Selectively Targeting LTA4H Aminopeptidase Activity for the Development of Novel Anti-inflammatory Drugs

Schroeder M. Noble[1], Kyung Hyeon Lee[1], Elaine Cagnina[2], Hoyoung Lee[2], Marie Burdick[2], Y. Michael Shim[2] and Mikell Paige[3]

[1]Department of Wound Infections, Bacterial Diseases Branch, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, Maryland, USA, 20910.

[2]University of Virginia, Department of Medicine, Division of Pulmonary and Critical Care Medicine. Charlottesville, VA

[3]Department of Chemistry and Biochemistry, George Mason University, Manassas, VA

Leukotriene A_4 hydrolase (LTA4H) plays a critical role in inflammation, the immune response and host defense against infection. This bi-functional enzyme possesses epoxy hydrolase (EH) activity in inflammatory pathways and aminopeptidase (AP) activity in anti-inflammatory pathways. LTA_4H EH activity catalyzes hydrolysis of leukotriene A_4 (LTA_4) to LTB_4, a pro-inflammatory lipid mediator that contributes to pulmonary inflammation, irritable bowel syndrome (IBS), COPD, and adult respiratory distress syndrome (ARDS). The LTA_4H AP activity catalyzes the hydrolysis of the peptide proline-glycine-proline (PGP), a chemotactic peptide resulting from breakdown of collagen. One of the hallmarks associated with inflammatory lung diseases is high concentrations of PGP, which maintains neutrophilic inflammation. Given its role in inflammation, several groups have developed compounds for the non-selective inhibition of LTA_4H's hydrolytic activity, which resulted in the simultaneous inhibition of both pro-inflammatory and anti-inflammatory pathways. Although the EH and AP functions of LTA_4H share the same catalytic site, recently, we and others have sought to selectively target each function. We propose a novel therapeutic strategy of selectively augmenting LTA_4H AP activity with *de novo* preservation of the EH activity as treatment for inflammatory diseases. We have recently designed and tested a new molecule in two murine *in vivo* models to demonstrate potentiation of LTA_4H AP activity as an effective therapeutic approach. This anti-inflammatory compound was evaluated for enhancement of LTA_4H AP activity in kinetic assays, and the crystal structure of LTA_4H bound to the compound was determined. The anti-inflammatory compound increased catalytic efficiency and substrate binding 10-fold. For structure determination, LTA_4H crystal drops were overlaid with the anti-inflammatory compound to achieve co-crystals that diffracted to 2.9Å. The structure was determined using molecular replacement and revealed that the anti-inflammatory compound was stabilized by van der Waals interactions and hydrophobic interactions within the aromatic

binding pocket of LTA_4H. Neutrophilic pulmonary inflammation and acute lung injury (ALI) were induced by intra-nasal lipopolysaccharide in the presence or absence of intra-nasal anti-inflammatory treatment which selectively enhanced the LTA_4H AP activity. This treatment protected murine lungs from ALI by significantly reducing lung edema and infiltration of neutrophils into the lungs. In conclusion, we have demonstrated enhancement of LTA_4H AP activity by an anti-inflammatory compound *in vivo* and *in vitro*, and determined the crystal structure of LTA_4H bound to this compound. This structure will aid in the design of more potent small molecule compounds effective in potentiating LTA4H AP activity while preserving LTA4H EH activity.