# Resolvins: A novel approach to resolving inflammation

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ABSTRACT

Inflammation is the first response of the immune system to infection or injury, but excessive or inappropriate inflammatory responses contribute to a range of acute and chronic human diseases. Clinical assessment of dietary supplementation of ω-3 polyunsaturated fatty acids (i.e., eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) indicate that they have beneficial impact on these diseases, although the mechanisms are poorly understood at the molecular level. In this decade, it has been revealed that EPA and DHA are enzymatically converted to bioactive metabolites in the course of acute inflammation and resolution. These metabolites were shown to regulate immune cell functions and to display potent anti-inflammatory actions both in vitro and in vivo. Because of their ability to resolve an acute inflammatory response, they are referred to as proresolving mediators, or resolvins.

## Introduction

It is an established fact that chronic inflammatory illnesses such as diabetes mellitus, arthritis and heart diseases share a two way relationship with the periodontium i.e. these diseases have a negative impact on the periodontium and vice versa the periodontal diseases also pose a reciprocal effect on the progression of these diseases. Common pathways lead to the inflammatory disorders in the human body, successful management of these common pathways would lead to a better understanding of the disease processes and also to the development of novel treatment strategies.

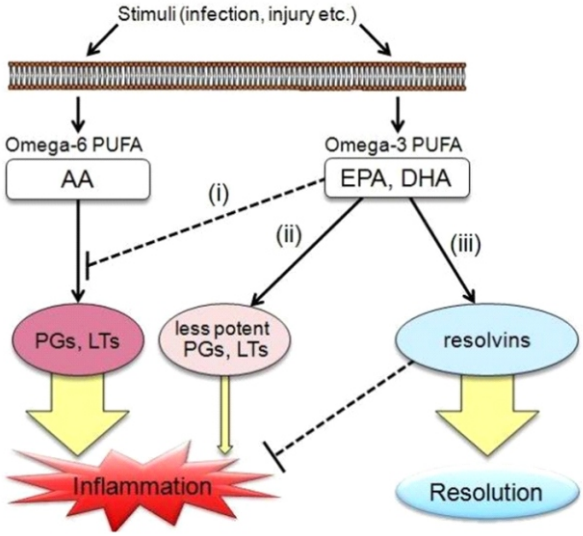
A considerable focus has been laid by nutritionists on the omega-3 fatty acids because of the perceivable health they exert on the health of consumers. Although their mechanisms are still a matter of controversy, it is likely that oxygenated metabolites derived from eicopentaenoic acid (EPA) and docosahexanoic acid (DHA), the resolvins and (neuro) protectins, must play a significant part as they have potent anti inflammatory and immunoregulatory actions at concentrations in the nanomolar and picomolar range. Resolvins and protectins are novel lipid derived mediators, they control the duration and magnitude of inflammation, the mapping of these resolution circuits may provide newer ways of understanding the molecular basis of many inflammatory diseases.[1]

The term 'resolvins' or 'resolution of inflammation' was coined by Professor Charles N. Serhan and colleagues as these compounds were first encountered in resolving inflammatory exudates. Compounds derived from EPA are designated as resolvins of the E series, while those formed from the precursor DHA are denoted as either resolvins or protectins ('neuroprotectins') of the D series.[2]

## What is Inflammatory Resolution?

It has been a misunderstanding that once the inflammation response has neutralized as injurious stimulus; the inflammation somehow fizzles out, this possibly from pro-inflammatory mediator catabolism. On the contrary, the resolution of acute inflammation is highly coordinated and active process that is controlled by endogenous 'pro-solving' mediators. It is believed that these factors lead to the restoration of the inflamed tissue to its prior physiological function by bringing about the safe disposal of inflammatory leukocytes, exudate and fibrin. Also, these resolvins give little opportunity for the development of chronic, immune mediated inflammation and also control the excessive tissue injury.[3] Varying degrees of tissue damage occurs only when the host is unable to neutralize the injurious agent and/or there is a failure of endogenous pro-solving mediators and there is perpetuation of the acute inflammation. A process called as regeneration occurs if the tissue injury is mild and new cells of the same type replace the parenchymal cells. However when the tissue damage is extensive and when the fibrin clot is not cleared after the initial phase of acute inflammation and the process of healing takes place by repair. The process of repair results in the formation of granulation tissue which involves the in-growth of from the surrounding connective tissue of an initially vascular tissue containing capillary loops, fibroblasts and leukocytes. The term "Organization" is given for the repair by granulation and fibrosis which occurs in many parts of the body where a deposit of clot, exudate or dead tissue occurs.[4] This explains that this form of inflammatory resolution is well associated with tissue damage. For example, in rheumatoid arthritis and asthma the ongoing tissue damage is a result of the continuous or repeated bouts of acute inflammation. Granulomatous tissue formation, angiogenesis, fibrosis and scar formation; all occur concurrently and

are a result of wound healing attempts. This chronic inflammation might be termed as a continuous inflammatory disease state which is driven by the development of an immune response to an endogenous antigen also known as autoimmunity.[5-8]




**Resolvins and Protectins**

Resolvins and protectins represent two new families of compounds which have been identified to play a role in the resolution of inflammation.[9-13] Resolvins are resolution phase interaction products which are endogenous compounds made from omega 3 fatty acids eicosanpentaenoic acid (EPA) and docosahexaenoic acid (DHA); termed as E series resolvins (RvE) and D series resolvins (RvD) respectively. In the presence of aspirin the AT(arpirin triggered) forms of resolvins are also produced by the COX-2 pathways.[13] In the absence of aspirin resolvins are biosynthesized by the Lipoxygenase initiated mechanisms. It has been proved that resolvins possess potent anti-inflammatory and immunoregulatory actions; they block the production of pro inflammatory mediators and regulate the traffcking of inflammatory cells and mediators to sites of inflammation. For example, resolvins stop PMN infltration and transmigration [13,14] and reduce cytokine expression on microglia cells.[11]

A separate pathway may also generate protectins from DHA and 22 carbon backbone with six double bonds, three in conjugated triene structure. These factors are anti -inflammatory and protective actions in neural tissues and systems where their biosynthetic location is designated with the prefix neuro- or is termed neuroprotectins. Like resolvins these also stop PMN infiltration and in addition also reduce retinal and corneal injury, reduce stroke damage, and improve wound healing.

A study by Sun Yp et al, compared the actions of RvD1 to 17 R-AT- RvD1 (0 to 1,000 nM) on human PMN trans-endothelial migration. RvD1 and AT-RvD1 stopped PMN transmigration in a concentration dependent manner. The potency of these compounds should be noted; a reduction in PMN transmigration; 50% was observed with concentrations as low as 10 nM.[14]

**Target Cells of Resolvins**

*Neutrophils (PMNs)*

The first line of immune defense is provided by the migration of the PMNS's at the site of injury of infection. In response to the invading pathogens the transmigrating PMN's undergo a potent respiratory burst and degranulation. Tissue damage occurs which contributes to numerous inflammatory diseases when this reaction becomes inappropriate or excessive.[15-19]

The pivotal event in the PMN recruitment is the transendothelial migration. RvE1 inhibits the LTB4-stimulated PMN migration across microvascular endothelial cell monolayers with an IC50 of ~10 nM.[12] Also RvE1 inhibits the LTB4-stimulated PMN transmigration across a monolayer barrier consisting of choroid-retinal endothelial cells (CRECs).[20] Transepithelial migration of the PMN's is a pathological hallmark of active mucosal inflammation and also represents an important component of the innate immune response. RvE1 potently attenuated f-METLeu-Phe (fMLP)-induced PMN transmigration across KB oral epithelial cells in a concentration-dependent manner.[21] RvE1 interacted with the LTB4 receptor BLT1 that is expressed on PMNs with a Kd value of ~50 nM, and attenuated proinflammatory signals by LTB4.[22] Resolvins also enhanced the effective functions of PMNs. RvE1 (10 nM) enhanced PMN phagocytosis and killing of Candida albicans.[23] RvD2 (10 nM) also enhanced PMN phagocytosis of Escherichia coli that was accompanied by an increase in intracellular reactive oxygen species generation.[24] These actions of resolvins are important for limiting invasion of pathogens and resolving inflammation.

**Dendritic Cells (DCs)**

DC's are immune cells which play an important role in the initiation of adaptive immunity and also in innate immunity.[25] During inflammation, they migrate into inflamed peripheral tissues where they capture antigens. In their immature state, DCs are distributed in tissues that are in contact with the external environment, such as the mucosal surfaces or the skin. . After they mature, they migrate to lymph nodes where they activate naive T cells and provide cytokines required for T-cell proliferation/differentiation. Intraperitoneally administered Soluble Toxoplasma gondii tachyzoite antigen (STAg) activated the mobilization of splenic DCs to T-cell enriched areas and production of IL-12. Treatment with RvE1 blocked DC migration and IL-12 production triggered by STAg.[10]

The deletion of effector T cells is a major mechanism for resolving an inflammatory process. The resolution is enhanced by RvE1 which induces the apoptsis of T cells. This response was consistent with the migration pattern, i.e., DCs exposed to RvE1 did not migrate in response to CCL19/21 and strongly migrated toward CCL3/5.[26] In addition, RvE1 induced apoptosis of antigen-specific activated CD4+ T cells through inducing DC expression of

indoleamine 2,3-dioxygenase,[26] which catabolizes the amino acid tryptophan and plays an important role in T-cell apoptosis.[27] These results imply that DCs exposed to RvE1 and pathogens remain at the inflammatory site instead of migrating to lymph nodes, and induce apoptosis of effecter T cells at the inflammatory site.

**Macrophages**

The clearance of apoptotic PMNs and tissue debris is the key histological event in tissue resolution.[28] In vitro, RvE1 (100 nM) stimulated murine peritoneal macrophages to ingest apoptotic PMNs.[29] RvE1 interacts with ChemR23, which is expressed in macrophages as well as DCs.[14] RvE1 induced human monocyte-derived macrophages to ingest opsonized zymosan, and this enhancement was blocked by an antibody against ChemR23.[30] RvE1 stimulated Akt and ribosomal S6 phosphorylation signals in macrophages. In vivo, in zymosan-induced peritonitis, RvE1 (300 ng/mouse) given at the peak of inflammation enhanced the removal of zymosan by phagocytes and its transport to lymph nodes and spleen.[29] These results indicated that RvE1 helped to resolve inflammation by activating macrophages to clear apoptotic cells and debris from inflammatory sites.

**Platelets**

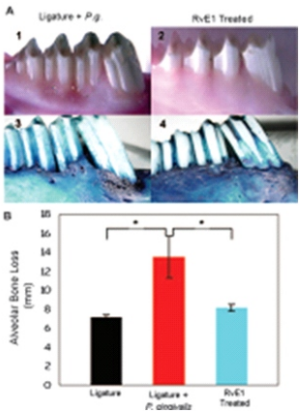
Platelets play important roles in blood coagulation, wound healing, and inflammation. aggregation, which has further effects on platelet-leukocyte and platelet-endothelium interactions, is essential in thrombosis. The connection between thrombosis and inflammation is provided by the cell-cell intercartions. RvE1 at nanomolar range blocked ADP-stimulated platelet aggregation in a concentration-dependent manner.[31] In addition to ADP, RvE1 also blocked platelet aggregation stimulated by U46619 (thromboxane receptor agonist), with similar kinetics.[31] Of interest, RvE1 did not block collagen-induced platelet aggregation. These results suggest that RvE1 acts to block excessive platelet aggregation rather than disrupt the physiologic coagulation induced by collagen.

**Leukocyte-Endothelial Cell Interactions**

A variety of adhesive and migratory molecular events including low-affinity transient and reversible rolling adhesions, integrin-dependent firm adhesive interactions, and migration of leukocytes thorough the endothelium are involved in the interaction between leukocytes and the endothelium.[32,33] Intravital microscopy demonstrated that intravenous administration of RvE1 (100 ng) rapidly reduced leukocyte rolling by approximately 40% in cremaster muscle venules of mice.[31] RvD2 (1 nM) decreased platelet activating factor (PAF)-stimulated leukocyte adherence and emigration in the microcirculation in the cremaster muscle of mice. RvE1 inhibited LTB4-stimulated PMN migration across microvascular endothelial monolayers with an apparent IC50 of ~10 nM.12 RvD2 reduced PAF and complement-stimulated capture and adhesion of PMN's by human umbilical vascular endothelial cells (HUVECs) under flow.[9] These actions of resolvins are mediated in part by modulating the expression of adhesion molecules on leukocytes and the production of anti adhesive mediators by endothelial cells. Addition of RvE1 (30 nM) to whole blood reduced CD18 expression on both PMN's and monocytes approximately by 50%. RvD2 diminished PAF-stimulated CD62L (L-selectin) shedding on isolated human PMNs and CD18 surface expression. Topical administration of RvD2 (100 pg/ear) increased endothelial-dependent nitric oxide production. Corroboratory results were obtained with HUVECs, whereby RvD2 dose dependently stimulated nitric oxide generation.[24]

**Resolvin E1 (rve1) Prevents Inflammation and Bone Loss in Experimental Periodontitis.**

Following characterization of the P. gingivalis -induced rabbit model of periodontal disease , the possibility that topical treatment with RvE1 prevents inflammation induced tissue and bone loss was explored. Rv E1, prepared by total organic synthesis, was topically applied to P. gingivalis-ligatured teeth (4 m g/tooth) three times a week in the rabbit model over a 6-week study period. Control groups received the same frequency of topical application of vehicle (ethanol) together with P. gingivalis application to ligatured teeth or placement of ligature alone.[26] Macroscopic evaluation at the end of the 6-week period demonstrated frank inflammation, as well as tissue and bone loss, in the buccal and lingual mandibular region of rabbits receiving non-RvE1 vehicle control, suggesting significant progression of periodontal disease. In addition, a profound increase in bone resorbing osteoclasts was observed histologically, with osteoclast proliferation increasing with closer proximity to the ligature, as well as prominent leukocyte infiltrates and collagen loss. This was in contrast to findings with RvE1 application, in which the




RvE1 protects from soft tissue destruction and bone resorption in rabbit periodontitis. Periodontitis was induced in New Zealand White Rabbits with 3-0 silk ligature and topical application of the human periodontal pathogen P.gingivalis (P.g.) for 6 wk. A) Topical application of 4 µg of RvE1 three times per wk prevented soft tissue inflammation and destruction (panel 1 vs. panel 2) and bone loss (panel 3vs. panel 4). Direct measurements of alveolar bone loss for all animals were quantified in B. RvE1 treatment significantly inhibited bone loss.

development and progression of periodontal disease seemed to be prevented, with essentially no neutrophils or tissue damage observed.

There was a remarkable absence of inflammatory changes and osteoclast proliferation, and bone remained largely intact. Correspondingly, mean alveolar bone loss was significantly reduced with RvE1 treatment or ligature alone compared to vehicle control. Radiographic evidence also demonstrated a significantly reduced percentage of bone loss associated with RvE1 treatment (<5%) over the 6 -week period compared to vehicle control s (<35% ) or ligature alone (<12%) (P<0.05).[34]

## Conclusion

We can thus conclude that resolvins; which are omega 3 PUFA derived mediators regulate the immune system and controls cellular function. EPA-derived E-series resolvins (i.e., RvE1 and RvE2) and DHA-derived D-series resolvins (RvD1 and RvD2) have potent anti-inflammatory and proresolution properties. Lipoxins, resolvins, and docosatrienes are novel families comprised of separate chemical series of lipid-derived mediators, each with unique structures and apparent complementary anti-inflammatory properties and actions. These families of compounds can also be generated in their respective epimeric forms when aspirin is given in mammalian systems. It is likely that these compounds and their AT-related forms may play roles in other tissues and organs, since they are involved in physiological and pathological processes. These novel lipid mediators are evolutionally conserved molecules that are host protective. In view of the important roles of their precursors DHA and EPA in human biology and medicine, it is likely that these novel pathways and compounds are responsible in part for the beneficial impact of omega-3 essential fatty acids in complex systems and could be of use in periodontal treatment. We now understand that the resolution of acute inflammation is not passive; it is actively regulated. Proresolving lipid mediators, such as lipoxins, protectins, and resolvins, can accelerate this process.

## Future Directions

Developing drugs that mimic the actions of mediators that are essential for resolution to treat chronic inflammatory diseases, such as rheumatoid arthritis, asthma and encephalo-myelitis and periodontitis has been suggested. Such therapeutics might be based on the mechanisms of action of cyPGs, lipoxins or lipoxin-receptor agonists, or on factors that trigger leukocyte apoptosis and stimulate macrophage phagocytosis of apoptotic cells. This mechanism could help to trick the inflammation into resolution. Further investigation into the mediators and mechanisms that are central to resolution will bring us into a new era of inflammation research, which, if approached with creativity and persistence, might provide numerous benefits for those suffering from inflammation-mediated diseases.

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