6 NOVEL BRD4 INHIBITORS BLOCK THE PATHOLOGICAL ACTIVATION OF BRD4-NFkB SIGNALING AND SUPPRESS COLONIC INFLAMMATION IN IBD MICE MODELS

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Uncertain Colitis (UC) and Crohn’s Disease (CD) are two major types of inflammatory bowel disease (IBD), with recurrent symptoms and significant morbidity. Long-term persistence of chronic inflammation in IBD is among the major factors contributing to neoplasic transformation and development of colitis-associated colorectal cancer. There is a lack of efficient medications for IBD, due to either limited efficacy or side effects. Antibodies against TNFs are effective therapies; however, they are expensive, and up to 40% of patients are non-responders. Thus, improved therapy with higher efficacy, enhanced safety and better patient affordability is urgently needed.

The master regulator of innate immune response NFκB plays a critical role in the initiation and chronicity of IBD mucosal inflammation. We demonstrated that bromodomain-containing protein 4 (BRD4), an epigenetic reader, is required for stabilization of NFκB binding on the promoters of inflammatory genes, activation of RNA polymerase II, and histone H3 lys12 acetylation (H3K122ac) to permit high levels of inflammatory gene expressions by sentinel epithelial cells and stromal fibroblasts. Our data using human IBD patient samples suggest that BRD4 activation is critical to the initiation of colonic inflammation.

We have successfully identified highly potent and specific BRD4 inhibitors, demonstrating that inhibition of BRD4 suppresses expression of inflammatory cytokines TNFα, IL-6, IL-17A, and IL-8. Administration of our lead inhibitors (ZL0454 & ZL0420) significantly reduces initiation of the mucosal inflammation in both dextran sulfate sodium (DSS)-induced and Cbor1 T cell transfer colitis models in vivo, but with low toxicity and much safer than the generic NFκB/IKK inhibitor BMS345541. The levels of NFκB and BRD4 activation were also compared using immunofluorescence staining of NFκB/RelA translocation, phospho-Ser276 RelA formation, and induction of the BRD4 marker H3K122ac. The BRD4 inhibitors reduced DSS-induced NFκB-BRD4 activation in colon tissue, demonstrating the target specificity in vivo.

Our data suggest that BRD4 activation is critical to the initiation of the colonic inflammation during IBD. BRD4 inhibition disrupts its interactions with acetylated histone lysine residues, thereby blocking the pathological activation of the BRD4-NFκB signaling and suppressing colonic inflammation. Therefore, inhibition of the BRD4 activation is a novel attractive target for the development of novel IBD therapy and in prevention of CAC. Using our novel BRD4 inhibitors, we further seek to evaluate the benefit of long-term BRD4 inhibition on UC-induced neoplastic transformation and development of CAC.

Funding support: Crohn’s & Colitis Foundation Entrepreneurial Investing (EI) initiative and a research fellowship award from the Crohn’s & Colitis Foundation of America.

P154 THE ANTI-INFLAMMATORY EFFECT OF A PLANT-BASED DIET IN DSS-INDUCED COLITIS

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Background: Inflammatory Bowel Disease (IBD) results from an imbalanced immune response toward the gut microbiota. Disruptions in the gut microbiota are proposed as critical elements that could foster pro-inflammatory imbalances and cause active flares IBD. Diet has been shown to have a pro-inflammatory effect in Crohn’s disease (CD) but not much is known on the anti-inflammatory effect of a plant-based diet. Gut microbial modulation via diet is a needed strategy for the therapeutic management of CD. However, no specific recommendations exist for dietary modification.

We hypothesized that plant-based diet may have a protective effect on the severity of acute dextran sodium sulfate (DSS)-induced colitis.

Methods: 20-wk old age-sex matched C57BL/6 mice were fed a plant-based diet or standard chow for 7 days. Mice were then administered 3% DSS for 7 days and sacrificed 2 days later. Mice were individually caged and maintained on Aspen bedding. A fecal homogenization protocol was performed prior to the start of the experiment. We monitored body weight, fecal myeloperoxidase activity, water and food intake in all mice. Epithelial permeability (FITC-dextran) and endoscopic assessment were performed before and after DSS treatment. Post mortem blood, mesenteric lymph node, colon and liver were collected. Colon morphology was evaluated using 3D-stereomicroscopy. Histological assessment was performed in a blinded manner. Analysis of samples by Illumina 16S protocols, real-time quantitative PCR (qPCR), flow cytometry.

Results: Mice fed the plant-based diet had decreased DSS-induced weight loss, myeloperoxidase activity and mortality compared to Chow-fed mice (P<0.05). Plant-fed mice also had significantly lower colonoscopy and histological scores, with colonoscopic images showing decreased transparency and increased presence of erosion and strictures in chow-fed mice. Compared to the chow-fed mice, the concentration of FITC-D in the plasma of plant-fed mice was significantly lower (Mann-Whitney P=0.004, power 0.98). Bacterial quantification qPCR CT-values (ΔΔCt) showed increased abundance for Lactobacilli and decreased abundance for Faecalibacterium prausnitzii. Further studies are needed to evaluate the promise of the pectin-containing prebiotic Psyllium husk in the prevention of DSS-induced colitis. Further studies are needed to evaluate the potential underlying mechanisms. These findings may have great potential for therapeutic dietary interventions in individuals with IBD.

Background: Crohn's disease (CD) is a form of chronic inflammatory bowel disease (IBD). Although CD is an immune-mediated condition of unknown etiology, many studies suggested that abnormal immune responses against certain intestinal bacteria triggered the development of chronic inflammation. The T cell-mediated colitis model is a well-characterized adaptive transfer murine model of chronic small bowel and colonic inflammation which resembles human CD (e.g., diarrhea, a heavily inflamed colon, loss of mucus from goblet cells, Th1/Th17 dominated cytokine profile). OPS-2071, a novel agent synthesized by Otsuka Pharmaceutical Co., Ltd, has broad and strong anti-bacterial activity with low systemic absorption. In this study, we have evaluated the therapeutic effect of OPS-2071 on murine T cell-mediated colitis and in vitro activity on T cell activation, cytokine production and antibacterial activity.

Methods: OPS-2071 was administered via naive T cell (CD4+CD62L+CD44-) naive T cell activation and cytokine production and suppressed T cell proliferation, the in vitro experiments, OPS-2071 or vehicle solution was administered orally for 3 weeks, followed by harvesting of colon tissue and assessment of efficacy evaluation by histological score and inflammatory index (colon weight/length). In vitro activity for TNFα production was assayed by ELISA using human peripheral blood mononuclear cells. The effect on T cell activation and cytokine production (TNF-α, IFN-γ), was examined using human peripheral blood mononuclear cells and, mouse splenocytes which were stimulated by anti-CD3/CD28 antibody-loaded beads. In vitro activity against bacteria was considered to be the cause of IBD was also tested.

Results: In the murine T cell transfer model, OPS-2071 significantly reduced both histological score (control: 9.2, OPS-2071: 1.1, p<0.001) and inflammatory index (control: 54.7±2.2 cm², OPS-2071: 27.8±1.7 cm², p<0.001) at a dose of 10 mg/kg. In the in vitro experiments, OPS-2071 suppressed TNF-α production produced by LPS in human whole blood dose-dependently. OPS-2071 also suppressed human and mouse T cell activation and cytokine production and suppressed T cell proliferation. At high OPS-2071 concentrations, these effects were comparable to prednisolone. The Minimum Inhibitory Concentration against IBD-related bacteria for OPS-2071, ciprofloxacin, and metronidazole were 0.015 - 0.5, 0.25 - 8, and 0.03 - >128 mg/mL, respectively.

Conclusion: OPS-2071 demonstrated significant therapeutic effects on colonic inflammation in the murine T cell-mediated colitis model, suppressed TNF-α production in vitro and showed in vitro activity against bacteria related to CD. The dual effect of anti-inflammatory effects and antibacterial activity of the novel agent OPS-2071 demonstrated in this study, provide rationale for exploring the impact of this compound on human CD in clinical trials.

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