The Novel Lipid Mediator $PD1_{n\text{-}3\ DPA}$: An Overview of the Structural Elucidation, Synthesis, Biosynthesis and Bioactions

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Abstract

Resolvins, protectins and maresins are individual families of specialized pro-resolving mediators biosynthesized from the dietary n-3 polyunsaturated fatty acids eicosapentaenoic acid and docosahexaenoic acid. These enzymatically oxygenated polyunsaturated lipid mediators were first elucidated during the resolution phase of acute inflammation in animal models of self-limited inflammation. Specialized pro-resolving mediators display potent bioactions when administrated in vivo. Biosynthetic pathway studies have revealed that individual lipoxygenases and cyclooxygenase-2 converts eicosapentaenoic acid and docosahexaenoic acid into distinct families of the resolvins, protectins and maresins. Recently n-3 docosapentaenoic acid was found to be a substrate for the biosynthesis of several novel families of specialized pro-resolving mediators. One example is $PD1_{n\text{-}3\ DPA}$. During the 6th European Workshop on Lipid Mediators, Frankfurt, Germany, the structural elucidation, total organic synthesis, studies on the biosynthetic pathway, as well as the potent anti-inflammatory and pro-resolving properties of $PD1_{n\text{-}3\ DPA}$ were presented. Herein, we provide an overview of these topics for the new member $PD1_{n\text{-}3\ DPA}$ of the super-family of pro-resolving mediators.

Keywords

n-3 docosapentaenoic acid; specialized pro-resolving mediators; protectin $D1_{n\text{-}3\ DPA}$; lipoxygenase; pro-resolution; anti-inflammatory; human leukocytes.

Contents

[To be inserted by Editorial Office]

1. Introduction

1.1 Polyunsaturated fatty acids are enzymatically converted into specialized pro-resolving lipid mediators

Polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA, 20:5(n-3), (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-5,8,11,14,17-eicosapentaenoic acid) and docosahexaenoic acid (DHA, 22:6(n-3), (4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosa-4,7,10,13,16,19-hexaenoic acid), play a major role in the physiology of living organisms [1], see Fig. 1 for chemical structures. Omega-3 fatty acids are abundant in marine organisms and fish oil commercial products that are widely used as dietary supplements [1]. These essential fatty acids have long been associated with potential beneficial roles in human health and in the prevention of various diseases [2, 3], immunomodulation [4], autoimmune diseases [5], rheumatoid arthritis [6], cardiovascular diseases [7], Alzheimer's disease [8], type-2 diabetes [8], and certain cancers [9].

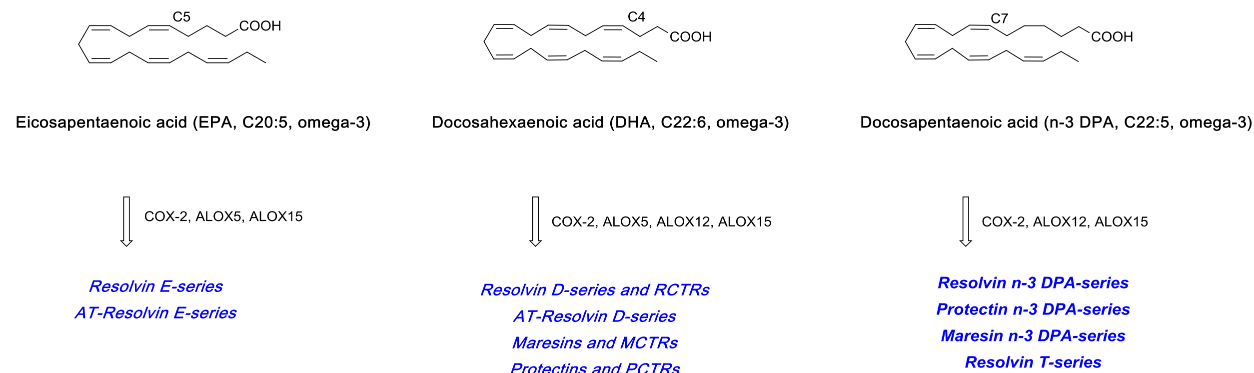

Fig. 1. The chemical structures of eicosapentaenoic acid, docosahexaenoic acid, n-3 docosapentaenoic acid and an outline of the individual families of SPMs biosynthesized from these n-3 PUFAs.

The omega-3 PUFAs EPA and DHA have for a long time believed to possess anti-inflammatory properties [10, 11] by competing with arachidonic acid (AA), resulting in a reduction of the biosynthesis of pro-inflammatory eicosanoids derived from AA [11]. Examples of eicosanoids are the prostaglandins, thromboxanes and leukotrienes [12]. However, the detailed molecular mechanisms and cellular events behind the aforementioned competition are still unclear. Recent efforts have shown that resolving inflammatory exudates use EPA and DHA to produce distinct families of oxygenated PUFA products [13]. The resolvins [14, 15], protectins [15-17] and maresins [18], as well as the recently described sulfido-conjugates RCTRs (resolvin conjugates in tissue regeneration), PCTRs (protectin conjugates in tissue regeneration), and MCTRs (maresin conjugates in tissue regeneration [19-21], are examples of distinct families of PUFA derivatives coined specialized pro-resolving lipid mediators (SPMs) [22]. Both DHA and EPA are substrates for the lipoxygenase (LOXs) enzymes as well as cyclooxygenase-2 (COX-2) (Fig. 1). The individual biosynthetic pathways and enzymatically controlled processes in the biosynthetic formation of many dihydroxylated SPMs includes the following common steps: a) an antarafacial hydrogen abstraction at the C3 position in a *Z,Z*-1,4-diene moiety of EPA or DHA; b) stereoselective insertion of molecular oxygen with a concomitant formation of a carbon-oxygen bond resulting in the formation of a hydroperoxide intermediate; c) a second hydrogen abstraction followed by an intramolecular nucleophilic attack by the oxygen in the reactive hydroperoxide intermediate resulting in the formation of an epoxide; d) hydrolase assisted nucleophillic addition of H_2O to a *Z*-double bond in an epoxide intermediate [22]. The biosynthetic conversion of the omega-3 PUFAs EPA and DHA into SPMs is of particular interest towards elucidating the many health benefits attributed to supplementing these PUFAs in the diet [22]. Recent efforts have provided the first detailed molecular mechanistic insights in endogenous anti-inflammatory and pro-resolution actions of the SPMs that may, at least in part, explain the beneficial health effects of EPA and DHA, disease and aging associated with excessive inflammation [22]. Moreover, intake of the non-steroidal anti-inflammatory drug aspirin together with omega-3 supplement has also been reported to be an advantage for human health [23]. Notably, in the presence of aspirin and COX-2, metabolically more stable *R*-epimers of some SPMs are formed [24].

1.2 SPMs are agonists towards GPCRs resulting in potent anti-inflammatory and pro-resolving bioactions

The SPMs play essential roles in the return to homeostasis or a normal physiological state [25]. The molecular, biochemical and cellular events involved in the return to homeostasis have been coined catabasis [26]. The SPMs display potent nanomolar agonist effects in vivo [27] and act as ligands for individual G-protein coupled receptors (GPCRs) [28]. The activation of one or several GPCRs induces cellular functions that explain the bioactivities of the SPMs [27, 28]. At current time, it is unclear whether other DHA-derived SPMs participate in GPCR signaling [28]. The molecular identification of the GPCRs that protectin D1 (PD1) or $PD1_{n\text{-}3\ DPA}$ elicit signaling responses has not yet been fully characterized, nonetheless initial studies on protectin D1 have shown cell-type specific activity that was structure dependent, lending support for at least one receptor [29]. This was also supported by using radiolabelled protectin D1, showing that there is specific binding to leukocytes [29]. In Table 1, an overview of the receptors that SPMs act on is provided.

Table 1. Specialized pro-resolving mediators and their receptors.

Specialized pro-resolving mediator	Receptors	Human	Mouse
Lipoxin A_4	ALX/FRP2; GPR32	Yes; Yes	Yes; n/a*
Resolvin E1	BLT1; CMKLR1; ERV	Yes; Yes	Yes; Yes
Resolvin D1	ALX/FRP2; GPR32; DRV1	Yes; Yes	Yes; n/a*
Resolvin D2	GPR18, DRV2	Yes; Yes	Yes; Yes

* Not available

SPMs display potent anti-inflammatory and pro-resolving bioactions that are key events in the promotion of the termination of ongoing inflammation. In this setting, SPMs also limit further neutrophil recruitment to the inflammatory sites, accelerate the resolution time in vivo and increase the clearance of bacteria and apoptotic cells. For a review on the cellular events involved in resolution of acute inflammation and recently introduced signs of resolution of inflammation, see reference [25]. These bioactions have attracted a considerable interest in the biomedical and health care communities as efforts on n-3 PUFA supplementation and lead compounds for the development of new drugs against many human diseases [25]. The most studied SPMs of the DHA class are protectin D1 (PD1), maresin 1 (MaR1), resolvin D1 (RvD1) and resolvin D2 (RvD2) [22], see Fig. 2 for chemical structures.

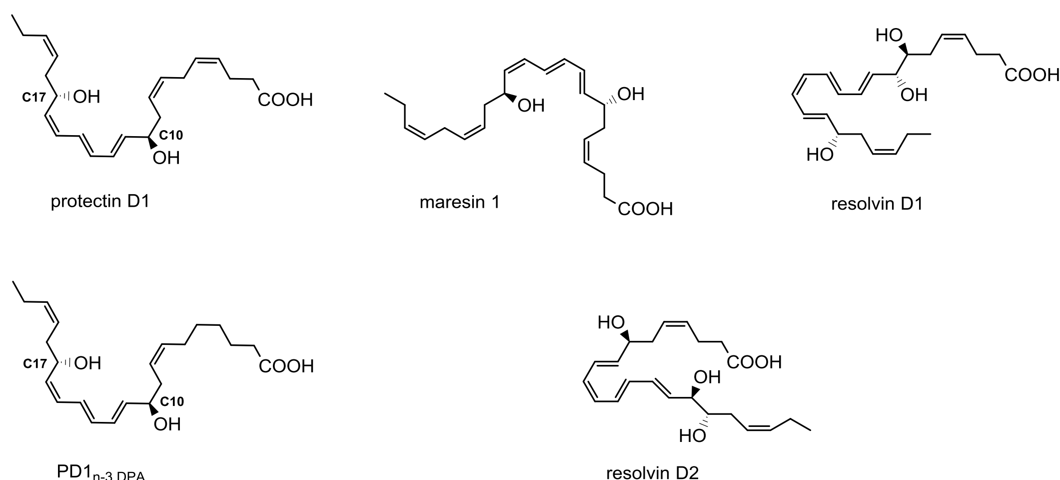

Fig. 2. Chemical structures of protectin D1 (PD1), maresin 1 (MaR1) and resolvin D1 biosynthesized from DHA, and the novel SPM $PD1_{n-3\ DPA}$ biosynthesized from n-3 DPA.

More recent findings have disclosed that SPMs protect the host from bacterial infections [30] and govern tissue regeneration, as reviewed by Serhan and co-workers [22]. In addition, protectin D1 also display potent neuroprotective effects in neuronal systems [17], and is sometimes referred to as (neuro)protectin D1. Some SPMs have entered clinical trial development programs [27].

1.3 n-3 Docosapentaenoic acid (n-3 DPA) is also a substrate and precursor for SPM formation

It has been shown that the all-*Z* PUFA n-3 docosapentaenoic acid (n-3 DPA, 22:5, n-3) participates in several fundamental cellular processes and is an intermediate in the metabolic pathway of DHA [31]. The first double bond in n-3 DPA is at C7 while in DHA the first double bond is at C4, see Figure 1. These differences give rise to different biophysical properties resulting in different functional and biological roles of n-3 DPA and DHA, for example in neuronal systems [32]. The PUFA n-3 DPA is part of our normal diet through fish and lean red meat [33]. In recent years, studies have shown that intake of n-3 DPA provides potential beneficial health effects, which include anti-inflammatory actions, antiplatelet aggregation, and improved plasma lipid profile [32]. Moreover, an enhancement in circulating levels of n-3 DPA is followed by a simultaneous decrease in the level of DHA due to single nucleotide polymorphisms observed in the gene encoding for the fatty acid elongase 2 (ELOVL2) enzyme [34]. It has also been reported that the biosynthesis of thromboxane A_2 (TxA_2) [35] and

prostacyclin [36] was reported altered by n-3 DPA in platelets. In addition, 11- and 14-hydroxy-docosapentaenoic acid metabolites [35] are also produced in platelets from this PUFA. Previously, the synthesis of chemically pure n-3 DPA was achieved [37].

The biological observations mentioned spurred an interest in investigating if n-3 DPA is enzymatically converted during inflammation-resolution processes in mice and by human macrophages. Towards such aims, Dalli, Colas and Serhan reported in 2013 the isolation, structural elucidation and biological evaluations of several novel SPMs biosynthesized from n-3 DPA [38]. Herein, a review of the structural elucidation, the total organic synthesis and the identified biosynthetic pathway of one of these novel SPMs, namely $PD1_{n\text{-}3\ DPA}$ is provided [38, 39]. In addition, the potent in vivo anti-inflammatory and pro-resolving properties are also presented.

2. Results and discussion

2.1 Structural elucidation using LC/MS-MS and matching experiments with material obtained from total synthesis

SPMs are formed in pico- to nanogram amounts in vivo. To obtain the complete configuration assignment of $PD1_{n\text{-}3\ DPA}$, a stereoselective total synthesis was needed. The key steps in the total synthesis are two highly Z-selective Lindlar reductions of internal alkynes, a stereocontrolled Evans-acetate aldol reaction with Nagao's chiral auxiliary, a Wittig reaction and the use of a chiral pool derived glycidol-ether. The chirality of the alcohols at C10 and C17 as well as the double bond geometry of the triene were assured using these synthetic protocols, see reference [39] for details.

In order to determine whether synthetic prepared material matched the endogenous $PD1_{n\text{-}3\ DPA}$, authentic SPM material from murine self-resolving exudates as well as human macrophages was obtained. Then LC/MS-MS metabololipidomics experiments [40] using multiple-reaction monitoring in the negative detection mode for selected ion pair *m/z* 361-183 were performed. These experiments gave clear results that demonstrated an identical retention time (T_R) at 12.4 min for $PD1_{n\text{-}3\ DPA}$ from resolving inflammatory exudates and human macrophages, as well as synthetic material. Moreover, co-injection of synthetic and endogenously obtained material added at essentially equal amounts showed a perfect match at T_R = 12.4 min. The MS/MS spectra for both natural and synthetic material showed similar *m/z* fragmentation patterns that

added additional structural evidence supporting that the exact chemical structure of $PD1_{n\text{-}3\ DPA}$ is (7*Z*,10*R*,11*E*,13*E*,15*Z*,17*S*,19*Z*)-10,17-dihydroxydocosa-7,11,13,15,19-pentaenoic acid (Fig. 2). Identical UV spectra for both synthetic and endogenously produced $PD1_{n\text{-}3\ DPA}$ were also observed with λ_{max}^{MeOH} 262, 271 and 282 nm, as expected for a *E*,*E*,*Z*-triene [25] providing further evidence matching the physical properties of the synthetic material with endogenous $PD1_{n\text{-}3\ DPA}$.

2.2 Biosynthetic studies of $PD1_{n\text{-}3\ DPA}$

The LOXs belongs to a family of non-heme iron-containing dioxygenases that catalyze the insertion of molecular oxygen into PUFAs containing one or more *Z*,*Z*-1,4-pentadiene moieties [41], also called a skipped *Z*,*Z*-diene. Previously it has been reported that ALOX-15 enables the formation of 17(*S*)-H*p*DHA in human whole blood, human leukocytes, human glial cells, and mouse brain [16]. The reactive hydroperoxide 17(*S*)-H*p*DHA is formed after an antarafacial hydrogen abstraction has occurred at C15 in DHA with concomitant insertion of molecular oxygen at C17. Hence, it is likely that a similar antarafacial hydrogen abstraction at C15 to occur with n-3 DPA, leading to the formation of 17(*S*)-H*p*HDPA, see Fig. 3. The intermediate dioxygen radical is most likely reduced by NADH. As seen for this type of insertion process with a PUFA containing one or more *Z*,*Z*-1,4-pentadiene moieties, a conjugated *E*,*Z*-diene is formed [41]. This double bond system is UV-active and is thermodynamically more stable than a skipped *Z*,*Z*-diene. In the next biosynthetic step, the reactive and short-lived 17(*S*)-H*p*HDPA intermediate first undergoes a second hydrogen abstraction and then undergoes an intramolecular attack at C16 by the oxygen atom at C17, resulting in the formation of an epoxide with the loss of water. This epoxide was named 16,17-$PD_{n\text{-}3\ DPA}$ epoxide, based on the structural similarities with the 16*S*,17*S*-PD epoxide [42]. Both epoxides contains the 10*Z*,12*E*,14*E* triene geometry, counting from C1. The bond angels of an epoxide deviate from the tetrahedral angel of other sp^3-hybridized ether-carbon atoms, leading to so-called bent bonds. This result in a high amount of ring-strain in epoxides which accounts for the high chemical reactivity of epoxides [43]. A nucleophilic addition of H_2O at C10, catalyzed by a hydrolase, results in the opening of the 16,17*S*-$PD_{n\text{-}3\ DPA}$ epoxide, culminating in the formation of $PD1_{n\text{-}3\ DPA}$. The triene geometry in $PD1_{n\text{-}3\ DPA}$ was determined to be 11*E*,13*E*,15*Z*, and not 11*E*,13*E*,15*E*, by matching experiments with synthetic material. The configuration at C17 is kept in the *S*-configuration, but the absolute

configuration at C16 in the epoxide intermediate is not yet established. However, based on the structural similarities with the congeneric 16*S*,17*S*-PD epoxide, the absolute configuration at C16 is most likely *S* [42].

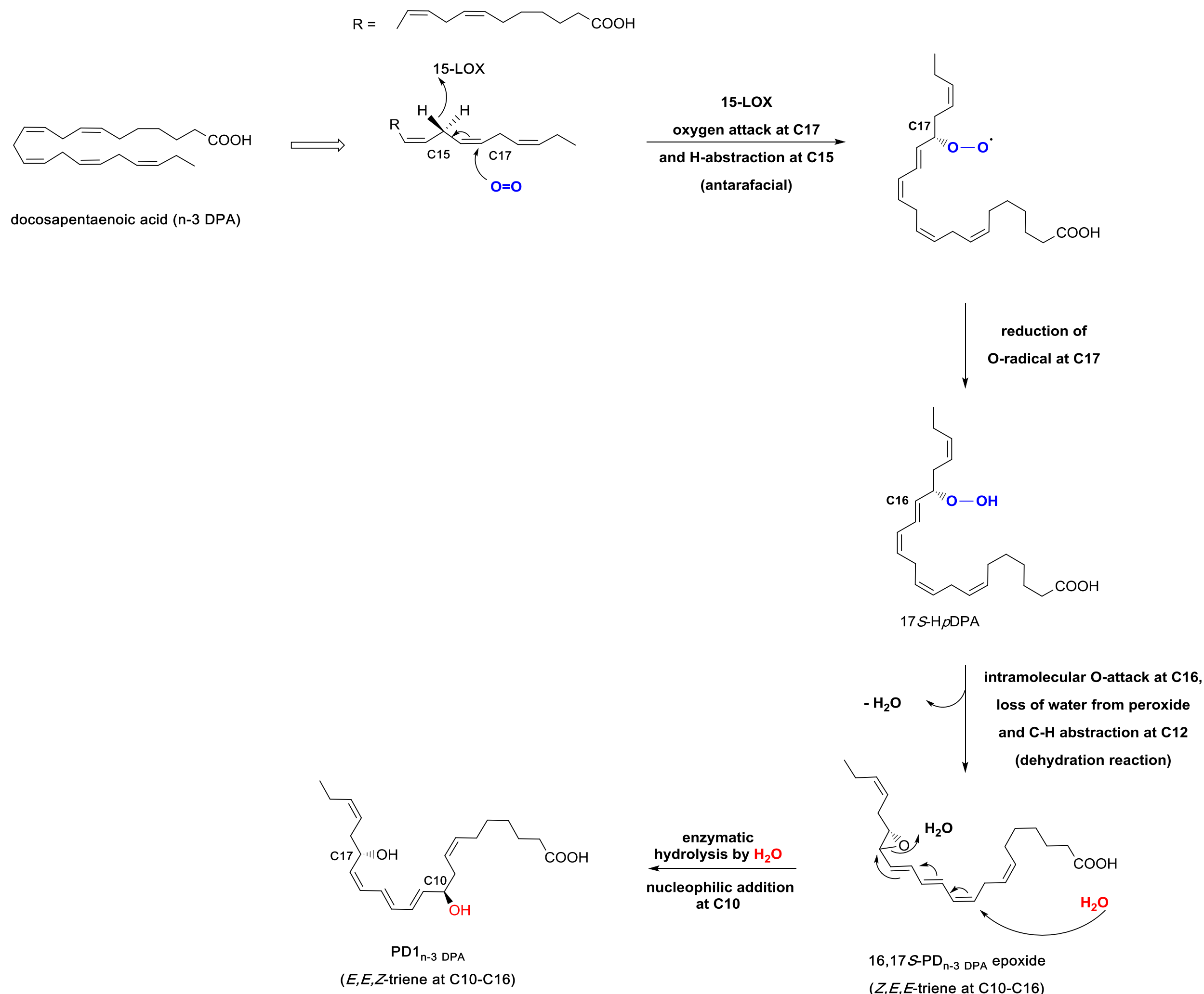

Fig. 3. Biosynthetic pathway for PD1$_{n\text{-}3\ DPA}$. For details on the individual enzymatic steps in the biosynthesis of oxygenated PUFAs, the readers should consult reference [24] and [41].

2.4 The identification of methanol trapping products renders support for involvement of an epoxide intermediate in the biosynthesis of PD1$_{n\text{-}3\ DPA}$

Experiments designed to probe the tentative biosynthetic pathway outlined in Fig. 3 were then performed. The results from these experiments support the proposed pathway [39]. Soybean lipoxygenase-15 (50 U/100 mL) was incubated with n-3 DPA (0.2 μM in 200 μL) in borate buffer (pH = 8.2) at ambient temperature. To this excess acidified MeOH (pH ~3.5) was added in

order to quench the enzymatic activity, but also to promote opening of any epoxide intermediate. The role of the 16,17*S*-$PD_{n\text{-}3\ DPA}$ epoxide as a true intermediate, generated from the immediate 17*S*-hydroperoxy-DPA precursor, in the biosynthesis was supported by results obtained from LC/MS-MS assisted experiments. The product mixture was subjected to lipid mediator metabololipidomics analysis with *m*/*z* values in the 375-277 range [39]. These efforts allowed the structural assignment of four methyl-ether products, see Fig. 4.

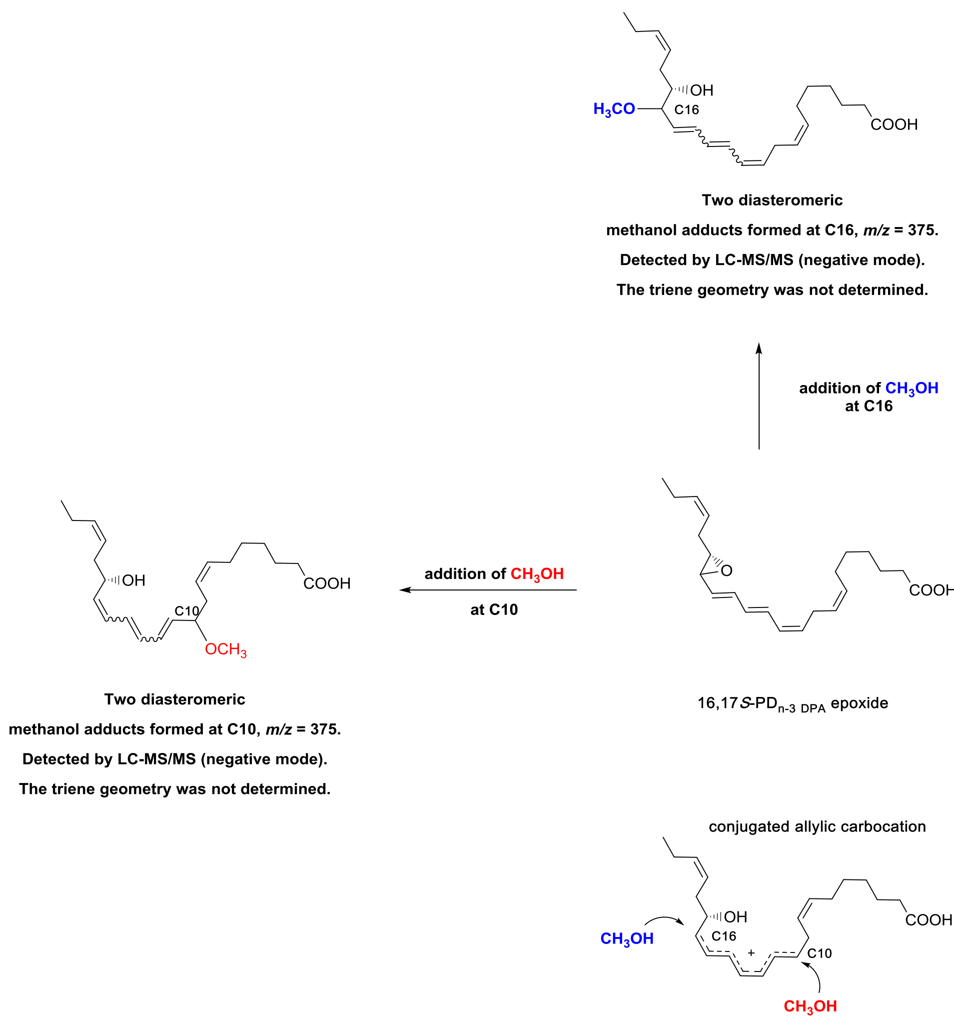

Fig. 4. Outline of the formation of the four methanol trapping products formed, their chemical structures and the putative carbocation intermediate involved.

Treatment of the solution mentioned above with acidified methanol (CH_3OH) resulted in a nucleophilic addition of methanol at C10. This addition mimics the biosynthetic step of H_2O-addition at C10 to the proposed 16,17*S*-$PD_{n\text{-}3\ DPA}$ epoxide. The structures of these two geminal

diastereomeric methyl ethers were elucidated based on data obtained from the fragmentation patterns of the MS-MS spectra. The LC/MS-MS profiles obtained from the same biosynthetic material generated from n-3 DPA and soybean lipoxygenase-15, also allowed the structural identification of two additional diastereomeric geminal methyl-ethers, see Fig. 4. The formation of these two methanol adducts are explained formed by a nucleophilic opening at the more reactive allylic C16 position (compared to C17). The addition of CH_3OH at C10 occurs most likely via a conjugated allylic carbocation (see Fig. 4). Such species have been rationalized to be involved in the biosynthesis of other oxygenated lipid mediators [44, 45]. Regarding the unassigned configuration at C16 in the $PD_{n\text{-}3\ DPA}$ epoxide, it was recently reported that 17(*S*)-H*p*DHA was converted into protectin D1 via an epoxide intermediate [42]. This epoxide intermediate was assigned the 16*S*,17*S*-absolute configuration based on material obtained from stereoselective total synthesis and coined 16*S*,17*S*-PD epoxide [42]. Of note, n-3 DPA is the congener of DHA lacking the *Z*-double bond at C4, see Fig. 1 for chemical structures. This double bond is not involved in the biosynthesis of protectin D1. Of special note, Serhan and co-workers proposed and investigated by the use $^{18}O_2$ incorporation experiments the involvement of an epoxide intermediate [46] to participate in the biosynthesis of protectin D1. The role of this epoxide intermediate in PD1 biosynthesis as well as its complete stereochemistry were recently established using a total organic synthesis approach and matching of biogenic material with synthetic material. Here the complete stereochemistry of the PD intermediate was determined to be 16*S*, 17*S*-dihydroxydocosa-4*Z*,7*Z*,10*Z*,12*E*,14*E*,19*Z*-hexaenoic acid (16*S*,17*S*-epoxy-PD) [42]. Therefore, a similar biosynthetic pathway with the involvement of a 16*S*,17*S*-epoxide derived from n-3 DPA should be anticipated. However, final proof would be to perform a stereoselective synthesis of 16*S*,17*S*-$PD_{n\text{-}3\ DPA}$ epoxide and then subject this synthetic material to biosynthetic studies.

2.5 Epoxide intermediates are also involved in the biosynthesis of other lipid mediators

Epoxides have also been reported earlier as intermediates in the biosynthesis of oxygenated PUFAs, including lipid mediators derived from EPA and DHA [47, 48]. The collaboration between Professors Bengt Samuelsson, Karolinska Institutet, and Elias J. Corey, Harvard University, both Nobel Laurates, is a classic example. Their collaborative efforts resulted in the structural elucidation [49] by the aid of total synthesis [50] as well as investigations of the

biological roles of leukotriene A_4 (LTA_4) [51]. LTA_4 is the 5*S*,6*S*-epoxide biosynthesized from AA, see Fig. 5. Further biosynthesis of LTA_4 produces leukotriene B_4 and the cysteinyl leukotrienes LTC_4, LTD_4 and LTE_4 [51]. The lipoxins are a class of SPMs biosynthesized from AA via a common epoxide intermediate, namely 5*S*,6*S*,15*S*-epoxytetraene [52]. The E-series and D-series are both biosynthesized via novel epoxide intermediates produced by human leukocytes demonstrated by trapping and $^{18}O_2$ incorporation [14, 15, 53, 54]. In 2013 Serhan, Petasis and co-workers reported that the DHA-derived SPM maresin 1 is also biosynthesized via the epoxide intermediate named 13*S*,14*S*-MaR epoxide [55], see Fig. 5. This epoxide was stereoselectively prepared and utilized in the biosynthetic studies to produce maresin 1. Interestingly, these authors reproted that the 13*S*,14*S*-MaR epoxide inhibited the LTA_4-hydrolase enzyme [55]. As already mentioned, the biosynthesis of protectin D1 was established to occur via the 16*S*,17*S*-PD epoxide [42]. Both protectin D1 and $PD1_{n\text{-}3\ DPA}$ share common structural features, namely two chiral secondary alcohols separated by the six carbon atoms of an *E*,*E*,*Z*-triene moiety, reflecting a common biosynthetic pathways with the involvement of ALOX15. Such moieties are also present in maresin 1 and the leuoktrienes. Overall, the discussion presented above for the leukotrienes, maresin 1 and protectin D1, suggested the involvement of an epoxide intermediate in n-3 DPA derived protectin D1.

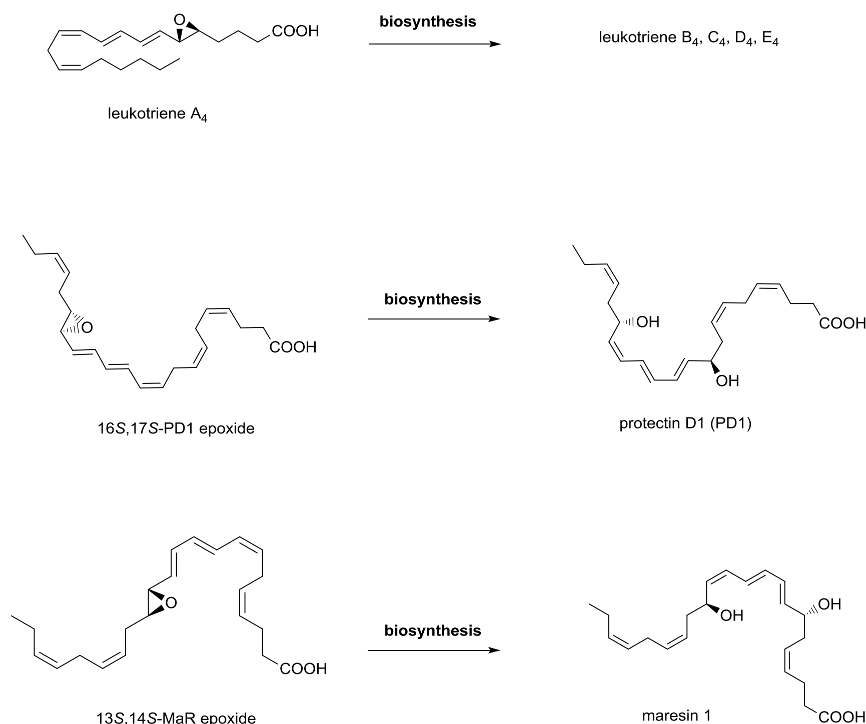

Fig. 5. Examples of biosynthetic pathways for mediators in inflammation-resolution in which epoxides are intermediates. Only the chemical structures of the DHA derived examples are depicted; for structures of the AA derived, see references [41, 51, 52].

2.6 Biological evaluations

To obtain further evidence for the complete structure of $PD1_{n\text{-}3\ DPA}$ it was essential to assess that the synthetic material carried the potent biological actions described for $PD1_{n\text{-}3\ DPA}$. Administration of synthetic material using 10 ng per mouse significantly reduced neutrophil recruitment during peritonitis, following zymosan A challenge, as determined by light microscopy and flow cytometry. These bioactions were comparable to those displayed by the DHA derived SPM protectin D1. Human macrophage phagocytosis, macrophage efferocytosis and the clearance of apoptotic neutrophils are all key processes in the resolution of inflammation. Altogether, such actions are defining for a true SPM. Therefore the abilities of synthetic material of $PD1_{n\text{-}3\ DPA}$ to stimulate human macrophage phagocytosis and efferocytosis were investigated. Incubation of synthetic material with human macrophages with increasing concentrations of synthetic material of $PD1_{n\text{-}3\ DPA}$ gave rise to an increase in macrophage phagocytosis of both fluorescence labeled yeast cell wall particles (zymosan A) as well as apoptotic neutrophils. Similar effects were also observed for protectin D1. In addition, $PD1_{n\text{-}3\ DPA}$ potently, at concentrations in the pico- to nanomolar range, increased macrophage efferocytosis of apoptotic human neutrophils, a key pro-resolving biological action. The trend towards a bell-shaped dose-response curve with these isolated human cells was seen. Overall, these results verified that $PD1_{n\text{-}3\ DPA}$ exhibited both potent anti-inflammatory and pro-resolving actions, confirming the potent immunoresolvent properties of this SPM, but also established the complete structure of this novel mediator as (7*Z*,10*R*,11*E*,13*E*,15*Z*,17*S*,19*Z*)-10,17-dihydroxydocosa-7,11,13,15,19-pentaenoic acid.

Summary

Herein the new evidence recently obtained on the structure-function of the growing numbers of the endogenously formed specialized pro-resolving mediator super-family is provided for a novel member, namely $PD1_{n\text{-}3\ DPA}$. The distinct families of SPMs are chemically characterized by two or three chiral, secondary alcohols, separated by either an *E*,*E*,*Z*-triene or an *E*,*Z*,*E*,*E*-tetraene

moiety. As of today, structural elucidation of these oxygenated PUFA derivatives using LC-MS/MS based metabololipidomics is necessary along with derivatives to deduce endogenous structures of the bioactive products [56], since only pico- to nanogram amounts of biosynthetic material are formed. Matching with synthetic material obtained by stereoselective total synthesis is essential for the exact configuration assignment of SPMs, but also for thorough biological evaluations.

Resolution of inflammation is now held to be an active process where SPMs playing an important role in maintaining homeostasis. Studies in humans are contributing new knowledge that supplementation with n-3 PUFAs is increasing the biosynthesis of SPMs [47, 48, 57]. Further biological studies of the n-3 DPA derived SPMs continue to be of interest [58].

The different families of SPMs display high structural complexity due to the presence of several stereogenic centers, both in the form of chiral, secondary alcohols and conjugated *E*- and *Z*-double bonds [24], reflecting their biochemical origins, functions and stereospecific bioactions towards individual GPCRs [27]. Hence, acquired knowledge and distinct care must be exercised when working with SPMs. Failure of such diligent operations will not reveal the correct chemical structures or the exciting bioactions that SPMs possesses as resolution agonists. Elucidating the role of PUFAs and their oxygenated products at the cellular and molecular level in health is of current interest [23], [47, 48], [58]. The SPMs are among the most exciting small molecules currently under investigations towards drug development and elucidating the biology of resolution of inflammation [59]. Only prudent handling of each SPM, due to their delicate chemical structures, will be met with success within this interesting field of biomedical research.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Declaration of interest

J. D. and C. N. S. have filed patents on $PD1_{n\text{-}3\ DPA}$ and related compounds. C. N. S.'s interests are reviewed and are managed by BWH and Partners HealthCare in accordance with their conflict of interest policies.

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