

## Lipoxins and lung diseases

with

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### What is known about Lipoxins?

**Bruce D. Levy:** Lipoxins are lipoxigenase interaction products that are generated via biosynthetic circuits engaged during cell-cell interactions at sites of inflammation(1). Lipoxins (LXs) are trihydroxy derivatives of arachidonic acid with a conjugated tetraene that are structurally and functionally distinct from other eicosanoids, such as prostaglandins (PGs) and leukotrienes (LTs). 15-lipoxygenase (15-LO) is present in airway epithelial cells, cytokine-primed alveolar macrophages and other leukocytes, and capable of both initiating LX biosynthesis as well as converting leukocyte 5-LO derived LTA<sub>4</sub> to LXs(2). In addition to this 15-LO pathway, 12-LO can also serve as a LX synthase(1). In an interesting biosynthetic switch, aspirin therapy redirects eicosanoid metabolism by acetylating cyclooxygenase-2 (COX-2) so that it no longer produces PGs, but remains catalytically active in cells to generate 15(R)-hydroxyeicosatetraenoic acid (15(R)-HETE), a substrate for leukocyte 5-LO mediated conversion to the 15-epimer-LXs. 15(R)-HETE can also be generated in the lung in the absence of aspirin by cytochrome P450s for conversion to 15-epi-LXs.

Lipoxins are rapidly generated to act locally and then rapidly inactivated via enzymatic pathways shared with other eicosanoids(1). Compared to native LXs, 15-epi-LXs are metabolized less efficiently, leading to an approximate doubling of their biologic half-life that enhances their ability to evoke bioactions.

LXA<sub>4</sub> is a potent stop signal for neutrophil (PMN) functional responses, including chemotaxis, transmigration, azurophilic granule degranulation, and superoxide anion generation(1). In sharp contrast, LXA<sub>4</sub> stimulates monocytes, but in a non-phlogistic manner to adhere, move and clear apoptotic PMNs by phagocytosis. Similar to PMNs, eosinophil activation, dendritic cell mobilization and IL-12 release, T-lymphocyte cytokine release and NK cell cytotoxicity are all inhibited by LXs. Thus, LXs' cell-type specific actions provide regulation of both innate and acquired immunity to promote resolution of inflammation.

Lipoxins interact with one or more specific receptors, including their own specific receptor, a subclass of LTD<sub>4</sub> receptors (i.e., CysLT1), and additional intracellular recognition sites. The LXA<sub>4</sub> receptor (termed ALX) is a G-protein coupled protein that binds LXA<sub>4</sub> with high affinity ( $K_D = 1.7$  nM) (3). ALX was the initial receptor identified to bind both lipid and peptide ligands. Of interest for asthma therapy, corticosteroids induce expression of annexin 1 that can also interact with ALX to initiate anti-inflammatory signals(4). In PMNs, ALX signals in part via polyisoprenyl phosphate remodeling (5) and inhibition of leukocyte-specific protein-1 phosphorylation, a downstream regulator of the p38-MAPK cascade(6).

### Are there any effects on epithelial cells?

**Bruce D. Levy:** Epithelial and non-myeloid cell functional responses are also potently regulated by LXs. Human bronchial epithelial cells (NHBE) express ALX(7). LXA<sub>4</sub> blocks proinflammatory mediator release and gene expression via NF $\kappa$ B in epithelial cells(8). Acid injury increases NHBE ALX expression and LXA<sub>4</sub> promotes restitution by increasing basal NHBE proliferation and inhibiting pro-inflammatory events in differentiated NHBE,

such as cytokine release and PMN transmigration (9). Despite potent regulation of epithelial cell and leukocyte function, LX bioactions are distinct from immunosuppressive compounds in that LX signaling regulates pathogen mediated inflammation (10) and promotes mucosal bacterial killing via expression of bacterial permeability-inducing protein in epithelial cells(11). Thus, in addition to anti-inflammation, LXs are also host protective.

### Is there a therapeutic concept regarding modulation of Lipoxins?

**Bruce D. Levy:** Lipoxins and 15-epi-LXs have been identified in a wide range of lung diseases involving leukocyte activation(12). Severe forms of human respiratory illness, including aspirin-exacerbated respiratory disease, severe steroid-dependent asthma, and cystic fibrosis, are characterized by diminished formation of these counter-regulatory lipid signals(13-15). Decreased production would predispose the host to more severe and protracted inflammatory responses. In one clinical trial, LXA<sub>4</sub> was administered to individuals with asthma, leading to protection from LTC<sub>4</sub>-induced bronchoconstriction(16).

To block rapid inactivation, LX structural analogs have been created with specific modifications to the native structures of LXA<sub>4</sub> and LXB<sub>4</sub>(17). Bioactive LXA<sub>4</sub> analogs have been prepared with a phenoxy group bonded to carbon-16 to replace the  $\omega$ -end of the molecule to protect from dehydrogenation *in vivo* and addition of halide to the *para*-position of the phenoxy ring to further hinder degradation. A 15-epi, 16-*para*-fluorophenoxy-lipoxin A<sub>4</sub> analog displays potent biological activity and signals via ALX receptors. Thus, these modifications to native LXA<sub>4</sub> serve to prolong *in vivo* half-life in the circulation and enhance biological properties.

In a murine experimental model of asthma, a LX stable analog markedly inhibits allergen-driven airway hyper-responsiveness and inflammation(18). Targeted expres-

sion of human ALX to murine leukocytes in transgenic mice also dramatically inhibits allergic airway inflammation. Aspiration of gastric acid is a common cause of acute lung injury and frequently worsens asthma control. LX and 15-epi-LX generation and ALX signaling promote spontaneous resolution of hydrochloric acid-initiated acute airway injury(7). With an ED<sub>50</sub> of less than 0.05 mg/kg in these murine models, bioactive LX analogs compare favorably to both CysLT1 receptor antagonists and synthetic glucocorticoids. Thus, ALX activation by endogenous ligands or a LX stable analog evokes potent stop signals for airway responses. The recent development of new LX stable analogs that are topically and orally active should enable further investigation on LX regulation of human illness.

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