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Resolvins and protectins: Natural pharmacophores for resolution biology

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A B S T R A C T

Dietary ingestion of fish is associated with a reduced risk for many common human illnesses. Fish oils are enriched with n-3 polyunsaturated fatty acids eicosapentaenoic acid and docosahexaenoic acid. Resolvins and protectins are newly discovered mediators that are enzymatically generated from these n-3 fatty acid precursors to orchestrate inflammation resolution. These natural compounds and their mimetics are providing intriguing evidence in model systems and translational research for cellular and molecular mechanisms that are active during catabasis. This review provides information on the biosynthesis and actions of these recently identified chemical mediators with particular reference to resolution of mucosal inflammatory responses.

1. Introduction

The resolution of acute inflammation is a complex active process that involves several distinct molecular and cellular mechanisms. In most cases, acute inflammation spontaneously resolves to restore homeostasis. The events directing resolution are not merely a passive dilution of pro-inflammatory signals and effectors. Rather, tissue resolution is a tightly orchestrated and multifaceted host response [1]. It has its origins early in acute inflammation. In the first few minutes to hours in acutely injured, infected or inflamed tissues, biosynthetic circuits are created for chemical mediators that have anti-inflammatory properties and promote resolution [2]. These counter-regulatory signals provide tissue level control over the intensity of the inflammatory response, and ultimately lead the process of catabasis. Specific pro-resolving mediators are generated that serve as agonists at specific receptors [3]. Disruption of their biosynthesis or sites of action by genetic susceptibility or pharmacologic intervention can prove to be harmful to resolution, delaying the restoration of tissue homeostasis [4,5]. In addition, many common diseases are characterized by chronic inflammation that fails to resolve. Recent identification of pro-resolving mediators has uncovered a shared pathobiology in several of these conditions of a defect in the generation of these protective compounds [6–11].

Acute tissue inflammation is characterized by specific cellular events, including increased endothelial and epithelial permeability, infiltration of polymorphonuclear leukocytes (PMNs) and inflammatory macrophages and lymphocytes [12]. As the tissue resolves, barrier integrity is reestablished and further infiltration of PMNs is blocked. Cell clearance from the inflamed tissue is critical to resolution and is driven both by apoptosis of leukocytes [13–15] and egress from tissues [16]. Clearance of the inflammatory site is mediated in part via the non-phlogistic recruitment of monocytes that, as macrophages, participate in the phagocytosis of apoptotic cells and microbes [5,17]. In addition, mechanisms unique to mucosal surfaces exist to release PMNs from the apical surfaces of epithelial cells into the lumen for tissue clearance [18]. These cellular events can be tracked by microscopy or tissue histology, leading to the development of several resolution indices to determine the extent of inflammation [19]. Most common in current use are the maximal number of leukocytes (ϕ_{max}) and the resolution interval (R_i), which is the interval of time from the ϕmax to the half-maximal response during resolution [19]. These metrics are particularly useful when determining the impact of gene expression or pharmacologic intervention on the pace of resolution. If the resolution interval decreases, then resolution has been accelerated. In contrast, if the resolution interval lengthens then there has been an adverse impact on leukocyte resolution — independent of the intervention's impact on early pro-inflammatory events.

As cell numbers decline in resolving tissues, levels of pro-inflammatory cytokines decrease and the metabolism of polyunsaturated fatty acids (PUFAs) changes, with a class switching in lipid mediator generation that changes from enzymatic conversion of pro-inflammatory mediators (e.g., leukotrienes (LTs) and prostaglandins (PGs)) to pro-resolving mediators (e.g., lipoxins (LXs), resolvins (Rvs) and protectins (PDs)) [20–22]. This review will focus on rapidly emerging information on the generation and actions of recently identified resolvins and protectins that are

Abbreviations: COX, cyclooxygenase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LOX, lipoxygenase; LT, leukotriene; LX, lipoxin; MS, mass spectrometry; PD, protectin; PG, prostaglandin; PMN, polymorphonuclear leukocyte; PUFA, polyunsaturated fatty acid; Rv, resolvin

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enzymatically derived from the n-3 PUFAs eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3).

2. Resolvins

Resolvins are stereospecific small molecules that are enzymatically derived from EPA and DHA, n-3 PUFAs that are abundant in cold water fish ([21, 22] and reviewed in [1]). These naturally occurring bioactive mediators were first identified by physical methods during lipidomic system-based analyses of spontaneously resolving murine exudates. They were arbitrarily termed resolvins to reflect their identification as resolution phase interaction products. As compounds were isolated, retrograde analysis was carried out using biogenic as well as total organic synthesis to elucidate structures and characterize stereochemistry [21–26]. Using these methods, several classes of resolvins were identified. Resolvins are now categorized as either E-series (from EPA) or D-series (from DHA) (Fig. 1) and aspirin-triggered epimers of these compounds have also been identified (as reviewed in [3]).

2.1. E-series resolvins

The E-series resolvins currently comprise Resolvin E1 (RvE1) and Resolvin E2 (RvE2). Each of these molecules is enzymatically derived from EPA and was originally isolated *in vivo* from murine resolving exudates [21]. Transcellular formation of RvE1 can occur during inflammation when endothelial cells interact with leukocytes. For example, in the presence of aspirin, EPA can be enzymatically transformed to 18R-hydroxyeicosapentaenoic acid (18R-HEPE) by endothelial cell cyclooxygenase-2 (COX-2). Aspirin acetylates COX-2 and the acetylated enzyme no longer generates prostaglandins, but can still convert EPA to 18R-HEPE, a biosynthetic precursor for RvE1. During cell–cell interactions, 18R-HEPE is released to neighboring leukocytes for subsequent

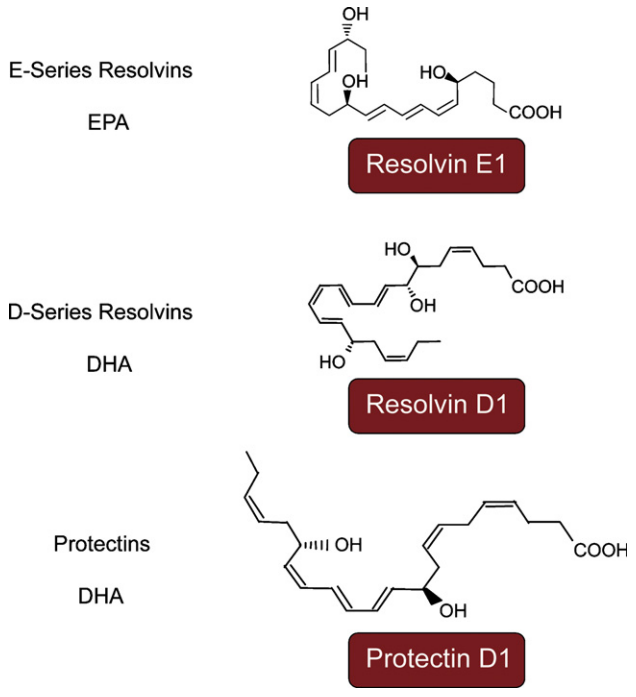

Fig. 1. Resolvins and protectins. Resolvins and protectins have been identified in spontaneously resolving exudates. They are enzymatically derived from n-3 fatty acids: E-series resolvins are from EPA, while D-series resolvins and protectins are from DHA. The chemical structure of representative members of these families of natural compounds is shown.

conversion by 5-lipoxygenase (ALOX5) to RvE1 via a 5(6) epoxide-containing intermediate [21,23]. RvE1 is present in human whole blood and can be increased by ingestion of aspirin [23]. The structure of RvE1 was elucidated as 5S,12R,18R-trihydroxy-6Z,8E,10E,14Z,16E-EPA and the complete stereochemistry confirmed [21,23].

The bioactivity of RvE1 is highly stereoselective both *in vivo* and *in vitro*. Anti-inflammatory actions for RvE1 include potent regulation of PMN trafficking and activation. RvE1 blocks PMN transendothelial migration [21], and PMN functional responses to inflammatory stimuli, such as NF-κB activation [27] and superoxide anion generation [28]. In addition to these anti-PMN actions, RvE1 promotes PMN removal by clearance from the apical surface of mucosal epithelial cells [18] and macrophage-mediated phagocytosis of apoptotic PMNs [5]. RvE1 also regulates the levels of pro-inflammatory peptide mediators by inhibiting dendritic cell migration and cytokine release [23,29] and upregulating leukocyte CCR5 expression [30]. This complex array of bioactions provides both anti-inflammatory and pro-resolving properties for this compound.

RvE1 displays potent *in vivo* regulation of mucosal inflammation at many levels of the aerodigestive tract. In a rabbit model of periodontitis, RvE1 both dampens inflammation and promotes restoration of periodontal tissues, including bone [31,32]. RvE1 also markedly decreases the inflammatory sequellae of 2,4,6-trinitrobenzene sulfonic acid-induced colitis [33] and can facilitate the resolution of allergic airway inflammation ([29]; Fig. 2). In comparison with other agents that are clinically available to decrease inflammation, RvE1 proved log-orders more potent than dexamethasone or aspirin in a murine dorsal air pouch model of dermal wounding and inflammation. In nanogram quantities, RvE1 decreases leukocyte infiltration by 50–70%, whereas the IC_{50} for dexamethasone and aspirin in this model are in the microgram and milligram range, respectively [21,23]. RvE1 also displays important actions on structural cell functional responses, including the facilitation of wound healing by epithelial cells [34]. Thus, RvE1 displays characteristics *in vitro* and *in vivo* of a mediator for catabasis.

RvE1 is metabolically inactivated in a rapid and regiospecific manner. The first step in its inactivation is a regiospecific conversion at carbon 18 to generate 18-oxo-RvE1 [35]. RvE1 inactivation is complex and shows species-, tissue- and cell-type specific pathways [35]. Rapid inactivation of RvE1 in a regulated manner provides local control for cells and tissues in catabasis. In addition, elucidation of these metabolic pathways has informed the design of structural analogs of RvE1 that resist inactivation [35].

Structure–activity assays for RvE1 and related compounds indicate that the mechanism of action of RvE1 relies on specific receptors [23]. Use of [^{3}H]-labeled RvE1 confirmed specific binding and the molecular identification of RvE1 cognate receptors has followed. The first RvE1 receptor identified was a G-protein coupled receptor termed ChemR23, a seven-membrane spanning receptor expressed on dendritic cells and monocytes [23]. RvE1 serves as an agonist at this receptor to block cytokine-initiated NF-κB activation and stimulate specific MAP kinases [23]. ChemR23 was initially characterized as a receptor for the peptide chemerin that also transduces anti-inflammatory signals [36]. Thus, similar to the lipoxin A_4 receptor ALX [37], ChemR23 can interact with both lipid or peptide ligands. Distinct from dendritic cells and monocytes, RvE1 also displays high affinity specific binding to human PMN membranes with a K_d of 48.3 nM at 4 °C. Competitive displacement of [^{3}H]-labeled RvE1 binding to PMN membranes is observed with unlabelled RvE1 (K_i=34.3 nM), LTB_4 (K_i=0.08 nM), and the LTB_4 receptor 1 (BLT1) selective antagonist U-75302 (K_i=1.5 nM). These findings suggest binding