Lipoxin Metabolism in Inflammatory Bowel Disease

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**Medical and Scientific Background:** The etiology of IBD is not known but chronic mucosal inflammation of unknown origin is hallmark. Most normal inflammatory responses start and stop and proceed in three distinct phases:

1. Initiation
2. Escalation and Maintenance
3. Resolution.

Normal local chemical mediators are responsible for orchestrating these phases, which can promote cell healing after inflammation during the resolution phase. However, IBD seems to run continuously after initiation without resolution. While some of these mediators initiate and maintain the inflammatory response (leukotrienes, PAF, Th1 cytokines), others reverse and resolve the inflammatory response (*lipoxins*). Without resolution, an initiated inflammatory response, no matter what the cause, would go indefinitely. Since this in fact characterizes IBD, the hypothesis that IBD may be caused, in part, by a failure of the bowel to resolve an existing inflammation by releasing anti-inflammatory signals should be considered. Therefore, the overall objective of this study was to test this hypothesis by elucidating lipoxin metabolic pathways in the colon of patients with IBD (ulcerative Colitis) and to test if these aberrations are causal for the disease.
Lipoxin Route (Black Arrows): Lipoxins (LXA4 and LXB4) are anti-inflammatory mediators produced by TWO independent enzymatic pathways (15-lipoxygenase and 5-lipoxygenase). Lipoxin synthesis usually involves the interaction of both neutrophils with either epithelial or endothelial cells.

ATL Route (Red Arrows): Another route of lipoxin synthesis arises from aspirin acetylated COX-2, which produces a non-naturally occurring lipoxin isomer called ATL (Aspirin Triggered Lipoxin) instead of prostaglandins. ATL is also an active anti-inflammatory mediator.
**Hypothesis:** The hallmark of IBD is persistent inflammation and unresolved inflammatory cycles. The particular hypothesis of this proposal is that IBD is caused, in part, by a failure to resolve an initiated inflammatory response by failure to synthesize or transduce biologically active chemical “stop signals” (*Lipoxins*) that are a necessary component of the normal course of inflammation and tissue repair.

We will test this hypothesis by conducting experiments to:

1. Determine colonic lipoxin metabolism in patients with IBD (UC), relative to colon from patients without IBD.
2. Determine lipoxin receptors on colonic epithelial cells and neutrophils from patients with IBD compared to patients without IBD
3. Determine causation of lipoxin deficiency to the IBD phenotype by altering lipoxin synthesis in a mouse model of ulcerative colitis.

The clinical significance is the possibility to alter the course of IBD by altering lipoxin activity in the colon with stable lipoxin analogs or by transfection of the missing components of the complete lipoxin metabolic pathway.
METHODS

**Human Colon**

Group 1: Experimental
- a. IBD patients (Meds)
- b. IBD patients off steroids

Group 2: Controls
- a. Organ Donors
- b. Slow transit-time Constipation

**Colon Resection**

**Tissue Culture**

**Lipoxin ELISA**

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**Murine Model**

Group 1: 15-LOX Knock out
Group 2: C57BL/6 Wild Types

**Drinking Water Control**

**Water + 5% Dextran Sodium Sulfate (DSS)**

1. Intensity of IBD Phenotype
2. Colonic Lipoxin Synthesis
Lipoxin synthesis by colonic mucosa from patients with IBD (colitis) and patient controls (Organ Donors) measured in tissue culture media after incubation. Some IBD patients were steroid free. Some tissue cultures contained aspirin (ASA, 1 mM) or calcium ionophore (A23187, 10 μM).

* P< 0.05, relative to corresponding IBD groups
ATL (15-epi-LXA4) synthesis by colonic mucosa from IBD patients compared to the synthesis of native lipoxin. Some tissue cultures were treated in-vitro with aspirin (ASA, 1 mM).
15-Lipoxygenase protein levels from colonic mucosa obtained from patients with IBD and from organ donors (Controls). Two isotypes exist in humans: 15-LOX-1 is expressed in leukocytes and 15-LOX-2 is expressed in airway epithelium. The 15-Lipoxygenase is necessary for native lipoxin synthesis.
Figure 4. Mouse body weight with DSS colitis

Weight loss due to colon malfunction during DSS-induced colitis in wild type and ALOX-15 mice (-/-) deficient for murine leukocyte 12/15-Lipoxygenase
Mouse colon inflammation as indexed by the colon weight-to-length ratio.

Figure 5. Mouse Colon Inflammation

Mouse colon inflammation as indexed by the colon weight-to-length ratio.
Colon capillary permeability changes induced by DSS colitis in wild type and ALOX-15 mice.
Colonic lipoxin synthesis in wild type and ALOX-15 knock out mice with DSS colitis. ALOX-15 knock out failed to prevent mucosal lipoxin synthesis suggesting the presence of another 15-lipoxygenase isozyme in the colon of these mice.
Significance to Human IBD

These data suggest a marked deficiency in lipoxin synthetic capability in IBD patients. However, failure of the 15-lipoxygenase knock out animal model prevents a causal nexus between lipoxin deficiency and IBD phenotype from being proposed at this time. If lipoxin deficiency in humans with IBD is causal for the chronic bowel inflammation associated with the disease, then restoring lipoxin activity may be therapeutic. This may be done through activating the ATL pathway, which is active in IBD, or through natural lipoxin replacement therapy.