International Journal of Research in Pharmacy and Life Sciences

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Review Article **Open Access**

A Review on Resolvins -Therapeutic Role

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A B S T R A C T

This review gives information on resolvins and related substances, which are important both as endogenous proresolvers and as therapeutic candidates preventing deterioration of inflammation and pathologic pain. Inflammation is the first response of the immune system to injury or infection. This way the immune system protects the body from infection or injury. Inflammation is an essential biological process for maintenance of homeostasis and recovery from tissue injury or foreign pathogens. Resolvins block the production of pro-inflammatory mediators and regulate leukocyte trafficking to inflammatory sites as well as clearance of neutrophils from mucosal surfaces. Also limitation of polymorph nuclear leukocyte migration and infiltration across the endothelium is done by resolvins. Pain an important aspect of inflammation, may be strongly affected by the actions of resolvins. Resolvins may also directly regulate the function of the sensory neural circuit, independent of their control mechanisms for the inflammatory state**.** RvE1 is a local acting autacoid that has proved to display pro-resolving activity when treatments were given either by topical, intra venous, or intra peroneal administration**.**

Keywords: Resolvins, Inflammation, pharmaceutical, pain

A R T I C L E I N F O

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Article History: Received 10 September 2016, **Accepted** 24 October 2016, **Available Online** 24 November 2016

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Manuscript ID: IJRPLS3076

PAPER-QR CODE

Citation: Krishnaveni Manubolu, *et al.* A Review on Resolvins -Therapeutic Role. *Int. J. Res. Pharm, L. Sci.*, 2016, 4(2): 102-111.

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1. Introduction

Inflammation is the first response of the immune system to injury or infection. This way the immune system protects the body from infection or injury. Inflammation is an essential biological process for maintenance of homeostasis and recovery from tissue injury or foreign pathogens. For a immediate host defence, acute inflammation is an important process but the inflammation has to cease after the stimulus is removed and if the inflammation still persists for a long time then it may leads to arthritis and cardiovascular diseases. These inflammations persisting for a long time is termed as chronic inflammation. This chronic inflammation is a symptom in many chronic degenerative disorders such as arthritis, low back pain and inflammatory bowel disorders. Many COX inhibitors or opiods are used to control the pain in these patients. But these COX inhibitor may be ineffective on long therapy or they may have certain side effects such as unselective COX inhibitors may cause gastrointestinal bleeding and kidney damage[1].Despite many recent advances in the treatment of inflammatory diseases ,mechanisms for the resolution of inflammation are still poorly understood. Thus now it has become a great interest for both doctors and patients to find an alternative, powerful treatment for chronic inflammation[2].

Tissue injury not only produces pro-inflammatory mediators but also generates novel local mediators that are both anti-inflammatory and pro-resolvins, thus resulting in a spontaneous or self limited recovery of acute inflammation and acute pain[3].It was historically believed that resolution of inflammation was a passive process (cottran and collis, 1999), driven primarily by the declining levels of pro-inflammatory mediators over time and 'fizzling out' of the acute inflammatory response. Recent studies have demonstrated, however, that effective resolution of inflammation, including timely clearance of leukocytes and return of host stromal/parenchymal cells to a 'non inflammatory' state, a process known as CATABASIS, is indeed an active process and is similar in complexity to the onset of inflammation[2] .

Resolvins:

Among the pro-resolving mediators mentioned above important are the resolvins. Resolvins were discovered at Serhan laboratory in Bringham and Women's hospital at Harvard Medical School when they were looking for potential endogenous bioactive compounds derived from omega_3fatty acids. Resolvins are the endogenously generated pro-resolving mediators. The first exposure for resolvins was at the 11th international conference on Advances in Prostaglandin and leukotriene Research, Italy, 2000. The term 'Resolvins' or 'resolution phase interaction products' was coined by Professor Charles Serhan and colleagues because these compounds were first encountered in resolving inflammatory exudates. They're derived from omega-3 fatty acids[1].These are lipids that are enzymatically biosynthesized from PUFAs by multiple types of lipoxygenases[5]. As mentioned earlier resolvins are synthesized from omega 3 fatty acids such as EPA and DHA, and thus given as E series (RvE) and D series (RvD) respectively[5]. EPA and DHA have long been known to have beneficial effects in several diseases including atherosclerosis, asthma, cardiovascular disorders, and cancer[3].Resolvins were originally isolated from in exudates formed in the resolution phase of acute inflammation in both rodents and humans[4].Resolvins block the production of pro-inflammatory mediators and regulate leukocyte trafficking to inflammatory sites as well as clearance of neutrophils from mucosal surfaces. Also limitation of polymorph nuclear leukocyte migration and infiltration across the endothelium is done by resolvins[1].

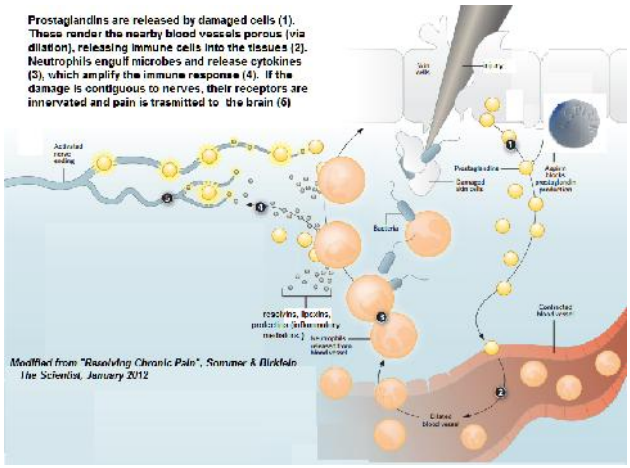

Figure1: Resolvins generation

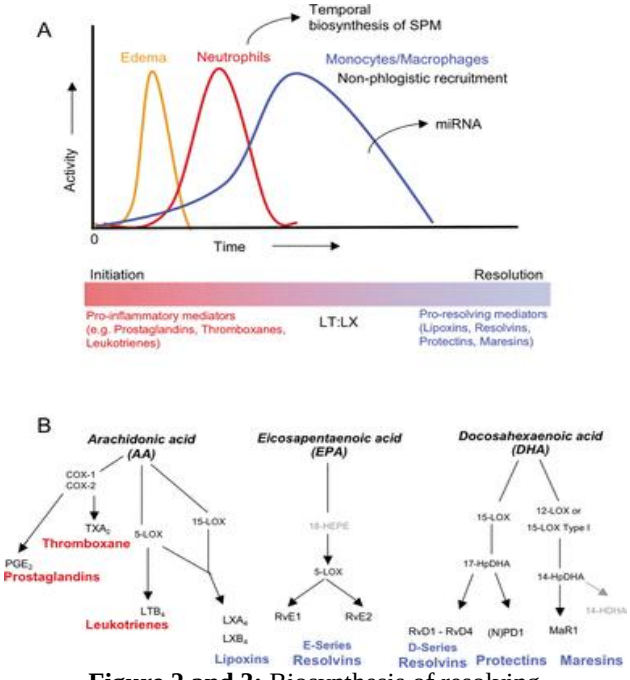

Figure 2 and 3: Biosynthesis of resolving

2. Biosynthesis

Distinct series of pro-resolving mediators are generated, depending on the parent substrate and the presence or absence of aspirin. A series of novel compounds derived from 5Z,8Z,11Z,14Z,17Z-EPA and 4Z,7Z,10Z, 13Z, 16Z, 19Z-DHA, the most abundant omega -3 poly unsaturated fatty acids in cold water marine fish oils, have recently been discovered in resolving murine inflammatory exudates.

These naturally occurring bioactive lipid mediators are termed as resolvins (resolution phase interaction products)(figure2 and 3)[6].

EPA and DHA enzymatically converted into RvE1 and RvD1/ AT-RvD1 in a multi-step reaction that proceeds through a transcellular biosynthetic endothelial/ neutrophil cell-cell interactions[7,8]. The resolvins has recently biosynthesized. Breifly describing RvE1 (5S, 12R, 18R-trihydroxy-4Z, 8E, 10E, 14Z, 16E-eicasopentanoic acid) requires several enzymes such as COX-2 or cytochrome p450, and 5-LOX for its biosynthesis from EPA[9]. RvD1 (7S, 8R, 17S-trihydroxy-4Z, 9E, 11E, 13Z, 15E, 19Z-docosohexaenoic acid) utilizes 15-LOX and 5-LOX for its biosynthesis from DHA (Fig-4).

E-series resolving

RvE1 is highly potential in treating inflammation-related diseases in various animal models. In a murine peritonitis model, RvE1 at nanomolar levels shows a significant reduce dermal inflammation, peritonitis, neutrophil cell migration, and also modulate the expression of cytokines and chemokines.RvE1 potently protected against neovascularisation in a mouse model of oxygen induced retinopathy, in part by reducing TNF -alpha production in microglia associated with retinal vessels[10].RvE1 also protected against intestinal inflammation and colitis in mice by increasing the survival rates and decreasing leukocyte infiltration. It also mediates the resolution of allergic inflammation, via regulating natural killer (NK) cell migration *in vivo* and NK cell cytotoxicity *in vitro*[11].Stromal keratitis, a chronic immunopathological disease after herpes simplex virus infection often causes blindness in humans. RvE1 treatment significantly reduced the extent of angiogenesis and stromal keratitis lesions, by reducing the numbers of inflammatory cells including T helper cells and neutrophils in the cornea, increasing the production of anti-inflammatory cytokine IL-10, and inhibiting the production of pro-inflammatory mediators in mice[12].Most cases of pneumonia can spontaneously resolve which also engages resolvins. In a murine model of aspiration pneumonia, RvE1 treatment decreased neutrophil accumulation and enhanced clearance of E.coli in the lung[13,14].

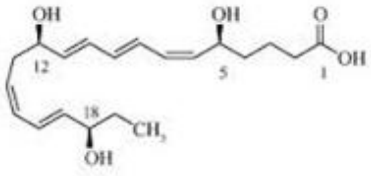

Figure 4: Resolvin E1

Biosynthesis of E-series resolvins *EPA* is oxygenated at the carbon-18 position via the action of aspirin-acetylated COX-2 to form 18R-HEPE, which is subsequently oxygenated to form 5S-hydroxy (peroxy)-18R-HEPE via the 5-LOX activity. The 5S-hydroperoxy group is either converted to a 5,6-epoxide intermediate to form RvE1 or reduced to a 5S-hydroxyl group to form RvE2.

D-series resolvins

RvD1 and AT-RvD1 (the aspirin triggered form)also exhibit potent anti-inflammatory and pro-resolution actions in rodent models pro inflammation. Mouse kindey produces RvD1 in response to bilateral ischemia /reperfusion injury, and RvD1 adminstration before the ischemia alleviated functional and morphological kindey injury reduced intestinal fibrosis and leukocytes infiltration, and blocked TLR-mediated activation of macrophages[15]. RvD1 also regulates microRNA expression in self-limited murine peritonitis. In murine dorsal skin air pouch model, in which resolvins were originally indentified, RvD1 blocked neutrophil recruitment. RvD1 also controls inflammation after oxidative stress[14,16].

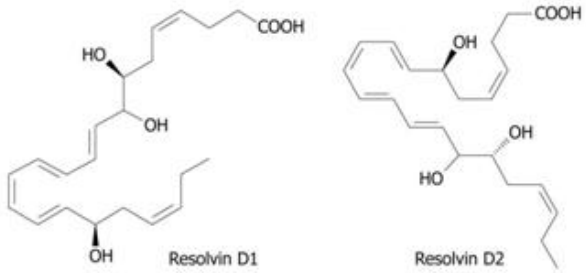

Figure 5: D series resolvins

Biosynthesis of D-series resolvins *DHA* is the substrate for two groups of resolvins produced by different biosynthetic routes, referred to as the 17S and 17R D Series resolvins, during the resolution of inflammatory exudates. Endogenous DHA is converted *in vivo* via lipoxygenase initiated mechanisms to the 17S-hydroxy-containing series of four resolvins, known as resolvins D1 to D4. In the presence of aspirin, the oxygen at C13 switches to C17 with an R configuration that is a precursor for the aspirin triggered 17R D Series resolvins(serhan et al.,2002;Hong et al., 2003)[17]

3. Chemistry and Biology

The omega-3 essential fatty acids are currently the focus of considerable interest among nutritionists. The resolvins are synthesized either from EPA or from DHA. The basic structure of this potent bioactive product generated from EPA proved to be 5, 12,18R-trihydroxyeicasopentanoic acid. RvE1 possesses an interesting and novel distinct structure consisting of a conjugated diene plus a conjugated diene chromophore present within the same molecule. Both biogenic and total organic syntheses were achieved and its complete stereochemical assignment was established along with that of several related natural isomers (claria and Serhan, 1995). RvE1 proved to be 5S, 12R, 18R trihydroxy-6Z, 8E, 10E, 14Z, 16E-eicasopentanoic acid. Human micro vascular endothelial cells, when treated with aspirin in hypoxia, release 17 RHDHA produced from DHA. Human recombinant COX-2 converts DHA to k13 hydro (peroxy)-DHA, which is monitored as 13-hydroxydocosa-hexaenoic acid. With aspirin, these switch to 17R- oxygenation to give a group of AT resolvins (AT-RvD1 to RvD4) that were also found in brain. Using the mediator lipodimics-informatics approach, and employing tandem LC-PDA-MS-MS, it was

exciting to find that neither aspirin nor exogenous DHA was required to monitor the production of these structures *in vivo*. The endogenous DHA was converted in vivo to a 17S alcohol containing a series of resolvins via LOX initiated mechanisms. The stereochemistry of both 17R and 17S series of resolving D1 was established and total organic synthesis achieved. RvD1 proved to be 7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid, and AT-RvD1 matched 7S,8R,17R-trihydroxy-4Z, 9E, 11E, 13Z, 15E,19Z-docosahexaenoic acid[18].

Functions: These mediators are generated endogenously during defined intervals within the acute inflammatory response, the view emerges that they may not necessarily serve solely to block and inhibit inflammation, but may also activate resolution within the inflammatory exudate and thus promote the return to homeostasis. The inflammation gets investigated through various steps.pro-inflammatory mediator catabolism was sufficient for inflammation to switch off and the response subsequently just "fizzled out". This is only part of the process at the tissue level, as polymorph nuclear leukocyte (PMN) or eisonophils, if left unchecked, could do untold ha to an already inflammed site and must be disposed of in a controlled and effective manner. Next is the cell clearance either by systemic circulation or apoptosis or by phagocytosis. The last for tissue resolution and homeostasis is that the parenchymal and stromal cells that hosted the inflammatory event revert back to a non-inflammatory phenotype.

- RvE1 displays potent counter-regulatory and tissue-protective actions *in vivo* and *in vitro*. Circulating omega-3 fatty acids rapidly appear in the inflammatory sites which require conversion to resolvins that control excessive neutrophil infiltration, protect organs, and faster resolution.
- Resolvins regulate macrophage and dendritic cell functions by controlling the production of cytokines or by regulating phagocytosis.
- Intravenous administration of resolvins in nanogram quantities dramatically reduces the PMN infiltration in a murine acute inflammation model. Resolvins are not anti microbial but alteration of microbial flora induced by RvE1 is intriguing.
- They reduced the pain induced by inflammation caused by intraplantar injection of formalin.
- Topical application of the eicasapentanoic acid derived RvE1 helps prevent soft tissue inflammation and destruction, as well as bone loss associated with periodontal disease. Resolvins regulate the immune system by controlling functions of specific cell types
- RvE1 can reduce neuropathic pain by several mechanisms. In human platelet rich plasma, RvE1 selectively blocked the Adenosine diphosphate stimulated and thromboxane stimulated cells.

Effect of Resolvins on Inflammatory Pain

In a variety of forms of inflammation, D and E series resolvins known to lead tissue situation to the resolving phase. Pain an important aspect of inflammation, may be strongly affected by the actions of resolvins. Resolvins may also directly regulate the function of the sensory neural circuit, independent of their control mechanisms for the inflammatory state[19].A large number of inflammatory pain models have been tested to determine whether resolvins affect pain states, and as a result surprising analgesic effects have been consistently reported(table 3). JiJab study gave a pioneering result, that focussed on inflammatory pain and commonly on multiple types of experimental animal models such as inflamed animals using complete Freund's adjuvant, carrageenan, and TNF-Alpha and on an acute pain model using capsaicin, a pain receptor TRPV1-specific agonist; RvE1 administration drastically decreased pain symptoms even at nanogram levels. The underlying mechanisms that realize such powerful analgesic outcomes might be complex and still require further elucidation, but clear evidence for some downstream signaling was provided. First, a typical mechanism as shown above in the inflammation research field also works in the inflamed tissues of pain models. When intraplantular injections were given into inflamed paws, RvE1 distributed neutrophil infiltration and paw edema formation and reduced expression of proinflammatory cytokines and chemokines macrophage inflammatory protein. Another action is through the direct action on the neuronal circuit .RvE1 has been shown to exert direct effects on the primary nociceptor neurons. TRPV2 is a major pain receptor present in the tissue-innervating terminals of nociceptor neurons, detecting and electrically transducing damage signals including heat, anisotonocity, acid, and capsaicin, in normal and inflammatory conditions. Thus, TRPV1 has been a key peripheral analgesic target for nearly two decades. RvE1 blocked capsaicin-evoked acute pain and TRPV1- mediated heat pain. The microglia located in the spinal cord dorsal horn play a critical role in maintaining pathologic plasticity in the nociceptive synapses between DRG and dorsal horn neurons. The monocytic origin of spinal microglia leads to a strong possibility that the microglia may transcellularly synthesize and secrete resolvins and also express the resolvin-specific GPRs- as already shown in tje monocytes and macrophages. It is also noteworthy that intrathecal RvE1 had a greater efficacy in dampening the second pain phase in formalin-induced models. The analgesia seems to follow an indiscriminate manner, for example, when administered intrathecally, RvE1 or RvD1 alleviates both heat and mechanical hypersensitivity.For resolvins' analgesic actions, not only the mechanism in the spinal synapses, but also the peripheral mechanisms seem to depend on the GPR signaling. In DRG neurons, RvD2 inhibited both of activities of TRPV1 and TRPA1. The effect of RvD2 was prevented by the pretreatment t of pertussis toxin which is able to block G-coupled signaling. This suggests that the TRP channel blockade is mediated by GPR action. Identities of RvD2-specific GPRs remain obscure. Accordingly, pain inhibitory effects of resolvins might be limited to the sensory modalities (e.g.,heat, cold, pressure, and poking) that the target TRP channel conveys. AT-RvD1 has shown different specificity; it only has an inhibitory effect on TRPV3. This was consistent with behavioural phenotypes seen following the use of TRPV3 agonists. In addition, AT-RvD1 was also effective at

inflammatory heat responses triggered by CFA or carrageenan injections. The results suggest that it is not likely that inhibitory effects of RvDs depend on GPR signaling. Furthermore the potency of RvD actions on TRP channels is different from the known potency of RvD actions on leukocytes that likely involve GPR signaling, indicating that different receptors with different affinities are engaged[20].

In a further series of experiments, Xu et al. also showed that RvE1 reduced the potentiation of N-methyl-D-aspartic acid (NMDA) glutamate receptor currents by TNF, again via an(extracellular signal-regulated kinase pathway) ERK-mediated pathway. Hyperactivity of spinal NMDA receptors is a well-known mechanism of mechanical allodynia (hypersensitivity to innocus mechanical stimuli). After application of RvE1, the NMDA glutamate receptor currents were back to normal, thus the spinal plasticity implicated in pain hypersensitivity was reverted[21].

Analgesic actions of resolvins

To explore the effects of resolvins in animal models of pain Xu and colleagues performed a comprehensive study. They tested two different resolvin molecules RvE1 and RvD1, for their ability to reduce inflammatory pain behaviour in mice, and found both molecules to be effective. They reduced pain induced by inflammation caused by intraplantar injection of formalin. In the formalin model, only the second phase of pain behaviour (which is likely mediated by spinal cord mechanisms) was attenuated, indicating a central action of the molecules via the chemerin receptor 23(chemR23; also known as chemokine receptor like 1), which is a G-protein coupled receptor for resolvin, present on the nociceptive neurons in the dorsal horn of the spinal cord. These neurons also express the transient receptor potential vanilloid 1(TRPV1), also known as the capsaicin receptor. RvE1 did not alter the responses to painful stimuli in mice that had not undergone inflammation or injury. This means that resolvins affected only pathological pain sensations, not normal sensations evoked by painful stimuli. RvE1 was also effective in reducing pain behaviour after direct injection of the irritant capsaicin and the proinflammatory cytokine tumour necrosis factor- alpha into the tissue. Also, RvE1 reduced pain behaviour in a model of tissue injury and in a model of neuropathic pain (spinal nerve ligation)[20].

Postoperative pain caused by skin/muscle incision normally resolves within 1 week, and such pain is commonly recapitulated in rodent models of paw incision. Postoperative pain after prolonged muscle retraction lasts 3–4 weeks in humans and rodents. It is of great significance to effectively and aggressively manage acute postoperative pain and shorten the duration of patients' hospital stay. Indeed, fast-track surgery has been strongly advocated in an effort to optimize surgical outcomes and control ever-increasing health care costs. Effective management of acute postoperative pain may also reduce the incidence of chronic postoperative pain, which can persist 3–6 months after surgery. Resolvins have also displayed potent analgesic actions in rodent models of postoperative pain. RvE1 and RvD1 prevented paw incision-induced postoperative pain in mice and rats. In a recently developed skin-muscle retraction model (SMIR), surgery-induced mechanical hypersensitivity can last up to 4 weeks, yet a single treatment of RvD1 on postsurgical day 2 largely prevented this postoperative hyperalgesia in rats. Of note, RvD1 treatment at later time points (e.g., postoperative day 9) only produced transient pain relief (< 1 day). Suggesting a time-dependent efficacy of resolvin treatment. Nevertheless, resolvin still displays efficacy in treating late-phase pain.

Importantly, resolvins do not interfere with normal pain perception. Thus, intraplantar, intrathecal, or systemic injection of resolvins was not observed to affect either thermal and mechanical pain sensitivity in rat and mice. In sharp contrast, the classic analgesics such as morphine dramatically decrease pain sensitivity. Physiological pain has evolved as a protective response. For people who can not feel pain, due to genetic mutation of the pain genes encoding neurotrophic tyrosine kinase receptor type A (TrkA) and sodium channel Nav1.7, life is often tragic, with self-mutilation and short life span . Thus, resolvins may serve as a new class of analgesics that act to block abnormal pain and restore normal sensitivity, rather than blocking pain transmission like classical analgesics[20]

Mechanisms underlying the analgesic action of resolvins

Resolvin's analgesic actions are thought to be mediated by specific GPCRs. The specific binding of RvE1 to the Gαi-associated ChemR23 receptor has been demonstrated using synthetic [^{3}H]-labeled RvE1. Several lines of evidence indicate that ChemR23 mediates RvE1's analgesic action in formalin-induced 2ndphase pain in mice. First, RvE1's action in this pain model was abolished by pertussis toxin treatment, a specific inhibitor for Gi-coupled GPCRs, but not by naloxone, an opioid receptor antagonist. Second, RvE1's analgesic effect was abrogated by knockdown of ChemR23 with specific siRNA treatment. Third, RvE1's analgesic action could be recapitulated by chemerin, a natural peptide ligand for ChemR23. Similarly to RvE1, chemerin reduces transepithelial migration of neutrophils and promotes apical clearance of neutrophils. Chemerin also inhibits the production of the pro-inflammatory mediators (e.g., TNF-α, IL-1β, IL-6 and IL-12) and induces the expression of anti-inflammatory cytokines such as transforming growth factor-beta (TGF-β) and IL-10 in macrophages, in a pertussis toxin–sensitive manner.

A broad expression of ChemR23 in various cell types may explain the versatile actions of RvE1. Earlier studies demonstrated ChemR23 expression in macrophages, microglia, and dendritic cells. Recent findings have revealed that ChemR23 is also expressed by primary sensory DRG neurons. In particular, ChemR23 is heavily localized with TRPV1, a heat sensor in nociceptors. Chem R23 synthesized in DRG cell bodies is transported axonally to central terminals in the spinal cord–and presumably, to peripheral terminals. ChemR23 is also expressed in spinal cord. Furthermore, inflammation induces ChemR23 expression in skin macrophages. In addition, AT-RvD1 and

RvD1 are known to activate the same GPCRs, GPR32 (human) as well as the LXA_4 receptor ALX/FPR2 (murine), and the latter is expressed in spinal astrocytes . However, the expression patterns of these receptors and their co-existence with ChemR23 awaits further investigation.

The RvE1 receptor ChemR23 is widely expressed in immune cells, glial cells, and neurons in mouse tissues. (a) Double staining of ChmR23 and ED1 shows that ChemR23 is largely colocalized with the macrophage marker ED1 in the dermis of the CFA-inflamed**.** Strikingly, different resolvins may differentially regulate TRP channels. RvE1 is known to block capsaicin-induced spontaneous pain, ERK activation in DRG neurons, and spinal cord synaptic plasticity, without affecting TRPA1-induced pain. In contrast, RvD1 inhibited TRPA1, TRPV3 and TRPV4, but not TRPV1 currents, in cultured human embryonic kidney 293 (HEK293) cells and DRG neuron cultures. Consistent with these findings, RvE1 is effective at low doses in reducing TRPV1-mediated heat hyperalgesia, whereas RvD1 and AT-RvD1 are very effective in inhibiting mechanical hyperalgesia, which is known to involve the activation of TRPA1/TRPV4. It should be noted that RvE1 can also reduce CFA-induced mechanical allodynia, but this occurs at much higher doses than those observed for the inhibition of heat hyperalgesia. Finally, a recent study demonstrated that 17(R)-RvD1, an analogue of RvD1, specifically inhibited TRPV3 *in vitro*. Thus, different resolvins may regulate different modalities of pain that are controlled through distinct TRP channels. Tissue injury-induced spinal cord synaptic plasticity (i.e. central sensitization) has been strongly implicated in the genesis of persistent pain[8]. Such plasticity is measured in part as changes in spontaneous excitatory postsynaptic currents (sEPSCs), which could indicate both presynaptic mechanisms (frequency changes) and postsynaptic mechanisms (amplitude changes). Perfusion of spinal cord slices with RvE1 does not alter basal synaptic transmission; however, it does abolish capsaicin- and TNF-α-induced sEPSC frequency increases in lamina II neurons. This indicates that RvE1 can normalize spinal cord synaptic plasticity, presumably by inhibiting ERK phosphorylation and glutamate release in presynaptic terminals. Activation of NMDA receptors in dorsal horn neurons is a key element for central sensitization and chronic pain development. TNF-α not only increases sEPSC frequency but also increases NMDA-induced currents in dorsal horn neurons in an ERK-dependent manner[71]. Notably, RvE1 blocks both the TNF-α-induced ERK phosphorylation and the correlated NMDA receptor activation in dorsal horn neurons. Thus, it is conceivable that RvE1 abrogates central sensitization via both presynaptic and post/extra-synaptic mechanisms. The underlying signaling mechanisms are largely unknown, although modulation of the ERK pathway - a critical pathway involved in central sensitization - is likely to be involved[20].

Resolvins and Immune System: The ω-3 PUFAs are appreciated for their beneficial actions in the immune system, for instance, the presence of DHA, EPA and their mediators are found at local sites of inflammation. During acute inflammation, PMN produce oxygen radicals and release hydrolytic and proteolytic enzymes. These byproducts are capable of killing bacteria and need to be removed from the site of inflammation. Therefore, failure of this mechanism might cause tissue damage and chronic inflammation. Apoptosis of PMN is a physiological process for removal of PMN from inflammatory sites by opsonization and recognition by macrophages. Abolition of inflammation is also mediated by secretion of anti-inflammatory cytokines, such as IL-10 and TGF-β. However, when there is a failure to resolve acute inflammation, there is necrosis of PMN. This may rupture cell membrane, release of intracellular content and cause tissue damage. The progress of these events results in chronic inflammation that includes abscess formation, scarring and autoimmunity.

Resolvins regulate the immune system by controlling functions of specific cell types. For instance, RvD1 differentially modulates primary human macrophage responses to lipopolysaccharides, depending on the context in which this molecule is presented to the macrophage. Resolvins and protectins have been shown to stimulate innate killing mechanisms to manage bacterial loads and stimulate clearance of bacteria. RvE1 is a potent inhibitor of leukocyte infiltration, dendritic cell migration, IL-12 production and PMN transendothelial migration. Furthermore, RvE1 was found to negatively regulate the development of an allergic inflammation *in vivo*. Other studies demonstrated that RvE2 stimulates host-protective actions throughout initiation and resolution of the innate immune responses. Additionally, RvE3 has proven to be a potent inhibitor of PMN chemotaxis *in vitro* and *in vivo*.Recently, it was demonstrated that in *E. coli* infections, the combination of RvD1, RvD5 and protectin D1 (a dihydroxy product formed in inflammatory exudates), together with antibiotics, increased antimicrobial responses in mouse peritoneum. The studies stated above indicate that resolvins block excessive inflammatory responses and promote resolution of inflammation as follows: (a) blocking cytokine production; (b) reducing PMN transendothelial migration and (c) increasing macrophage activity resulting in the clearance of apoptotic cells and debris from inflamed areas.

4. Resolvins and Pain

The precursor of resolvin D series, 17*S*-HpDHA, modulates both the genesis and the maintenance of mechanical hyperalgesia in an arthritis model in rats. This anti-hyperalgesic effect in acute inflammation seems likely to be mediated by inhibition of both NF-κB and COX-2 in the peripheral nervous system .These effects were partly related to decreased production of TNF-α and IL-1β in rat hind paw. RvE1 can reduce neuropathic pain by several mechanisms, which include inhibition of the following: (a) TNF-α synthesis release and downstream signaling (b) transient receptor potential ion channel signaling and (c) peripheral inflammation via enhancing the phagocytic activity of macrophages. Furthermore, treatment with RvE1, three weeks after nerve injury, transiently reduced

mechanical allodynia and heat hyperalgesia. The studies listed above suggest that resolvins could be used as a novel class of analgesics to treat inflammatory pain. Two advantages over current drug therapies to treat pain include; high potency and endogenous production in the body[22].

Resolvins and Coagulation

Blood clotting is an important mechanism to help the body repair injured blood vessels. This mechanism involves several steps, including: (a) constriction of blood vessels; (b) platelet aggregation and (c) stabilization of the blood clot. These processes are mediated primarily by activation of thromboxane and PG. In human platelet-rich plasma, RvE1 selectively blocked both adenosine diphosphates (ADP)-stimulated and thromboxane-stimulated platelet aggregation in a concentration-dependent manner. Another study demonstrated that RvE1 possesses regulatory actions, such as reduction of ADP-stimulated P-selectin surface mobilization and actin polymerization. The specific platelet actions of RvE1 selectively engaged with ADP activated platelets may contribute to both resolution of vascular inflammation and ADP-dependent platelet activation. RvE2 may also contribute to homeostasis, as it rapidly downregulates surface expression of human leukocyte integrins in whole blood. Additionally, it dampens responses to platelet-activating factor, a potent activator of platelets and leukocytes. Together, these results indicate that RvE1 selectively regulates platelets, which are critical cell components for blood coagulation[22].

Resolvins and Periodontitis

Periodontitis is a chronic inflammatory disease caused by the release of immune mediators, resulting in destruction of the alveolar bone and periodontal connective tissue. The process of bone resorption is a result of proteolysis and acid production mediated by osteoclasts. Additionally, in this process, there is a massive expression of vacuolar-type H^+-ATPase that enables bone degradation. The mechanism by which bone resorption is regulated involves different factors, including PGE_2, which activates osteoclastswhile influencing their number and function. In contrast, RvE1 was found to inhibit osteoclast growth and bone resorption by interfering with its differentiation. A previous study indicated that topical application of RvE1 to rabbit periodontal tissue conferred dramatic protection against tissue and bone loss associated with periodontitis. In that study, it was also demonstrated that PMNs from localized aggressive periodontitis were refractory to resolving molecules of the lipoxin series. However, PMNs responded to RvE1, which stopped superoxide anion generation by binding at a site that is functionally distinct from the aspirin-triggered lipoxin receptor .These studies revealed the potential of using resolvins for prevention and treatment of periodontal disease. Furthermore, they provide a new role for resolvin signaling in the pathogenesis of periodontal disease[22].

Resolvins and Salivary Gland Function: Sjögren's Syndrome (SS) is an autoimmune disease characterized by xerostomia (dry mouth) and Keratoconjunctivitis sicca (dry eyes). Such symptoms are clinically detectable only after salivary and lacrimal glands display chronic inflammation, a point at which current therapies have no benefit. RvD1 receptor activation promotes resolution of inflammation and tissue repair in salivary epithelium, which may have relevance in the restoration of salivary gland dysfunction associated with SS. It was demonstrated that RvD1 treatment in Par-C10 cells prevents TNF-α mediated disruption of salivary epithelial formation. Also, RvD1 enhanced cell migration and cell polarity via PI3K/Akt signaling in Par-C10 cells. These studies indicate that activation of ALXR/FPR2 with RvD1 could be used not only to block inflammation, but also to improve tissue repair and regeneration in damaged salivary glands[22].

Methods and formulations for administration of resolvin anti-inflammatory compounds

The resolvins may be administered in a variety of forms, including drug depots comprising polymers or lipids. The pharmaceutical formulations of the present invention may be used to treat a variety of conditions including acute pain and chronic pain. Resolvins used in the methods and compositions of the present invention may be prepared in vivo or in vitro and then substantially purified and isolated by techniques known in the art (see, for example, U.S. Pat. No. 6,670,396 which is incorporated by reference in its entirety.) Without limitation, the purity of the compounds is generally at least 90%, preferably at least 95%, and most preferably at least about 99%. Certain resolvins used in the present invention may be prepared by chemically modifying one or more of the purified compounds. For example, a purified compound may be chemically modified into a pharmaceutically acceptable salt or prodrug. Additionally or alternatively, one or more hydroxyl, thiol or amino groups of the molecule may be protected using methods well known in the art. Resolvins can also be manufactured independently using conventional methods. Resolvins are delivered via a suitable carrier to treat an inflammatory condition. Examples of inflammatory conditions or diseases that could be treated using resolvin therapy include, but are not limited to, rheumatoid arthritis (RA), dermatitis, multiple sclerosis, ankylosing spondylitis and osteoarthritis. Further, vascular inflammatory disease could be treated, including but not limited to, vasculitis, rheumatoid vaculitis, Buerger's Disease, and cryoglobulinemia. In addition, resolvins have activity as neuro-protectants and may be used for this purpose according to the methods of the present invention. The resolvins may also be used to treat conditions associated with acute or chronic inflammation. Chronic inflammation is associated with a number of conditions and diseases, including but not limited to, disc herniation, facet joint disorders, and periodontal disease.

The term "periodontal disease" as used herein includes all diseases of the periodontal tissue that surround and support the teeth (see, for example, D. M Williams et al., "Pathology of Periodontal Disease", 1992 Oxford University Press). These include gingival, cementum, periodontal ligament, alveolar process bone, and dental supporting bone. Periodontal diseases include, but are not limited to gingivitis and periodontitis. A "therapeutically effective amount" or "effective amount" is such that when administered, the drug results in alteration of the biological

activity, such as, for example, inhibition of inflammation, improvement of the condition, etc. It will be understood that the dosage administered to a patient can be as a single dose, or multiple doses, continuous doses (e.g. continuous infusion) or depot or multiple depots depending on a variety of factors, including the drug's administered pharmacokinetic properties, the route of administration, patient conditions and characteristics, extent of symptoms, concurrent treatment, frequency of treatment and the effect desired.

A "depot" includes but is not limited to capsules, microspheres, microparticles, microcapsules, microfibers particles, nanospheres, coating, matrices, wafters, pills, pellets, emulsions, liposomes, micelles, gels or other pharmaceutical delivery compositions. Suitable materials for the depot are ideally pharmaceutically acceptable biodegradable materials that are preferably FDA approved or GRAS materials. These materials can be polymeric or non-polymeric, as well as synthetic or naturally occurring, or a combination thereof. The depot may also comprise a drug pump. The term "biodegradable" includes that all or parts of the drug depot will degrade over time by the action of enzymes, by hydrolytic action and/or by other similar mechanisms in the human body. In various embodiments, "biodegradable" includes that the depot (e.g. microparticle, microsphere, gel, etc.) can break down or degrade within the body to non-toxic components after or while a therapeutic agent has been or is being released. By "bioerodible" it is meant that the depot and/or gel will erode or degrade over time due, at least in part, to contact with substances founds in the surrounding tissue, fluids or by cellular action. By "bioabsorbable" it is meant that the depot will be broken down and absorbed within the human body, for example, by a cell or tissue. "Biocompatible" means that the depot will not cause substantial tissue irritation or necrosis at the target tissue site[23].

Resolvins and their therapeutic value

Regeneration of lost periodontal tissues is considered to bone of the most challenging aspects of periodontal therapy. Our current understanding of the role of the host immune inflammatory response in periodontal diseases forms the basis of new therapeutic approaches. Many studies have been carried out to evaluate the efficacy of systemic administration of omega-3 polyunsaturated fatty acids as an adjunct treatment to routine periodontal therapy. The findings suggest that the combination therapy demonstrated successful reduction of gingival inflammation, reduction of pocket depth and attachment level gain, accompanied by a trend for modulation of the cytokine profile in gingival crevicular fluid (Elkhouli, 2011). Improved outcomes are attributed to the primary metabolites of omega-3 fish oils, eicosapentaenoic acid (EPA) and docoshexaenoic acid (DHA). In a 5-year longitudinal study of subjects 70 years of age at baseline, an inverse independent relationship was found between dietary DHA intake and periodontal disease events, after controlling for confounding factors. People with low DHA intake had an approximately 1.5 times higher incidence rate of periodontal disease progression (Iwasaki et al., 2010).Topical application of 4 mg resolvin E1 per tooth every other day for 6 weeks in cases of periodontitis was shown to reduce PMN infiltration, and stop inflammation-induced tissue and bone loss (Hasturk et al., 2006). The effect of dietary supplementation was evaluated by El-Sharkawy et al. (2010) in a double blind clinical study of parallel design. The control groups were treated with scaling and root planing (SRP) or a placebo while the test group received SRP followed by dietary supplementation of fish oil(900mg EPA1DHA) and 81mg aspirin daily. Results showed a significant reduction in pocket depth and attachment gain after 3 and 6 months in the test group compared with baseline and control group. In addition, supplementation with omega-31 aspirin resulted in a significant shift in the frequency pockets.

To evaluate the efficacy of systemic administration of omega-3 polyunsaturated fatty acids plus low-dose aspirin as an adjunctive treatment to regenerative therapy of furcation (bone loss) defects. Resolvins in the future It is clear that future care of trauma and surgical patients, as well those with periodontal diseases, will rely heavily on clinicians having a detailed map and fundamental appreciation of the temporal relationships involved in the resolution of local acute inflammation and tissue injury at the cellular, molecular, organ, and systemic levels. Novel lipid mediator pathways are very attractive for new therapeutic interventions because they: rely on small molecules (<500 MW); are amenable to total organic synthesis; and can be manufactured with currently available pharmaceutical facilities (Serhan et al., 2002, Navia and Peattie, 1993). It is too early to speculate on the intended use as a periodontal therapeutic. It is doubtful that an inflammation modulating agent will ever completely replace some form of mechanical therapy aimed at the control of the biofilm; even if the veracity of the hypothesis that inflammation impacts upon the composition of the biofilm is proven, the biofilm will still be there. Reduction of the mass of the biofilm as a means of reducing the inflammatory burden remains a viable approach. The use of these molecules may be an interesting preventative approach that will protect against the acquisition or overgrowth of intrinsically pathogenic bacteria (Maurizio et al., 2011). Resolvins offer an entirely novel biological approach to treating significant inflammatory diseases, with a decreased potential for immuno-suppression. Resolvins are potential candidates for drugs to treat a broad range of acute and chronic diseases caused by a failure to resolve the inflammatory response and restore immune homeostasis. Such diseases include autoimmune diseases (like Crohn's disease, psoriasis and rheumatoid arthritis), allergic diseases (like asthma) and chronic inflammatory diseases. Resolvins offer an entirely novel biological approach to treating significant inflammatory diseases, with a decreased potential for immuno-suppression.[25]

Pharmaceutical and clinical development - copies of resolvins: RvE1 is a local acting autacoid that has proved to display pro-resolving activity when treatments were given either by topical, intra venous, or intra peroneal administration (Bannenberg et al., 2005; Arita et al., 2006).

Resolvyx Pharmaceuticals is investigating a series of resolvin E1 analogs, including the eicosapentaenoic acid (EPA)-derived RX-10005 (RX-05) and RX-1001, for the potential treatment of acute and chronic inflammatory diseases including colitis, periodontitis, arthritis, asthma and dry eye disease. About RX-10001 The resolvin known as RX-10001 is a naturally occurring, small molecule lipid mediator. It acts to protect healthy tissue during an inflammatory response to an environmental insult and to resolve inflammation once the environmental insult has passed. In pre-clinical tests, RX-10001 has shown to be active with a very high potency across a range of inflammatory disease models, including asthma, colitis, rheumatoid arthritis, atherosclerosis, dry eye and retinopathy, active by oral, intravenous and subcutaneous routes of administration. Reolvyx Pharmaceuticals, Inc., a resolvin therapeutics company, has announced that it has initiated the first human clinical trial evaluating RX-10001 in a phase I clinical trial in healthy volunteers[25]. Although the powerful potencies and negligible adverse effects of resolvins appear to promote their transitions to clinical settings, many aspects of the biological mechanisms remain to be clarified. Early termination of inflammation by resolvin treatment seems to be beneficial in that it prevents conversion into chronic inflammation and chronic inflammatory pain. On the other hand, if it is a premature termination, clearance of initial damaging insults or microorganisms might be incomplete. Surprisingly beneficial indices were obtained in this regards; RvDs and RvE1 contribute to bacterial clearance. In addition, resolvins appear to promote injury repair by elevating phosphoinositide 3 kinase-dependent migration and reducing apoptotic cell accumulation.

5. Conclusion

This review summarizes information on resolvins and related substances, which are important both as endogenous proresolvers and as therapeutic candidates preventing deterioration of inflammation and pathologic pain. Indeed, clinical applications related to inflammation are seriously being considered; a clinical phase II trial of a resolvin-derived synthetic analog RX-10045 for eye dryness was recently completed. A phase I study of oral administration of RvE1 was also completed, and its possible clinical applications will likely include rheumatoid arthritis, asthma, and colitis. Studies on the total synthesis processes of resolvins will help to establish a platform to generate more chemically and metabolically stable analogs which guarantees sufficient proresolving duration when clinically applied. Resolvin studies, as mentioned above, are also evolving into ones investigating practical utilities for various pain diseases.

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