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PERSPECTIVES IN BASIC SCIENCE

Lipoxins: Potential anti-inflammatory, proresolution, and antifibrotic mediators in renal disease

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Lipoxins: Potential anti-inflammatory, proresolution, and antifibrotic mediators in renal disease. Lipoxins are lipoxygenase-derived lipid mediators with both anti-inflammatory and proresolution properties that have been demonstrated in vivo and in vitro. The bioactivity profile of lipoxins in vitro suggests that they have therapeutic potential in acute renal failure and glomerulonephritis; predictions that have been borne out to date in experimental models of renal disease. We review recent developments on the molecular basis of lipoxin bioactions mediated through receptor crosstalk and the accumulating evidence that lipoxins may have potential as novel anti-inflammatory agents.

Eicosanoids play a key role in the initiation, propagation and termination of inflammatory cascades, which are vital for efficient host defense [1]. These responses are characterized initially by generation of proinflammatory lipid mediators (e.g., leukotrienes and prostaglandins) in a first-phase response and a subsequent switch, to "second phase" anti-inflammatory, proresolution mediators such as lipoxins and cyclopentenone prostaglandins, such as 15 deoxy$\Delta^{12,14}$PGJ$_2$ (reviewed in [1] and [2]). Lipoxins, an acronym for lipoxygenease interaction products, are endogenous anti-inflammatory mediators that promote resolution of inflammation in vivo (reviewed in [3]). Lipoxin generation has been demonstrated in a variety of human and experimental inflammatory, hypersensitivity and vascular diseases (reviewed in [3]). Here we shall review accumulating evidence for a role for lipoxins in limiting inflammation, by influencing key pathophysiologic events leading to the generation of a proresolution phenotype. These developments herald the promise of novel anti-inflammatory therapies.

Key words: Lipoxins, eicosanoids, anti-inflammatory, acute renal failure, glomerulonephritis.

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Overview of lipoxin biosynthesis

Lipoxins are produced locally at sites of inflammation by transcellular routes, involving interaction of neutrophils with platelets or resident tissue cells, such as epithelial cells, by one of at least three biosynthetic pathways (see Fig. 1). At mucosal surfaces, epithelial-granulocyte interactions via the action of 15-lipoxygenase (LO) on arachidonate generate intermediate eicosanoid products, which serve as substrates for polymorphonuclear neutrophil (PMN) 5-LO, generating lipoxin A$_4$ (LXA$_4$) and lipoxin B$_4$ (LXB$_4$).

Within the vascular lumen, platelet-neutrophil interactions, involving neutrophil 5-LO and platelet 12-LO, generate lipoxins. In this setting platelets convert neutrophil-derived leukotriene A$_4$ (LTA$_4$) to the lipoxin intermediate (5,6 epoxytetraene) through the action of platelet 12-LO (see Fig. 1) [4]. Platelet 12-LO essentially functions as a 15-LO when its substrate is LTA$_4$, augmenting lipoxin biosynthesis via transcellular pathways. Thus, within a multicellular inflammatory environment, LTA$_4$ can serve as key intermediate for both lipoxin and leukotriene formation. Maintaining the balance between lipoxin and leukotriene formation is likely to be a critical determinant of the resolution of inflammation.

The third major pathway for lipoxin generation is the aspirin-triggered lipoxin (ATL) pathway (Fig. 1). Expression of cyclooxygenase-2 (COX-2) can be induced rapidly in cells involved in inflammation such as fibroblasts, monocytes, and vascular endothelium, in response to growth factors, cytokines, hormones, and bacterial endotoxin [5]. Aspirin acetylates COX-2, shifting its activity from endothelial cell prostanoid production to favor 15 (*R*) HETE production. In the context of neutrophil interaction with endothelial or epithelial cells, this COX-2–derived 15 (*R*) HETE can then be converted by neutrophil-derived 5-LO to either 15-epi-LXA$_4$ or 15-epi-LXB$_4$ through cell-cell interactions [6]. These ATLs share many of the bioactions of the native lipoxins but typically demonstrate greater potency [7]. The production of ATL has led to the hypothesis that, in

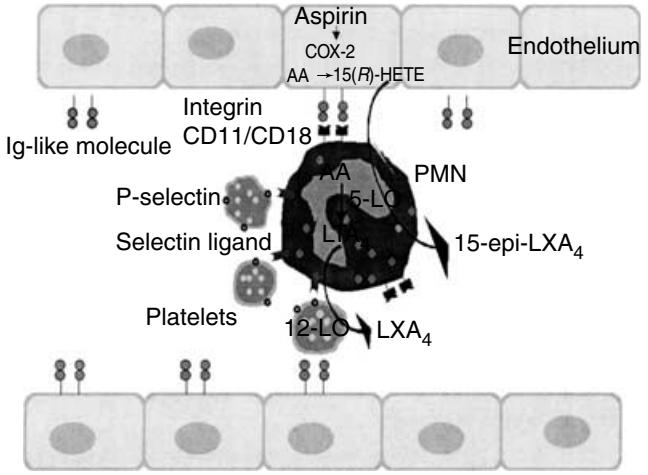

Fig. 1. Transcellular generation of lipoxin A_4 (LXA_4) and 15-epi-lipoxin A_4. P-selectin–mediated endothelial-polymorphonuclear neutrophil (PMN) interaction facilitates LXA_4 biosynthesis. In a cytokine primed milieu aspirin acetylation of endothelial cyclooxygenase (COX-2) facilitates the transcellular generation of 15-epi-LXA_4. Abbreviations are: LO, lipoxygenase; AA, arachidonic acid; LX: lipoxin, HETE: hydroxyeicosatetraneoic acid. (From McMahon B, Mitchell S, Brady HR, Godson C: Lipoxins: Revelations on resolution. *Trends Pharmacol Sci* 22:391–395, 2001, with permission.)

addition to inhibition of prostaglandin biosynthesis, further benefits of aspirin therapy may include promoting generation of lipid mediators such as ATL that act locally to down-regulate inflammatory cell activity [7]. In addition to transcellular biosynthetic pathways described above lipoxin precursors such as 15-HETE can be formed and incorporated into PMN cell membrane for subsequent release and transformation upon PMN activation [7, 8].

Lipoxin biosynthesis has been described in many human and experimental diseases associated with cell-cell contact, including glomerulonephritis, asthma, and rheumatoid arthritis [3]. Lipoxin production is enhanced in vitro by conditions that may be relevant to disease. The interaction of PMNs with platelets facilitates lipoxin generation, as mentioned above [4]. Activation of platelets with thrombin or platelet membrane disruption facilitates lipoxin generation during interaction with leukocytes [9]. Th-2–derived lymphokines interleukin-4 (IL-4) and IL-13, putative negative regulators of the inflammatory and immune responses, both induce 15-LO expression in blood monocytes and epithelial cells, and are potential enhancers of synthesis during cell-cell interactions [10, 11]. Other cytokines that enhance lipoxin synthesis include granulocyte macrophage-colony stimulating factor (GM-CSF), which stimulates 5-LO expression in granulocytes [12] and IL-3, which induces 5-LO expression in mast cells [13]. Hypoxia and proinflammatory mediators such as IL-1β, IL-6, and tumor necrosis factor-α (TNF-α) have been shown to induce COX-2, and may contribute to the generation of ATL in vivo [5]. Lipoxin production may also be affected by the local redox potential as may occur during ischemic oxidant or leukocyte-mediated stress. In this context reduced cellular glutathione levels favor lipoxin synthesis over that of the proinflammatory cysteinyl-leukotrienes [14].

Lipoxin metabolism. Similar to other autocoids, both lipoxins and ATL are rapidly inactivated by local metabolism. In monocytes, this involves dehydrogenation and ω oxidation mediated, in part, by prostaglandin dehydrogenase [15]. LXA_4 undergoes dehydrogenation at C15 and probably ω oxidation at C20 [15]. Stable synthetic analogues of both native lipoxins and ATL have been developed with modifications such as addition of methyl groups to C5 or C15 of LXA_4, phenoxyl groups at C16, or para-fluoro-phenoxyl groups at C16 to both LXA_4 and ATL [15, 16]. Such modifications render the lipoxin resistant to dehydrogenation and oxidation, extending their half-lives and enhancing bioactivity and bioavailability [17, 18]. The selectivity of the modifications has been highlighted by recent descriptions of reduced potency of C1 methyl ester of LXA_4 and ATL in vivo [19]. The enhanced stability and improved efficacy of stable analogues of lipoxins and ATL permits exploration of lipoxins stable analogues as potential therapeutic agents [16].

Novel anti-inflammatory compounds. The interaction of aspirin acetylated COX-2 with omega-3 polyunsaturated fatty acid generates novel bioactive compounds called resolvins [20, 21]. These endogenous lipid-derived mediators have impressive anti-inflammatory properties in vivo, mediated in part by modulating cytokine expression [21]. These novel compounds may in part explain the anti-inflammatory mechanism of omega-3 fatty acids, in addition to highlighting beneficial effects of the combination of aspirin and omega-3 polyunsaturated fatty acids. Together with lipoxin and ATL, these novel compounds expand the field of potent local endogenous mediators that control inflammation. Other endogenously produced anti-inflammatory mediators may include the prostaglandin metabolites 15 deoxy$\Delta^{12,14}PGJ_2$ [1] and annexin-1 [22–25]. Investigations on the cyclopentenone prostaglandins indicate that these may suppress proinflammatory macrophage function through modulation of nuclear factor-kappaB (NF-κB) activity, activated protein-1 (AP-1), and signal transducer and activation of transcription (STAT)–mediated transcription [1].

Major lipoxin bioactions

Vascular and bronchial smooth muscle tone. Lipoxins display vasodilatory roles in in vivo and in vitro disease models [3]. A direct effect on renal hemodynamics is seen with LXA_4, manifesting as dose-dependent increases in

renal plasma flow (RPF) and glomerular filtration rate (GFR) [26]. In in vivo studies in rats, LXA_4 antagonizes LTD_4-induced falls in GFR but not RPF, implying prevention of LTD_4-mediated reductions in the glomerular ultrafiltration coefficient, a consequence of mesangial cell contraction [27]. This action appears to be mediated, in part, through the ability of LXA_4 to act as a partial agonist at some peptidoleukotriene receptors.

Several studies have demonstrated LXA_4 inhibitory effects on bronchial smooth muscle contraction in vitro and in vivo [28, 29], including recently published inhibition of bronchoconstriction by LXA_4 analogue in response to methacholine in a dose-dependent manner in a murine model of asthma [30]. This bronchodilator effect is thought to be mediated by LXA_4 stimulation of nitric oxide generation, with inhibition of acetylcholine release, resulting in reduced vagal nerve–mediated contraction of airway smooth muscle [31]. Lipoxin also stimulates the formation of vasodilatory prostaglandins such as prostacyclin by endothelial cells [32]. Thus, the vasoactive properties of lipoxin are in part mediated by vasodilatory prostaglandins and nitric oxide and/or partial agonist activity at peptidoleukotriene receptors [31–33].

Leukocyte trafficking. Lipoxins are putative endogenous "stop signals" that inhibit neutrophil recruitment and promote resolution of inflammation [3]. Lipoxins display selective actions on specific leukocytes: inhibiting activation of PMNs and eosinophils and activating monocytes and macrophages [34–49].

Native LXA_4, LXB_4, ATLs, and several synthetic lipoxin analogues modulate key steps in PMN recruitment (Table 1), including inhibition of neutrophil chemotaxis [34], attenuation of CD11/CD18 up-regulation [35], inhibition of neutrophil-endothelial cell adhesion, and migration of neutrophils across endothelial cell monolayers [36] and transmigration through epithelial cells [37]. Chemotaxis of eosinophils in response to proinflammatory mediators is also inhibited by both lipoxin and ATL [38]. Both lipoxin and ATL antagonize many of the effects of proinflammatory leukotrienes, including PMN adhesion mediated by CD11/CD18 expression [39], endothelial neutrophil adhesion dependent on endothelial P-selectin [40], and PMN-mesangial cell adhesion [40].

A role for both lipoxin and ATL in limiting PMN trafficking and PMN-mediated damage is suggested by evidence of attenuated PMN-mediated second organ injury by lipoxin and ATL. Following hind limb-ischemia/reperfusion, transgenic mice over expressing LTB_4 receptor demonstrate markedly increased PMN infiltration of lungs and skin microabscesses [41]. Administration of both lipoxin and ATL attenuated this reperfusion-initiated second organ injury, with blunted PMN infiltration of lung tissue, and topical application of both agents was protective in acute skin inflammation. Abrogation by lipoxin of ischemia/reperfusion injury–mediated injury may not merely reflect lipoxin modulation of leukocyte trafficking as will be discussed below [42, 43].

Cytokine networks. Cytokines are integral to the coordinated response to injury. Lipoxin and ATL have been shown both in vivo and in vitro to play a key role in regulating cytokine-chemokine axes. Both lipoxin and ATL directly modulate the cytokine composition in the inflammatory environment favoring generation of proresolution mediators [44–48]. In cytokine-primed human synovial fibroblasts, LXA_4 was found to inhibit IL-1β-induced matrix metalloproteinase-3 (MMP-3) expression and IL-6 and IL-8 release while stimulating the synthesis of tissue inhibitors of metalloproteinase (TIMP)-1 and -2 proteins [44]. LXA_4 and stable analogues of lipoxin attenuate IL-8 release from TNF-α–primed colonic cell lines [45], human colon ex vivo [46], and from intestinal epithelia in response to challenge with *Salmonella typhimurium* [47]. Additional involvement of lipoxin in regulatory cytokine loops is demonstrated by suppression of TNF-α–stimulated release of IL-1β and macrophage inflammatory peptide-2 and superoxide production and by increased production of the potent anti-inflammatory cytokine IL-4 by both native LXA_4 and ATL [48].

Host defense. Modulation of proinflammatory responses during microbial infection by lipoxin in vivo has been described [49, 50]. Lipoxin inhibits IL-12 production in vivo by murine splenic dendritic cells stimulated with extract of *Toxoplasma gondii*; furthermore, mice deficient of 5-LO developed more severe infection with the parasite [49, 50]. Additional powerful immunomodulatory effects of lipoxin include regulation of T-cell responses to TNF-α, as recently described [51]. Activated human peripheral blood T cells treated with ATL demonstrate inhibition of TNF-α secretion, an effect that is mediated through an LXA_4 receptor [51]. Further host protective effects of lipoxin include the recent report that epithelial cells treated with ATL stimulate production of mRNA for a host protective protein bactericidal/permeability-increasing protein (BPI), a protein that inhibits endotoxin signaling [52], thereby circumventing potential immunosuppressive effects of anti-inflammatory treatment.

Proresolution. Movement of monocytes to sites of inflammation and injury and subsequent clearance of apoptotic leucocytes by monocyte-derived macrophages are key steps in wound healing and resolution of inflammation [1]. Defective clearance of apoptotic cells by macrophages has been implicated in several chronic inflammatory diseases, including glomerulonephritis and systemic lupus erythematosis (SLE) [53]. The initial response to inflammation is characterized by leukocyte infiltration to an inflammatory focus under the influence of chemoattractants, generated by proinflammatory lipid

mediators, including leukotrienes and prostaglandins [2]. Subsequently, in an attempt to limit the inflammatory response, a second-phase response is observed, characterized by a switch in the biosynthesis of lipid mediators in favor of agents with proresolutory activities, including lipoxins [2]. Thus, in addition to attenuating effects on proinflammatory mediators and PMN recruitment, both lipoxin and ATL are involved in the dynamic regulation of the resolution-phase of PMN-mediated inflammation [53–57]. Lipoxin and ATL cause potent activation and increased adhesion of monocytes, without degranulation suggesting that these actions of lipoxin are host protective [15, 18]. Both lipoxins and ATL enhance phagocytosis of apoptotic PMN by monocyte-derived macrophages [53, 54], rat bone marrow–derived macrophages in vitro without proinflammatory cytokine release and in a murine model of peritonitis in vivo [54], highlighting the ability of lipoxin to promote clearance of apoptotic leukocytes by macrophages at an inflammatory site [53]. This effect seems to be coupled to lipoxin-mediated alterations of the macrophage actin cytoskeleton [55].

Antiproliferative. Growth factor and chemokine-triggered enhancement of cellular proliferation is a characteristic of many injury and regeneration responses, which typically subsides with resolution of inflammation. Excessive proliferation is a hallmark of inflammatory diseases (e.g., glomerulonephritis characterized by mesangial cell proliferation and psoriasis with keratinocyte hyperproliferation). Lipoxin modulates the proliferative responses of mesangial cells to prototypic mitogens, including platelet-derived growth factor (PDGF) [56]. In mesangial cells in vitro, LXA_4 inhibits LTD_4-induced mesangial cell proliferation by modulating LTD_4-induced transactivation of the PDGF receptor [56]. ATL is a potent inhibitor of vascular endothelial growth factor (VEGF) and LTD_4-stimulated angiogenesis in vitro, and also inhibits angiogenesis in an in vivo granuloma model [57]. Attenuated epidermal proliferation by topical ATL in an in vivo model of cutaneous inflammation has recently been described [58]. This antiproliferative effect contributes to the aforementioned anti-inflammatory mechanisms of lipoxin that can interfere with the activation and migration of inflammatory cells.

Lipoxin in disease. A physiologic role for lipoxin in vivo is supported by the demonstration of lipoxin production in diseases associated with cell-cell interaction, including asthma, sarcoidosis, pneumonia, rheumatoid arthritis, juvenile periodontal disease, and postcoronary angioplasty [3]. It may be suggested that susceptibility to chronic inflammation may reflect dysregulated generation of lipoxin [66] or end-organ responsiveness to these agents. With this in mind, attempts have been made to harness the lipoxin network in generating an anti-inflammatory phenotype in numerous diseases characterized by acute inflammation.

Efficacy of lipoxin analogues in acute renal failure and glomerulonephritis

Over the past few decades, the mortality associated with acute renal failure (ARF) due to acute tubular necrosis (ATN) has changed little, despite introduction and refinement of dialysis techniques and improvement in the supportive care of patients with ARF [59–61]. This lack of progress may be accounted for by increased age and comorbidity among patients with ARF [58–60]. The static mortality rates may also reflect the complete absence of therapies that influence the course of ATN and the possible contribution of renal injury to dysfunction in other organs (so-called "second-organ injury").

Lipoxins are potential therapeutics in ischemic ARF, as they can influence a variety of pathobiologic functions that are relevant to ATN, including vascular tone, epithelial cell injury and cytokine release, and leukocyte recruitment and clearance (Table 1, Fig. 2). We have recently demonstrated that the stable lipoxin analogue, 15-epi-16-(FPhO)-LXA_4-Me, is protective in experimental murine ARF in vivo [42, 43]. Administration of the ATL, prior to ischemia, resulted in significant functional and morphologic protection and attenuated chemokine and cytokine responses [42]. Using a transcriptomic approach to explore the events that underlie lipoxin renoprotection, we found that treatment with the ATL, prior to injury, modified the expression of many differentially expressed pathogenic mediators, including cytokines, growth factors, adhesion molecules, and proteases [43]. Lipoxin-modulated transcriptomic response included many genes expressed by renal parenchymal cells and was not merely reflective of a reduced renal mRNA load by blunted leukocyte recruitment. In aggregate, these results suggested that the lipoxin analogue modulated events at the core of ATN.

Acute glomeruluonephritis represents another important cause of ARF [61]. Current treatments for this disease are limited by life-threatening side effects. Lipoxins and ATL offer therapeutic potential, by switching the cellular response from inflammation in favor of resolution, with dissipation of local gradients of proinflammatory mediators, inhibition of further PMN recruitment, enhanced clearance of recruited inflammatory cells, inhibition of mesangial cell proliferation, and potential regulation of matrix accumulation in this context. In the concanavalin A-ferritin model of immune complex glomerulonephritis, treatment of rat neutrophils ex vivo with LXA_4 reduces their subsequent trafficking into inflamed glomeruli [4]. Decreased LXA_4 biosynthesis is associated with exaggerated neutrophil infiltration in nephrotoxic serum nephritis in P-selectin knockout mice [62]. Administration of wild-type platelets, that express P-selectin, restore lipoxin generation and equilibrate neutrophil infiltration between knockout and wild-type

Table 1. Key biologic actions of lipoxins

Cell type/tissue	Bioactivity of lipoxin	Reference
Polymorphonuclear neutrophil	Inhibits	
	Chemotaxis, adhesion, and transmigration	34, 37
	L selectin shedding	36
	Interleukin (IL)-1β, monocyte chemoattractant protein (MCP-1) and superoxide production and stimulates IL-4 production	48
	Peroxynitrite, attenuation of nuclear factor-kappa B (NF-κB) and activated protein-1 (AP-1), reduced IL-8 expression	82
	Down-regulation of CD11/CD18 expression	35, 36
Monocytes/ macrophages	Stimulates	
	Chemotaxis and adhesion	15, 18
	Phagocytosis of apoptotic PMN in vivo and in vitro	53, 54
	Actin reorganization	55
	Attenuates	
	Bone marrow–derived macrophages matrix metalloproteinase (MMP-2) activity	54
	L-selectin shedding	36
	CD11/CD18 expression	36
Eosinophils	Inhibits	
	Plasminogen-activated factor (PAF)–stimulated chemotaxis, IL-5 and eotaxin secretion	38
Endothelial cells	Inhibits	
	P-selectin mobilization	39, 40
	Adhesiveness for PMN	39
	Attenuates	
	CD11/CD18 expression	36
	$CysLT_1$-mediated vascular leakage + PMN trafficking	39
Fibroblasts	Inhibits	
	IL-6 and IL-8 release, reduction of MMP-3 and increased tissue inhibitor matrix metalloproteinase (TIMP)-1 and -2 expression	44
Enterocytes	Inhibits	
	IL-8, MCP-1, and RANTES release from pathogenic-stimulated cells	45, 47
	Apoptosis	46
	Stimulates	
	Expression of bactericidal permeability-increasing protein (BPI)	52
Mesangial cells	Inhibits	
	Proliferation, contractility and adherence for neutrophils	26, 40
Bronchial epithelium	Blocks bronchoconstriction	67–69
	Stimulates cytosolic calcium increase with resultant chloride release	70
Dendritic cells	Inhibit IL-12 production in response to stimulation by *Toxoplasma gondii*	49, 50

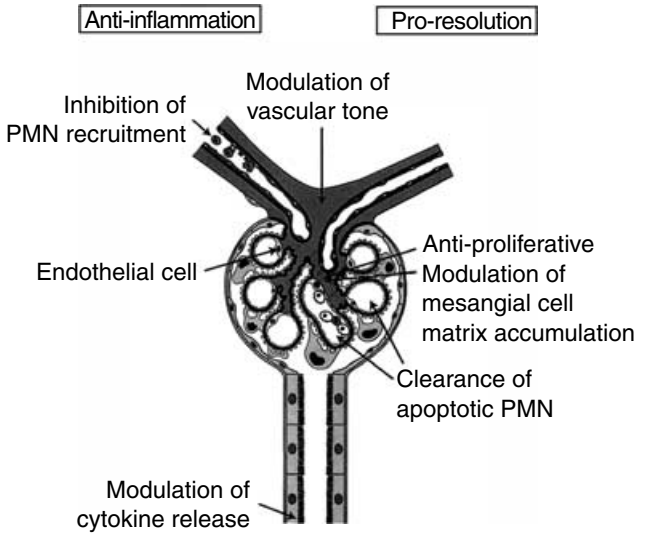

Fig. 2. Potential cellular targets of lipoxin in the kidney. Lipoxins demonstrate anti-inflammatory and proresolution actions within the kidney, modulating responses in numerous cell types, including endothelial cells, mesangial cells, polymorphonuclear neutrophils (PMNs), monocytes, and macrophages as described in the text.

mice [62]. Overexpression of 15-LO in rat kidney, protects in a model of antiglomerular basement membrane nephritis possibly related to increased lipoxin production [63]. Together, these results support the concept that it may be possible to harness the lipoxin network therapeutically in ARF and inflammatory glomerular disease.

Effects in other diseases

Asthma. A possible role for the involvement of lipoxin in asthma has been highlighted by the detection of LXA_4 in the bronchoalveolar lavage fluids of patients with pulmonary diseases, including asthma [64]. Levels of LXA_4 are significantly higher in the sputum of mild asthmatic patients compared to the levels measured in normal subjects or severe asthmatics [65]. In addition PMNs from mild asthmatics in vitro generate larger amounts of LXA_4 compared to normal individuals [65]. Aspirin-intolerant asthmatics display lower biosynthetic capacity for these potentially protective lipid mediators relative to aspirin-tolerant asthmatics or healthy subjects [66].

LXA_4 stable analogue attenuates both airway hyperreactivity and inflammation in a murine model of asthma [67], inhibiting generation of proinflammatory mediators IL-5 and IL-13. Native LXA_4 given to human asthmatics inhibits LTC_4-stimulated airway hyperresponsiveness [68] and blocks LTD_4-initiated constriction of airway smooth muscle in vitro [69]. Together these findings suggest that lipoxin plays a key physiologic role in asthma, regulating airway hyperreactivity via effects on key proinflammatory pathways and antagonizing cysteine-leukotriene–mediated actions on bronchial smooth muscle. Recent reports of LXA_4 stimulating rapid cytosolic calcium increase in human bronchial epithelium with resultant chloride release suggests that in addition to its anti-inflammatory role, LXA_4 maybe

Table 2. Actions of lipoxin and aspirin-triggered lipoxins in vivo in renal disease

Renal disease	Model	Lipoxin bioaction	Reference
Acute renal failure	Murine ischemia-reperfusion injury	Functional and histologic protection	42
		Modified transcriptomic response to injury	43
Glomerulonephritis	Concanavalin A-ferritin	Treatment of rat neutrophils ex vivo with lipoxin A_4 (LXA_4) blunts their subsequent trafficking into inflamed glomeruli	4
	Nephrotoxic serum nephritis in P-selectin knockout	Decreased LXA_4 biosynthesis is associated with exaggerated neutrophil infiltration, measures to restore lipoxin generation and reduce neutrophil infiltration	62
	Antigomerular basement membrane nephritis	Overexpression of 15-lipoxygenase (LO) in rat kidney protects from injury	63
Vascular tone	Mesangial cell contraction	LXA_4 antagonizes leukotriene D_4 (LTD_4)-induced falls in glomerular filtration rate but not renal plasma flow in rat kidney (native lipoxin)	27

involved in ionic transport regulation in the lung [70], thus offering potential for use in respiratory diseases associated with ion transport dysfunction such as cystic fibrosis.

Skin. The potential therapeutic efficacy of these agents has been evaluated in dermal inflammation. In vivo both native LXA_4 and aspirin triggered analogues inhibit PMN infiltration and vascular permeability in a model of dermal inflammation [71]. ATL modulates, in a dose-dependent manner, PMN infiltration, edema, and epidermal proliferation in several in vivo inflammatory dermatoses [72]. When compared to standard topical anti-inflammatory treatment (methylprednisolone) and LTB_4 receptor antagonists (LTB_4 R-Ant) in these cutaneous inflammatory models, topical ATL showed equivalent efficacy on most end points measured [72]. This further extends the therapeutic potential of these agents, without the detrimental local and systemic side effects associated with currently used corticosteroids.

Lipoxin and its stable analogues are currently being evaluated in other inflammatory diseases associated with PMN-mediated tissue injury such as periodontal disease [73] and intestinal inflammation (see Table 3). Several chronic inflammatory diseases, including SLE, are associated with accumulation of apoptotic leukocytes at an anti-inflammatory focus. Recent data suggest potential therapeutic gain for lipoxins in such diseases. In addition, a protective role for LXA_4 in protecting gastric mucosa from aspirin-induced damage has been recently highlighted [74]. LXA_4 in a dose-dependent manner attenuated aspirin-induced leukocyte adherence in in vivo studies, suggesting further anti-inflammatory effects [74].

Mechanism of action

Lipoxin receptors. Numerous cell types including PMN, monocytes, epithelia, endothelia, and fibroblasts express high affinity (K_d in subnanomolar range) G protein coupled receptors (GPCR) for LXA_4 (ALXR) [3]. Cloning of human, murine and rat receptors indicates that this receptor has homology with members of the chemokine receptor superfamily [56, 75]. A provisional nomenclature for the LX receptor has only recently been proposed [76]. The ALXR was originally identified as a low affinity N-formyl-methionyl-leucyl-phenyalanine (FMLP) receptor and there is accumulating evidence that the ALXR binds pleiotropic ligands: lipids, peptides, and proteins, including serum amyloid A, amyloid B, and prion proteins [22–25], the consequences of ALXR activation being dependent on the activating ligand.

Receptor expression may be induced by interferon γ (INF-γ) and IL-13, mediators thought to down-regulate inflammatory responses [45]. In several cell types, including mesangial and endothelial cells it has been shown that LXA_4 interacts with a subclass of peptido-leukotriene receptors (cysLT1) acting as a partial agonist, attenuating the pro inflammatory effects of LTD_4 in these cells [56]. We have shown that LXA_4, acting through distinct GPCR, inhibits LTD_4-induced mesangial cell proliferation by modulating LTD_4-induced transactivation of the PDGF receptor and subsequent phosphatidylinositol 3 (PI3)-kinase activation and mitogenic responses [56]. Studies in polarized intestinal epithelial cells have shown the LXA_4 receptor to be preferentially expressed on the basolateral cell surface, facilitating locally generated lipoxin to act rapidly on epithelial lipoxin receptor to down-regulate intestinal inflammation [77]. In addition to acting via GPCRs, there is evidence that LXA_4 can activate the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor, albeit at high (micromolar) concentrations [78].

ALXR signaling. Current understanding of the intracellular signaling pathways triggered on lipoxin receptor engagement remains incomplete. Controversy exists over whether LXA_4 binding to ALXR triggers distinct anti-inflammatory signals or whether receptor binding results in down regulation of proinflammatory signals [56].

Engagement of ALXR in both monocytes and PMNs results in a distinct profile of cell signalling events that may include guanosine triphosphate (GTP) hydrolysis, pertussis toxin-sensitive mobilization of intracellular calcium, activation of phospholipases A_2, C, and D, and arachidonic acid release [3].

Table 3. Lipoxins in other disease models

Disease	Lipoxin and aspirin-triggered lipoxin (ATL) bioactions	Reference
Asthma	Lipoxin A_4 (LXA_4) detected in the bronchoalveolar lavage fluids of patients with asthma	64
	Higher levels of LXA_4 in sputum of mild asthmatic patients compared to normal subjects or severe asthmatics	65
	Polymorphonuclear neutrophils (PMNs) from mild asthmatics in vitro generate larger amounts of LXA_4 compared to normal individuals	65
	Aspirin-intolerant asthmatics display lower biosynthetic capacity for LXA_4 than aspirin-tolerant asthmatics or healthy subjects	66
	LXA_4 stable analogue attenuates both airway hyperreactivity and inflammation in vivo, inhibiting generation of pro-inflammatory mediators interleukin (IL)-5 and IL-13	67
	Native LXA_4 given to human asthmatics inhibits leukotriene C_4 (LTC_4)-stimulated airway hyperresponsiveness and blocks LTD_4-initiated constriction of airway smooth muscle in vitro	68
Dermal inflammation	Native LXA_4 and ATL inhibit PMN infiltration and vascular permeability in vivo	71
	ATL modulates PMN infiltration, edema, and epidermal proliferation in several in vivo inflammatory dermatoses	72
Peridontal disease	Both topical LXA_4 analogues and ATL reduce *Porphyromonas gingivalis*–elicited PMN infiltration	73
Intestinal inflammation	ATL improves clinical features of DSS-induced inflammatory colitis	82
	Attenuated aspirin-induced gastritis	74
	Induces bactericidal permeability-increasing protein (BPI) expression, localized to cell surface	52

DSS, dextran sodium sulphate.

Lipoxin may activate specific anti-inflammatory signals as highlighted by identification of the polyisoprenyl phosphate-signaling pathway [79]. Presqualene diphosphate (PDSP), a component of this pathway, is a potent regulator of intracellular signals in PMN. Activation of the ALXR inhibits PDSP remodeling, resulting in accumulation of PDSP leading to inhibition of phospholipase D and superoxide anion generation and PMN activation [80].

In gastrointestinal epithelial cells and human leukocytes, lipoxin has been shown to inhibit the transcription factor NF-κB, which is a central regulator of inflammatory molecules and also is key for proliferation and antiapoptosis [82, 83]. In an in vitro model of acute inflammation, *Salmonella typhimurium*–induced colitis, LXA_4 analogue mediated down-regulation of proinflammatory gene expression via inhibition of the NF-κB pathway [81]. Inhibition of cytotoxic oxidant, peroxynitrite ($ONOO^-$) formation by activated leukocytes, concomitant with reduced activation of NF-κB and AP-1, and subsequent attenuation of proinflammatory IL-8 gene expression by pretreatment with both lipoxin and ATL, has been recently demonstrated [82].

In PMNs, stimulation by lipoxin stable analogue has been shown to up-regulate NAB1, a transcriptional corepressor identified previously as a glucocorticoid response gene [83]. NAB1 can counterregulate or "switch off" proinflammatory programs highlighting protective anti-inflammatory transcriptional signaling by lipoxin. In renal cells in vivo, we have shown that lipoxin modulates anti-inflammatory signals, up-regulating expression of suppressor of cytokine signaling 1 and 2 (SOCS1 and 2) [42]. Investigation of cDNA microarray data from lipoxin-treated and control samples may indicate transcriptional regulatory modules sensitive to modulation by lipoxin within the promoters of distinct cohorts of genes [43].

Additional signaling pathways have been highlighted in monocytes and macrophages, by the observation that LXA_4 and stable lipoxin analogues induce changes in actin cytoskeleton in these cells but not in PMNs [55]. Lipoxin-mediated cytoskeleton reorganization is dependent on monomeric GTPases RhoA- and Rac in THP-1 cells differentiated to a macrophage-like phenotype [55].

Transinactivation. A novel mechanism of action, namely receptor transinactivation by LXA_4, has been demonstrated in human mesangial cells [56]. LXA_4 inhibits PDGF activation and proliferative responses by PDGF and subsequent mitogenic responses as a consequence of receptor transinactivation [56]. This appears to be coupled to modulation of recruitment of SH2 domain containing proteins to the activated PDGF receptor. Given the significance of PDGF in regulating production of transforming growth factor-β (TGF-β) and other fibrotic agents [84], this suggests potential antifibrotic actions of lipoxins.

Receptor ligands. As is typical of the chemokine receptor superfamily, the ALXR binds pleiotropic lipid and peptide ligands, including *N*-formyl hexapeptides, serum amyloid A protein, prion protein, and the glucocorticoid-derived peptide annexin-1 [3, 22–25]. Interestingly, engagement of serum amyloid A protein with ALXR in human neutrophils, generates a proinflammatory phenotype, triggering PMN chemotaxis, IL-8, and TNF-α production as a consequence of NF-κB activation [22]. The production of this proinflammatory response in PMNs by peptide agonists can be blunted by LXA_4 [22]. This novel finding of a GPCR mediating disparate

functions dependent on ligand binding suggests the ALXR plays a key role in governing inflammatory and immune responses.

The human ALXR has recently been demonstrated to bind glucocorticoid-derived annexin-1 peptide [23]. Glucocorticoids, in addition to attenuating NF-κB activation and proinflammatory gene transcription, regulate the synthesis of the anti-inflammatory peptide annexin-1 [23–25]. Glucocorticoid-derived annexin-1 peptide has been demonstrated in vivo to attenuate leukocyte migration and both acute and chronic inflammation [24, 25]. Addition of ATL to this model results in synergistic anti-inflammatory effects [23]. Annexin-1 knockout mice, in models of acute inflammation, demonstrate exaggerated inflammatory responses, effects that are associated with resistance to the anti-inflammatory effects of glucocorticoids [25]. This novel mechanism of action of corticosteroids suggests that endogenous lipid and peptide anti-inflammatory mediators in binding to a common receptor may share similar intracellular signaling pathways. Thus, exploring the precise signals triggered on diverse ligand binding to this GPCR offers enormous potential for therapeutic gain.

CONCLUSION

The growing repertoire of powerful anti-inflammatory and proresolution actions of endogenous and aspirin triggered lipoxins coupled to their efficacy in vivo suggest these agents possess exciting therapeutic potential for use in human disease. As the cellular and molecular basis for these impressive actions continues to be explored and with new evidence that other anti-inflammatory mediators share lipoxin-evoked responses, exciting prospects for future therapies unfold.

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