzyme transforms its catalytic activity to a 15-lipoxygenase (15-LOX)-like reaction [4] that biosynthesizes 15*R*-hydroxyecosatetraenoate (15*R*-HETE) from arachidonic acid (AA) at the expense of prostaglandin production. This leads to the production of potent aspirin-triggered 15-epi-lipoxins (15*R*-LX) that are anti-inflammatory and pro-resolving mediators generated during cell-cell interactions in humans by transcellular biosynthesis. These aspirin-triggered lipoxins (ATLs) [5] bear *R* configuration at carbon 15 that is epimeric to the original 15- lipoxygenase-initiated lipoxin biosynthesis pathway that produces LX that carry their carbon 15 alcohol in the *S* configuration [6]. The ATLs are more resistant to metabolic inactivation than lipoxins [7] and also possess anti-inflammatory and pro-resolving actions in experimental animal inflammatory disease models, such as dermal inflammation [8], periodontitis [9] and inflammatory angiogenesis [10]. Their novel pro-resolving actions in vivo and biosynthesis as well as the resolvins were recently reviewed in ref. [11]. Also readers interested in the vascular actions of the pro-resolving mediators are directed to literature recently reviewed in [12] and in the pathophysiologic roles of these mediators and their potential role in inflammatory diseases, recently reviewed in ref. [13].

Bioactive SPM are also generated from essential omega-3 (n-3) polyunsaturated fatty acids (PUFA) such as EPA and DHA [1]. EPA and DHA are important in the human diet, and in a well-balanced inflammatory response [reviewed in refs. 14, 15]. The SPM including the resolvins, protectins and maresins together comprise a novel chemical genus of endogenous mediators characterized in this laboratory that exert potent anti-inflammatory and pro-resolution actions [reviewed in refs. 12, 13].

As in lipoxin biosynthesis triggered by aspirin, aspirin-triggered D-series resolvins (AT-RvDs) were also identified that carry position 17 in the *R* configuration [16]. In the case of EPA-derived E-series resolvins, the complete stereochemical structure of endogenous RvE1 was assigned and confirmed via total organic synthesis [17]. Also, in the original studies the E-series resolvins biosynthesis mechanism was partially elucidated in this laboratory [16, 18]. We recently reported the contribution of aspirin in E-series resolvins biosynthesis and identity of downstream enzymes involved in the biosynthesis of EPA-derived (E-series) resolvins with focus on the stereospecific and regiospecific products [19]. For this special issue, we review the LC-MS-MS-based lipidomics used to investigate the biosynthetic pathway and enzymes involved in the biosynthesis of novel E-series resolvins that were not apparent earlier without chiral lipidomics. Using chiral lipid mediator metabolomics, we

also analyzed samples obtained from subjects taking aspirin and EPA, which permitted the identification of 18*S*-HEPE as a precursor to novel 18*S* E series resolvins.

2. Chiral Lipidomics

Figure 1 shows the biosynthetic scheme for RvE1 biosynthesis [17, 18] and new 18*S*HEPE conversion to epimeric RvE1 and RvE2 identified and confirmed by using isolated human recombinant 5-LOX and LTA4H which is known as pro-inflammatory LTB₄-synthesizing enzymes. The original isolation and identification of RvE1 from the murine air pouch inflammatory exudate established the potent bioactions of this compound as a 5,12,18*R*-triHEPE biosynthesized from EPA that possessed endogenous anti-inflammatory actions with aspirin-treated mice [20]. The chirality at carbon 18 position was found to be 18*R* within the inflammatory exudates rather than 18*S* [16, 17, 20], likely because of its resistance to further inactivation of the 18*R* epimer and hence longer bio-half-life [19]. In addition to those findings, we recently reported that 5-LO-mediated oxygenation/epoxidation take place by using 18-HEPE as substrate, generating RvE2, RvE1 and their 18*S*-epimers by human PMN [18, 19]. Human 5- LOX is largely non-discriminatory to the stereochemistry at carbon 18 so it can convert both 18*R*- and 18*S*-HEPE. To investigate the molecular mechanism of actions for the new 18*S*-E series resolvins, we compared ligand-receptor interactions and metabolic inactivation of 18*S*RvE1 with RvE1. The pathophysiologic actions of 18*S*-E series resolvins were assessed using *E. coli* murine peritonitis and phagocytosis of *E. coli* as well as apoptotic PMN by human macrophages [19]. To elucidate the biosynthesis and functions of new E-series resolvins, we employed chiral column lipidomic profiling of the resolvins and lipoxin precursors (Figure 2), which are produced in mammalian and fish systems *vide infra*. Reverse-phase-based chiral HPLC-MS-MS lipidomics achieved baseline separation of all tested stereoisomer pairs (5-, 12-, 15- and 18-*R/S*-HEPEs) derived from EPA (Figure 2 and Table 1).

To profile E-series resolvins present within human samples, peripheral blood from healthy donors was obtained and subject to analysis (Figure 3). For these, we used both conventional high-sensitivity quantitation and chiral LC-MS-MS (to determine enantiomer ratios). Chiral lipidomic profiling gave separation of both 18*R*-HEPE and 18*S*-HEPE from serum (Figure 3A). In order to detect and quantify each positional isomer without ambiguity, a multiple reaction monitoring (MRM) method was established. The signature daughter ions for each standard HEPE (parent *m/z* 317) are as follows; 18-HEPE, 259; 12-HEPE, 219; 12-HEPE, 179; 5-HEPE, 115.

For each enantiomer pair illustrated in Figure 2, the *R* isomer eluted before *S* isomers using this column. Each signature ion for each species was unique; only two (*R* and *S* isomers) peaks are present on each extracted ion chromatogram. The two 18-HEPE enantiomers gave essentially indistinguishable tandem mass spectra with fragments at *m/z* 317 (*M-H*), 299 (*M-H*₂O), 273 (*M-CO*₂) and 255 (*M-CO*₂-H₂O) as well as the diagnostic ion at *m/z* 291 (Figure 3B).

Baseline serum 18-HEPE levels remained low (26.4±5.0 pg/mL) for healthy subjects without EPA supplementation and aspirin. Samples collected 3h later from volunteers taking 1 g of EPA showed elevated levels of 18-HEPE. Quantitative lipidomic profiling using chiral HPLC revealed that the 18*R*-HEPE isomer (see Figure 1) was dominant to 18*S*-HEPE in all human blood samples when only EPA was taken. In contrast, in those aspirinated individuals before EPA administration had more 18*S*-HEPE (27.7±7.8 pg/mL with EPA vs. 56.5±19.0 pg/mL with EPA and aspirin) [19]. These results suggested that aspirin might promote 18*S*-HEPE production as well as 18*R*-HEPE from ingested EPA.

To address this possibility, we tested human recombinant COX-2 role in this process. Aspirin-treated human COX-2 preferentially synthesized 15*R*-HEPE and 18*S*-HEPE, which were identified and quantified using chiral-LC-MS-MS (Figure 3C). These results were consistent with murine air pouch exudate lipidomic analysis, where aspirin treatment both increased total 18-HEPE biosynthesis (~6-fold) and shifted the ratio to more *S*-preferred (*R*/*S* ratio of 1.5:1 to 1:1) see Figure 4. These results indicate that aspirin-treated COX-2 is responsible for production of both 18*S*- and 18*R*-HEPE from EPA with isolated human recombinant COX-2 [19]. Actions of other NSAIDs on resolvins production have been tested at several different levels. Indomethacin and acetaminophen permit 18*R*-HEPE production by human recombinant COX-2 [20]. In contrast, selective COX-2 inhibitor blocks not only PGE₂ but also LXA₄ production in vivo; hence it is 'resolution-toxic' [21].

3. Role of 5-LOX in 18*S* E-Series Resolvins

We also assessed substrate preferences with 18*R*- and 18*S*-HEPE in the conversion to *E*-series resolvins. For analysis of the stereospecific preference, racemic mixtures of 18*R*- and 18*S*-HEPE were incubated with 5-LOX in the presence of required 5-LOX activators [18], followed by reduction of 5*S*-hydroperoxide to RvE2 (see Figure 1) and analysis by both conventional and chiral LC/MS/MS as in Figure 2. Two main 5-LOX products from racemic 18-HEPE were identified and assigned as 5,18-dihydroxyEPE on the bases of UV chromatogram at 236nm (presence of a conjugated diene chromophore) and selective ion monitoring *m/z* 333→199 (Figure 4). Both of these had essentially the same fragments at *m/z* 333 (*M-H*), 315 (*M-H-H*₂O), 297 (*M-H-2H*₂O), 271(*M-H-CO*₂) and 253 (*M-H-2H*₂O-*CO*₂) along with signature ions at *m/z* 275, 257 (275-*H*₂O) and 199 (217-*H*₂O).

The stereochemistry of the alcohol at carbon 18 was further confirmed by matching with biogenic RvE2 prepared from stereochemically pure 18*R*- or 18*S*-HEPE. These findings gave direct evidence that 18*S*-HEPE is utilized as a substrate by the isolated human recombinant 5- LOX to produce a hydroperoxy intermediate in 18*S*-resolvin biosynthesis (see Figure 1). Human 5-LOX can further convert this 5*S*-hydroperoxy,18-hydroxyeicosapentaenoate intermediate to a 5*S*(6)-epoxide-containing product [18], which becomes the direct precursor of RvE1. Hydrolysis of this allylic triene-epoxide is the key step for RvE1 biosynthesis to form the 6*Z*,8*E*,10*E*-triene conjugation present in RvE1 that is required for its bioactivity [17]. Without intact enzymes, the 5(6)-epoxide intermediate was converted to either 6*E*,8*E*,10*E*-isomers or 5,6,18- trihydroxyeicosapentaenoate by non-enzymatic hydrolysis [19] but not to RvE1 (Figure 5A). None of these matched the retention time of RvE1 also *cf.* Figure 5B and 5C.

4. The Role of LTA₄ Hydrolase in RvE Biosynthesis

As with other potent bioactive eicosanoids biosynthesized from arachidonate, the complete stereochemical structure of RvE1 is crucial for both ligand-receptor interactions and complete bioaction in vitro and in vivo [17, 20]. The synthetic geometric isomers of RvE1 give different chromatographic and biologic properties in that Δ6,14-*trans* RvE1 is significantly less active than RvE1 [17]. Bioactivity of other non-enzymatic hydrolysis products identified from these incubations such as 5,6,18-triHEPE (Figure 5) was assessed, and when directly compared to RvE1 at the same 20 ng/mouse doses in murine peritonitis, the isomer 5,6,18-triHEPE did not reduce neutrophil infiltration, whereas RvE1 reduced neutrophilic infiltration (39.9±5.1%). The conjugated addition-type hydrolysis of the 5*S*(6)-epoxy-6*E*,8*E*,10*E*-triene to 5*S*,12*R*-dihydroxy- 6*Z*,8*E*,10*E*-triene is a key step of RvE1 biosynthesis. Hence, given the structural similarity between the 5*S*(6)-epoxy-resolvin intermediate from EPA and that of LTA₄ from arachidonate (see Figure 1), we hypothesized that a specific enzymatic reaction is required, namely a hydrolase, as in LTB₄ biosynthesis

[22]. To this end, we next assessed the role and contribution of LTA₄ hydrolase (LTA4H) in the biosynthesis of RvE1.

This was addressed by obtaining several lines of evidence. First, since human PMN biosynthesize RvE1 and RvE2 from 18-HEPE [18], RvE1 production by PMN was tested in the presence of the LTA4H inhibitor bestatin [23]. When incubated with bestatin, RvE1 biosynthesis by isolated human PMN was decreased by ~65% (Figure 5). The combinatorial incubation of these two recombinant enzymes i.e. 5-LOX and LTA4H gave RvE1 that was assigned by matching with synthetic standard for RvE1. The retention time and diagnostic ions of *m/z* 291, 205 and 195 were confirmed from combinatorial incubation products (Figure 5C). In contrast, those incubations containing only 5-LOX generated nearly undetectable amounts of RvE1. These results indicate that LTA4H contributes to RvE1 biosynthesis [see ref. 19 for further details].

5. 18S-RvE1 with RvE1: Receptor Activation and Metabolic Inactivation

The actions and inactivation of both resolvins were assessed in systems where RvE1 is active. RvE1 interacts with at least two different GPCRs present on leukocytes, ChemR23 [17] and BLT1 [24], so the affinity of RvE1 and its 18S-epimer was directly compared using recombinant GPCR expressed in CHO cells. Employing a beta-arrestin overexpression system to examine these GPCRs, 18S-RvE1 gave an EC₅₀ of 6.33×10^{-12} M derived from fitted dose-response curves. This value is lower than that obtained with RvE1 which gives an EC₅₀ of 1.37×10^{-10} M with ChemR23-overexpressing cells. 18S-RvE1 also antagonized LTB₄-mediated BLT1 activation at higher potency and efficacy than that of RvE1. These results suggest that 18S-RvE1 can share the same site(s) of action as RvE1 *in vitro* with apparently higher affinity. In contrast to higher efficacy at the molecular level, 18S-RvE1 was more rapidly converted to the inactive 18-oxo-RvE1 (identified earlier in lung tissue and inflammatory exudates [25] by NAD-dependent dehydrogenase compared to RvE1 [19].

6. Bioactions of 18S-E Series Resolvins

To compare the actions of 18S-E series resolvins we directly assessed both chemically and biogenically prepared epimers including 18S-RvE1, RvE1, 18S-RvE2 and RvE2. Direct comparisons between 18R- and 18S- isomers were carried out in zymosan-initiated acute murine peritonitis. 18S-RvE1 reduced PMN infiltration at a similar potency as RvE1 and RvE2, whereas 18S-RvE2 gave a trend toward significantly reducing neutrophil infiltration in this *in vivo* system [19]. We also confirmed the bioactions of 18S-RvE1 in two additional settings. First, using doses from 1ng to 100ng, 18S-RvE1 was compared with RvE1 in murine peritonitis. At each dose, the 18S-epimer gave similar efficacy to RvE1. Second, using the murine dorsal air pouch skin model we also examined the counterregulation of TNF- α mediated neutrophil infiltration *in vivo*. At the same 20 ng doses, 18S-RvE1 displayed similar potency to RvE1 in this system giving 30% vs. 48% decreases between the two epimers. In addition to reducing PMN, RvE1 also stimulates resolution by enhancing phagocytosis and clearance of invaded microbes, apoptotic neutrophils and other cellular debris [1, 3, 21]. To assess macrophage-directed pro-resolving bioactions, 18S-RvE1 was again directly compared to RvE1-enhanced zymosan as well as *E. coli* phagocytosis using murine resident macrophages. The 18S-RvE1 gave a similar bell-shaped dose-response curve compared to RvE1. At lower concentrations, 18S-RvE1 was essentially equipotent to RvE1; at concentrations > 1nM, RvE1 was more potent than 18S-RvE1. Similar results were obtained for the phagocytosis of live *E. coli*: both RvE1 and 18S-RvE1 enhanced phagocytosis, showing maximal increases at 1nM [19].

These results were supported by *in vivo* results obtain from experiments with *E. coli*-initiated peritonitis. Enhancement of bacterial clearance was tested by two different time

intervals and using direct routes of administration during an inflammatory time-course. When both RvE1 and 18S-RvE1 were directly delivered to peritoneum, each enhanced phagocytosis of *E. coli* by resident macrophages. For example, 18S-RvE1 gave 61.4% vs. RvE1, which gave 55.0% enhancement. When RvE1 or 18S-RvE1 was given i.v. 3 hours after peritoneal injection of *E. coli*, both products reduced neutrophilic infiltration in the resolution phase. In this setting RvE1 gave more significant reductions than the 18S-RvE1 [19]. This difference in activity between RvE1 and 18S-RvE1 *in vivo* proved to be more apparent at longer time intervals during resolution. That is, when RvE1 was given at the maximal time point of PMN infiltration (i.e. 8h after zymosan injection), further decreases in PMN numbers were found 12 hours later [19]. In this system, the 18S-RvE1 only modestly expedited removal of PMN from the murine peritoneum compared to RvE1 that significantly reduced ~78% the number of remaining PMN at the inflammatory site *in vivo*. With *E. coli* peritonitis, RvE1 regulated inflammation and reduced pro-inflammatory cytokines *in vitro* and *in vivo* [19].

7. Marine Omega-3-Essential Fatty Acid-Derived Mediators

Earlier studies from Rowley and colleagues showed that, upon infection, rainbow trout produce substantial quantities of prostaglandins, leukotrienes and lipoxins from endogenous sources of arachidonic acid [26]. The rainbow trout also produce lipoxin A₅ from EPA and lipoxin A₄ from arachidonic acid as well as leukotriene B₄ and leukotriene B₅ from EPA [27]. These findings open the possibility that fish, in addition to utilizing arachidonic acid, also utilize essential fatty acids such as EPA and DHA to biosynthesize potent local-acting autacoids. Along these lines, following the original identification of the resolvins and protectins in murine and human systems, we also tested whether rainbow trout neural and hematopoietic organs produce endogenous resolvins and protectins from EPA and DHA. Indeed, rainbow trout organs, in addition to having EPA and DHA, also possess the biosynthetic pathways to produce both resolvins and protectins within bioactive levels found in other systems [28]. Since omega-3 fatty acids play an essential role in human health and nutrition [29] and humans obtain a substantial portion of their omega-3 essential fatty acids from marine oils [14, 30], we questioned whether other marine organisms rich in omega-3 fatty acids such as anchovies [31] also possess these potent bioactive mediators identified in mammalian systems from DHA. To this end, we obtained anchovies and extracted their lipids using solid phase C18 extraction. In Figure 6, we identify monohydroxy acids from DHA that are signatures for the lipoxygenase pathways, for example, 7-hydroxy-docosahexaenoic acid (a 5-LOX product in humans), 14-hydroxy-docosahexaenoic acid (a major 12-LOX product in human tissues) and 17-hydroxy-docosahexaenoic acid (a 15-LOX pathway marker in mammalian tissues) as well as several isobaric epoxides of DHA. In addition, Figure 6B shows the presence of both protectin D1 (NPD1/PD1) as well as resolvin D5 in samples obtained from anchovies. These results suggest that the pathways to produce resolvins, protectins as well as other potent bioactive mediators in mammalian systems are present in a marine organism that is widely used to isolate marine oils, namely anchovies. This suggests that, in addition to having potential bioactive roles within the organism, these mediators could also enter the food chain. Given their potent actions in many murine and human systems ranging in the picogram to nanogram range, it is possible that their dietary intake can have an impact in these systems. NPD1/PD1 has potent actions in regulating inflammatory response and its resolution as well as in ocular systems and retinal pigmented epithelial cells [32–34]. Together, these findings also open the potential to examine the role of EPA- and DHA-derived mediators in marine organisms.

8. Conclusions

The resolvins have been shown to have potent actions in many animal models of disease relevant to human disorders, as recently reviewed in refs. [11, 12]. To add to these recent findings, resolvin E1 regulates inflammatory pain by both central and peripheral actions [35]. Its receptor ChemR23 is present in dorsal root ganglion in addition to leukocytes. RvE1 signaling involves a series of phosphorylation events that can enhance, for example, phagocytosis, one of the key pro-resolving actions of RvE1 [36] and as also reviewed here for the 18S-RvE1. New actions of RvE1 have recently been uncovered, and it has been shown that RvE1 can regulate key antimicrobial peptides such as LL-37 [37] as well as control *Herpes simplex* virus lesions [38].

As reviewed recently [12], RvE1 has potent actions within the vasculature in addition to its actions on leukocytes. In this regard, RvE1 has been shown to protect against ischemia-reperfusion injury in rat heart [39]. In each of these settings, the actions of resolvin E1 have proven to be potent and selective. Together they add to the body of literature suggesting that stimulation of endogenous resolution pathways may represent a useful new therapeutic approach.

The many anti-inflammatory agents in use today have shown a dramatic impact in the treatment of human disease and daily ailments, yet many of them still have unwanted side effects [40], and as such new approaches are needed. In this regard, resolvins and pro-resolving mediators represent a new approach. The potent actions of RvE1 have been demonstrated in comparison with standard anti-inflammatory approaches in a number of laboratories in addition to the authors', including Navarro-Xavier et al. [41]. Also, the ability of aspirin to activate the endogenous biosynthesis of aspirin-triggered lipid mediators has been documented in humans in a trial setting; the endogenous aspirin-triggered lipid mediators in turn regulate leukocyte responses [42, 43]. Hence, the use of LC-MS-MS-based lipidomics to document novel pathways and the impact of therapeutics within metabolic pathways hold much promise in identifying novel mechanisms in the regulation of inflammation and in the new terrain of pro-resolving circuitry. The chiral LC-MS-MS-based lipidomics and its uses to evaluate the impact of aspirin and EPA reviewed here open a wide range of opportunities to investigate in human translation the cause-and-effect relationship between dietary omega-3 fatty acids, low-dose aspirin and a clinical endpoint and/or biomarker.

Along these lines, in earlier studies it was found that RvE1 and lipoxin A₄ regulate the inflammation associated with periodontal disease, stimulating resolution and tissue regeneration [44]. Such studies suggest that increasing dietary supplementation may lead to an increase in resolvin E1 *in vivo*. Recently, Van Dyke and colleagues have reported a preliminary clinical study, which suggests that dietary supplementation with omega-3 polyunsaturated fatty acids along with a daily dose of 81 mg aspirin reduces the clinical signs of periodontal disease [45]. These new clinical results are promising and together with chiral LC-MS-MS lipidomics reviewed here could provide direct cause-and-effect relationships in future studies of the *in vivo* production of omega-3-derived mediators, their origins and impact in resolution and the pathogenesis of disease mechanisms.

Acknowledgments

We thank Mary H. Small for skillful manuscript preparation. The study reviewed herein was supported in part by the National Institutes of Health Grants DE019938 and GM38765.

Abbreviations

RvE1	resolvin E1, 5 <i>S</i> ,12 <i>R</i> ,18 <i>R</i> -trihydroxy-6 <i>Z</i> ,8 <i>E</i> ,10 <i>E</i> ,14 <i>Z</i> ,16 <i>E</i> -eicosapentaenoate
RvE2	resolvin E2, 5 <i>S</i> ,18 <i>R</i> -dihydroxy-6 <i>E</i> ,8 <i>Z</i> ,11 <i>Z</i> ,14 <i>Z</i> ,16 <i>E</i> -eicosapentaenoate
PD1	protectin D1, 10 <i>R</i> ,17 <i>S</i> -dihydroxy-4 <i>Z</i> ,7 <i>Z</i> ,11 <i>E</i> ,13 <i>E</i> ,15 <i>Z</i> ,19 <i>Z</i> -docosahexaenoate
RvD5	resolvin D5, 7 <i>S</i> ,17 <i>S</i> -dihydroxy-4 <i>Z</i> ,8 <i>E</i> ,10 <i>Z</i> ,13 <i>Z</i> ,15 <i>E</i> ,19 <i>Z</i> -docosahexaenoate

References

1. Serhan CN, Chiang N. Endogenous pro-resolving and anti-inflammatory lipid mediators: a new pharmacologic genus. *Br J Pharmacol*. 2008; 153(Suppl 1):S200–215. [PubMed: 17965751]
2. Serhan, CN.; Ward, PA.; Gilroy, DW. *Fundamentals of Inflammation*. Cambridge University Press; New York: 2010.
3. Serhan CN. Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Annu Rev Immunol*. 2007; 25:101–137. [PubMed: 17090225]
4. Holtzman MJ, Turk J, Shornick LP. Identification of a pharmacologically distinct prostaglandin H synthase in cultured epithelial cells. *J Biol Chem*. 1992; 267:21438–21445. [PubMed: 1400457]
5. Claria J, Serhan CN. Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions. *Proc Natl Acad Sci U S A*. 1995; 92:9475–9479. [PubMed: 7568157]
6. Marcus AJ, Broekman MJ, Safier LB, Ullman HL, Islam N, Serhan CN, Weissmann G. Production of arachidonic acid lipoxygenase products during platelet-neutrophil interactions. *Clinical Physiology & Biochemistry*. 1984; 2:78–83. [PubMed: 6435929]
7. Serhan CN, Maddox JF, Petasis NA, Akritopoulou-Zanze I, Papayianni A, Brady HR, Colgan SP, Madara JL. Design of lipoxin A4 stable analogs that block transmigration and adhesion of human neutrophils. *Biochemistry*. 1995; 34:14609–14615. [PubMed: 7578068]
8. Takano T, Clish CB, Gronert K, Petasis N, Serhan CN. Neutrophil-mediated changes in vascular permeability are inhibited by topical application of aspirin-triggered 15-epi-lipoxin A₄ and novel lipoxin B₄ stable analogues. *J Clin Invest*. 1998; 101:819–826. [PubMed: 9466977]
9. Pouliot M, Clish CB, Petasis NA, Van Dyke TE, Serhan CN. Lipoxin A₄ analogues inhibit leukocyte recruitment to *Porphyromonas gingivalis*: A role for cyclooxygenase-2 and lipoxins in periodontal disease. *Biochemistry*. 2000; 39:4761–4768. [PubMed: 10769133]
10. Fierro IM, Kutok JL, Serhan CN. Novel lipid mediator regulators of endothelial cell proliferation and migration: Aspirin-triggered-15*R*-lipoxin A₄ and lipoxin A₄. *J Pharmacol Exp Ther*. 2002; 300:385–392. [PubMed: 11805195]
11. Bannenberg G, Serhan CN. Specialized pro-resolving lipid mediators in the inflammatory response: an update. *Biochim Biophys Acta*. 2010; 1801:1260–1273. [PubMed: 20708099]
12. Spite M, Serhan CN. Novel lipid mediators promote resolution of acute inflammation: impact of aspirin and statins. *Circulation Res*. 2010; 107:1170–1184. [PubMed: 21071715]
13. Serhan CN. Novel resolution mechanisms in acute inflammation: to resolve or not? *Am J Pathol*. 2010; 177:1576–1591. [PubMed: 20813960]
14. Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med (Maywood)*. 2008; 233:674–688. [PubMed: 18408140]
15. Calder PC. The relationship between the fatty acid composition of immune cells and their function. *Prostaglandins Leukot Essent Fatty Acids*. 2008; 79:101–108. [PubMed: 18951005]
16. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, Moussignac RL. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *J Exp Med*. 2002; 196:1025–1037. [PubMed: 12391014]

17. Arita M, Bianchini F, Aliberti J, Sher A, Chiang N, Hong S, Yang R, Petasis NA, Serhan CN. Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. *J Exp Med*. 2005; 201:713–722. [PubMed: 15753205]
18. Tjonahen E, Oh SF, Siegelman J, Elangovan S, Percarpio KB, Hong S, Arita M, Serhan CN. Resolvin E2: identification and anti-inflammatory actions: pivotal role of human 5-lipoxygenase in resolvin E series biosynthesis. *Chem Biol*. 2006; 13:1193–1202. [PubMed: 17114001]
19. Oh SF, Pillai PS, Recchiuti A, Yang R, Serhan CN. Novel 18S E-series resolvins, stereoselective biosynthesis and pro-resolving actions in human leukocytes and murine inflammation. *J Clin Invest*. 2011; 121:569–581. [PubMed: 21206090]
20. Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. *J Exp Med*. 2000; 192:1197–1204. [PubMed: 11034610]
21. Schwab JM, Chiang N, Arita M, Serhan CN. Resolvin E1 and protectin D1 activate inflammation-resolution programmes. *Nature*. 2007; 447:869–874. [PubMed: 17568749]
22. Shimizu T, Radmark O, Samuelsson B. Enzyme with dual lipoxygenase activities catalyzes leukotriene A4 synthesis from arachidonic acid. *Proc Natl Acad Sci U S A*. 1984; 81:689–693. [PubMed: 6322165]
23. Mueller MJ, Samuelsson B, Haeggstrom JZ. Chemical modification of leukotriene A4 hydrolase. Indications for essential tyrosyl and arginyl residues at the active site. *Biochemistry*. 1995; 34:3536–3543. [PubMed: 7893649]
24. Arita M, Ohira T, Sun YP, Elangovan S, Chiang N, Serhan CN. Resolvin E1 selectively interacts with leukotriene B4 receptor BLT1 and ChemR23 to regulate inflammation. *J Immunol*. 2007; 178:3912–3917. [PubMed: 17339491]
25. Hong S, Porter TF, Lu Y, Oh SF, Pillai PS, Serhan CN. Resolvin E1 metabolome in local inactivation during inflammation-resolution. *J Immunol*. 2008; 180:3512–3519. [PubMed: 18292578]
26. Pettitt TR, Rowley AF, Barrow SE, Mallet AI, Secombes CJ. Synthesis of lipoxins and other lipoxygenase products by macrophages from the rainbow trout, *Oncorhynchus mykiss*. *J Biol Chem*. 1991; 266:8720–8726. [PubMed: 1902832]
27. Rowley AF, Lloyd-Evans P, Barrow SE, Serhan CN. Lipoxin biosynthesis by trout macrophages involves the formation of epoxide intermediates. *Biochemistry*. 1994; 33:856–863. [PubMed: 8305432]
28. Hong S, Tjonahen E, Morgan EL, Yu L, Serhan CN, Rowley AF. Rainbow trout (*Oncorhynchus mykiss*) brain cells biosynthesize novel docosahexaenoic acid-derived resolvins and protectins -- mediator lipidomic analysis. *Prostaglandins Other Lipid Mediat*. 2005; 78:107–116. [PubMed: 16303609]
29. Bracco, U.; Deckelbaum, RJ. Polyunsaturated Fatty Acids in Human Nutrition. Raven Press; New York: 1992.
30. Salem N Jr, Litman B, Kim H-Y, Gawrisch K. Mechanisms of action of docosahexaenoic acid in the nervous system. *Lipids*. 2001; 36:945–959. [PubMed: 11724467]
31. Gunstone, FD.; Harwood, JL.; Padley, FB. The Lipid Handbook. 2. Chapman & Hall; London: 1994.
32. Ariel A, Fredman G, Sun YP, Kantarci A, Van Dyke TE, Luster AD, Serhan CN. Apoptotic neutrophils and T cells sequester chemokines during immune response resolution via modulation of CCR5 expression. *Nat Immunol*. 2006; 7:1209–1216. [PubMed: 17013391]
33. Sheets KG, Zhou Y, Ertel MK, Knott EJ, Regan CE Jr, Elison JR, Gordon WC, Gjorstrup P, Bazan NG. Neuroprotectin D1 attenuates laser-induced choroidal neovascularization in mouse. *Mol Vis*. 2010; 16:320–329. [PubMed: 20216940]
34. Bazan NG, Calandria JM, Serhan CN. Rescue and repair during photoreceptor cell renewal mediated by docosahexaenoic acid-derived neuroprotectin D1. *J Lipid Res*. 2010; 51:2018–2031. [PubMed: 20382842]

35. Xu ZZ, Zhang L, Liu T, Park JY, Berta T, Yang R, Serhan CN, Ji RR. Resolvins RvE1 and RvD1 attenuate inflammatory pain via central and peripheral actions. *Nat Med.* 2010; 16:592–597. [PubMed: 20383154]
36. Ohira T, Arita M, Omori K, Recchiuti A, Van Dyke TE, Serhan CN. Resolvin E1 receptor activation signals phosphorylation and phagocytosis. *J Biol Chem.* 2009; 285:3451–3461. [PubMed: 19906641]
37. Wan M, Godson C, Guiry PJ, Agerberth B, Haeggström JZ. Leukotriene B₄/antimicrobial peptide LL-37 proinflammatory circuits are mediated by BLT1 and FPR2/ALX and are counter-regulated by lipoxin A₄ and resolvin E1. *FASEB J.* 2011; 25:1697–1705. [PubMed: 21307335]
38. Rajasagi NK, Reddy PBJ, Suryawanshi A, Mulik S, Gjorstrup P, Rouse BT. Controlling Herpes simplex virus-induced ocular inflammatory lesions with the lipid-derived mediator resolvin E1. *J Immunol.* 2011; 186:1735–1746. [PubMed: 21187448]
39. Keyes KT, Ye Y, Lin Y, Zhang C, Perez-Polo JR, Gjorstrup P, Birnbaum Y. Resolvin E1 protects the rat heart against reperfusion injury. *Am J Physiol Heart Circ Physiol.* 2010; 299:H153–H164. [PubMed: 20435846]
40. Dinarello CA. Anti-inflammatory agents: present and future. *Cell.* 2010; 140:935–950. [PubMed: 20303881]
41. Navarro-Xavier RA, Newson J, Silveira VLF, Farrow SN, Gilroy DW, Bystrom J. A new strategy for the identification of novel molecules with targeted proresolution of inflammation properties. *J Immunol.* 2010; 184:1516–1525. [PubMed: 20032295]
42. Morris T, Stables M, Colville-Nash P, Newson J, Bellingan G, de Souza PM, Gilroy DW. Dichotomy in duration and severity of acute inflammatory responses in humans arising from differentially expressed proresolution pathways. *Proc Natl Acad Sci USA.* 2010; 107:8842–8847. [PubMed: 20421472]
43. Rajakariar R, Hilliard M, Lawrence T, Trivedi S, Colville-Nash P, Bellingan GJ, Fitzgerald D, Yaqoob MM, Gilroy DW. Hematopoietic prostaglandin D2 synthase controls the onset and resolution of acute inflammation through PGD2 and 15-deoxyDelta12 14 PGJ2. *Proc Natl Acad Sci USA.* 2007; 104:20979–20984. [PubMed: 18077391]
44. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat Rev Immunol.* 2008; 8:249–261.
45. El-Sharkawy H, Aboelsaad N, Eliwa M, Darweesh M, Alshahat M, Kantarci A, Hasturk H, Van Dyke TE. Adjunctive treatment of chronic periodontitis with daily dietary supplementation with omega-3 fatty acids and low-dose aspirin. *J Periodontol.* 2010; 81:1635–1643. [PubMed: 20572767]
46. Serhan CN, Yang R, Martinod K, Kasuga K, Pillai PS, Porter TF, Oh SF, Spite M. Maresins: novel macrophage mediators with potent anti-inflammatory and pro-resolving actions. *J Exp Med.* 2009; 206:15–23. [PubMed: 19103881]

Highlights

- Herein we review recent evidence obtained using chiral LC-MS-MS-based lipidomics to identify a novel 18*S*-series of resolvins derived from EPA.
- We review comparisons in E-series resolvins biosynthesis and action of 18*S*-resolvin E1 and 18*S*-resolvin E2.
- The biosynthesis and formation of both 18*S* and 18*R*-series are enhanced with aspirin treatment and involve the utilization of dietary EPA as well as recombinant human 5-lipoxygenase and LTA₄ hydrolase in their stereospecific biosynthesis.

Figure 1. Proposed biosynthetic pathways for conversion of EPA to 18S and 18R E-series resolvins and their bioactions.

Figure 2.

Chiral separation and lipidomics of monohydroxy-containing products. All samples were analyzed by LC-UV-MS-MS using an HPLC system (Shimadzu LC-20AC binary pump and autosampler) coupled with a linear ion trap quadrupole mass spectrometer (3200 QTRAP; ABSciex). For chiral lipidomic profiling, a Chiralpak AD-RH column (150 mm × 2.1 mm × 5 μm) (Chiral Technologies, West Chester, PA) was used with isocratic methanol:water:acetic acid = 95:5:0.01 (v/v/v) at 0.2 mL/min flow rate. Negative ion mode multiple reaction monitoring (MRM) was used to develop a method with signature ion fragments for each molecule (see refs. [19, 46] for further details).

Figure 3.

Chiral lipidomics with 18S- and 18R-HEPE.

A) In vivo production in murine air pouch with exogenous EPA and aspirin. B) MS-MS spectra of 18-HEPE enantiomers. C) 15-HEPE and 18-HEPE profiles with COX-2 incubated with different doses of aspirin. LC-MS-MS was performed as described in the Figure 2 legend, above.

Figure 4.

Human recombinant 5-LOX converts both 18*R* and 18*S*-HEPE to direct precursors of RvE2. A) Reverse-phase and chiral separation of 18*S*- and 18*R*-resolvin E2 after 5-LOX incubation and reduction. Diastereomeric mixture of RvE2 was isolated by conventional RP-HPLC separation and further resolved by chiral HPLC. B) Tandem mass spectra of 18*R*-RvE2, 18*S*-RvE2 and C) their corresponding MS-MS assignments (see insets).

Figure 5.

Enzymatic biosynthesis is essential for RvE1 production.

A) Profile of RvE1 and related isomers. RvE1 matching with synthetic RvE1. Non-enzymatic hydrolysis products e.g. 6-*trans*,5,12,18-triHEPE or 5,6,18-triHEPE (shown with solid line) display different retention times directly compared with synthetic RvE1-spiked profile (dashed line).

B) Activated human PMN incubated with 18-HEPE with or without a LTA4H inhibitor. Representative LC-MS-MS chromatograms, transition of 349→205 monitored.

C) RvE1 LC-MS-MS spectrum obtained with recombinant human 5-LOX and LTA4H in combinatorial incubations. Retention time of this product matched with synthetic RvE1. Note that significant amounts of RvE1 are not produced with 5-LOX alone or in the presence of bestatin-treated combinatorial incubations (see text for further details).

Figure 6A

Figure 6. Profiling metabolomics of marine-omega-3-derived bioactive mediators and pathway marker.
A) monohydroxy products of DHA (HDHAs); and B) dihydroxy products PD1 and RvD5 from DHA, identification in samples obtained from anchovies.

Table 1

List of lipid mediators used for multiple-reaction monitoring (MRM) based quantitation.

Name	Parent Ion	Signature Ion
<i>Internal Standards</i>		
d8-5SHETE	327.2	116.1
d4-PGE ₂	355.2	193.1
d4-LTB ₄	339.2	197.1
<i>Eicosanoids</i>		
LXA ₄	351.2	115.1
LXB ₄	351.2	217.1
PGE ₂	351.2	189.1
PGD ₂	351.2	233.1
PGF _{2α}	353.2	193.1
20-OH-LTB ₄	351.2	195.1
LTB ₄	335.2	195.1
5,15-diHETE	335.2	235.1
15HETE	319.2	219.1
12HETE	319.2	179.1
5HETE	319.2	115.1
<i>D-series resolvins, protectins and maresins</i>		
RvD1	375.2	215.1
RvD2	375.2	175.1
PD1	359.2	153.1
RvD5	359.2	199.1
Mar1	359.2	141.1
17HDHA	343.2	245.1
14HDHA	343.2	205.1
7HDHA	343.2	141.1
<i>E-series resolvins</i>		
RvE1	349.2	195.1
RvE2	333.2	199.1
LTB ₅	333.2	195.1
5,15-diHEPE	333.2	115.1
18HEPE	317.2	259.1
15HEPE	317.2	219.1
12HEPE	317.2	179.1
5HEPE	317.2	115.1