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Fatty acid amide hydrolase (FAAH) blockade ameliorates experimental colitis by altering microRNA expression and suppressing inflammation

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ABSTRACT

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), which is thought to result from immune-mediated inflammatory disorders, leads to high morbidity and health care cost. Fatty acid amide hydrolase (FAAH) is an enzyme crucially involved in the modulation of intestinal physiology through anandamide (AEA) and other endocannabinoids. Here we examined the effects of an FAAH inhibitor (FAAH-II), on dextran sodium sulphate (DSS)-induced experimental colitis in mice. Treatments with FAAH-II improved overall clinical scores by reversing weight loss and colitis-associated pathogenesis. The frequencies of activated $CD4^+$ T cells in spleens, mesenteric lymph nodes (MLNs), Peyer's patches (PPs), and colon lamina propiria (LP) were reduced by FAAH inhibition. Similarly, the frequencies of macrophages, neutrophils, natural killer (NK), and NKT cells in the PPs and LP of mice with colitis declined after FAAH blockade, as did concentrations of systemic and colon inflammatory cytokines. Microarray analysis showed that 26 miRNAs from MLNs and 217 from PPs had a 1.5-fold greater difference in expression after FAAH inhibition. Among them, 8 miRNAs were determined by reverse-transcription polymerase chain reaction (RT-PCR) analysis to have anti-inflammatory properties. Pathway analysis demonstrated that differentially regulated miRNAs target mRNA associated with inflammation. Thus, FAAH-II ameliorates experimental colitis by reducing not only the number of activated T cells but also the frequency of macrophages, neutrophils, and NK/NKT cell, as well as inflammatory miRNAs and cytokine at effector sites in the colon. These studies demonstrate for the first time that FAAH-II inhibitor may suppress colitis through regulation of pro-inflammatory miRNAs expression.
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1. Introduction

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), are associated with aberrant regulation of the mucosal immune system, resulting in recruitment of inflammatory cells to the intestinal tract. IBD affects over a million people in the United States alone; its symptoms include abdominal pain, diarrhea, rectal bleeding, weight loss, and poor ability to digest food. The precise cause of IBD is unknown, but common agreement among scientists has converged on environmental and genetic factors, as well as immune dysregulation and, particularly the role of commensal microbiota in the intestine (Braegger and MacDonald, 1994; Dohi et al., 2000; Podolsky, 2002; Sartor, 2006, 2008). Abnormalities in the gut immune microenvironment, enteric persistent infection, trauma, and inflammation may initiate and cause the progression of IBD (Mayer and Collins, 2002).

Intestinal inflammation is normally associated with infiltration of immune cells, including macrophages, neutrophils, and type 1 T helper (Th1) cells into the colon, which secrete the proinflammatory

Abbreviations: FAAH, Fatty acid amide hydrolase; DSS, Dextran sodium sulphate; MLN, Mesenteric lymph nodes; PPs, Peyer's patches; CB1-2, Cannabinoid receptors 1–2; AEA, Anandamide; TNF-α, Tumor Necrosis Factor-Alpha; IBD, Inflammatory Bowel Disease; UC, Ulcerative Colitis; CD, Crohn's Disease; TNBS, Trinitrobenzene sulfonic acid; miRNAs, MicroRNAs.

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cytokines TNF-α and IFN-γ (Strober et al., 2007, 2002). The available conventional treatments of IBD are useful and advance our understanding of the underlying pathologies, but patients are often resistant to these treatments and experience many side effects, justifying continued search for new, safe, and effective therapeutic approaches.

In recent years, there has been increasing recognition of the role of endogenous cannabinoid receptors 1–2 (CB1 and CB2) in the regulation of inflammation-associated with colitis. Anandamide (AEA) is the endogenous signaling lipid that binds and activates CB1 and CB2, receptors and AEA levels are tightly regulated by the catabolic enzyme FAAH. Further, FAAH is an integral membrane hydrolase with a single n-terminus transmembrane domain. Many distinct classes of FAAH inhibitors have been reported (Salaga et al., 2014). A study focusing on FAAH and intestinal inflammation demonstrated that CB1 receptor-deficient mice are more sensitive to experimental colitis than are control mice and that a CB1 antagonist increased the severity of experimental colitis (Massa et al., 2004). Other studies (Cluny et al., 2010; Engel et al., 2010; Kimball et al., 2006; Massa et al., 2004; Storr et al., 2008, 2009) have suggested that endogenous CB receptor activation may ameliorate colitis. Storr et al. (2008) showed that FAAH mRNA increased 3 days after trinitrobenzene sulfonic acid (TNBS) induction. Further, elevated levels of endogenous AEA resulting from deficiency or inhibition of FAAH are significantly more resistant to TNBS-induced colitis than are controls (Izzo and Sharkey, 2010). AEA can activate both CB1 and CB2 receptors and decrease the level of proinflammatory cytokines (Izzo and Camilleri, 2009).

MicroRNAs (miRNAs) are endogenous small RNA molecules 20–25 nucleotides in length; they regulate multiple genes by binding to target mRNAs, thereby controlling the stability and translation of protein coding mRNAs (Esteller, 2011; Guo et al., 2010). It has been established that cells of the innate and adaptive immune system express more than 100 miRNAs that are involved in mediating many functions, including cell proliferation and apoptosis. In addition, miRNAs are expressed in many developing tissues and are active in regulating inflammation (Ambros, 2004). Recent evidence suggests that miRNAs are differentially expressed in UC and have a key role as negative regulators of inflammation and innate immunity (Wu et al., 2008). miRNAs mir-21 and mir-216 are differentially expressed in active versus inactive UC. Further, it has been shown that induced miR-101b and miR-455 miRNAs mediates anti-inflammatory properties in colitis and associated colon cancer (Altamemi et al., 2014; Feng et al., 2012). However, the differential expression of miRNAs and their role in experimental colitis have not been investigated.

IBD is a major burden to both patients and society and there is an always need for safe and effective therapeutic options. The main hypothesis of this study is to determine the cellular and epigenetic mechanism of FAAH inhibitors mediated abrogation of experimental colitis. In this study, we examined changes in the severity of inflammation, cytokine levels, changes in the expression of miRNAs and immune function after FAAH inhibition using a dextran sodium sulphate (DSS) induced model of experimental colitis. Our studies demonstrated that FAAH-II inhibitor ameliorates colitis by suppressing inflammation through intervention of pro and or anti-inflammatory miRNAs at mucosal sites.

2. Materials and methods

2.1. Animals

Female wild-type mice on C57BL/6 background aged 8 ~ 10 weeks were purchased from Jackson Laboratories (Bar Harbor, ME). The animals were housed and maintained in micro-isolator cages under conventional housing conditions at the University of South Carolina School of Medicine animal facility. Care and use of animals was overseen and approved by the Animal Resource Facility (ARF). The ARF is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care-International (AAALAC). Experimental groups consisted of six mice each; all studies were repeated three times.

2.2. Colitis induction by DSS and FAAH treatment

The FAAH-II inhibitor was purchased from EMD Millipore (Billercia, MA). Experimental colitis was induced using DSS as described previously (Singh et al., 2014, 2010). In brief, eight-week-old C57BL/6 naive group of mice received water alone, while mice in the colitic group received drinking water containing 3% DSS (MP Biomedical, LLC, Ohio) *ad libitum* for seven days. In the following seven days, mice in the colitic group received water without DSS. Initially, we did a dose-response experiment using 5, 10, 20, or 40 mg/kg body weight doses of FAAH-II, determining that 10 mg/kg, as compared to higher or lower doses, effectively suppresses colitis symptoms. Therefore, 10 mg/kg doses of FAAH-II were used throughout this study. We not only used the same lot of FAAH-II in all experiments, but we used same lot and dose of DSS as described earlier in our experimental model of colitis (Singh et al., 2014, 2010).

Mice were given either 100 μl of FAAH-II (10 mg/kg body weight; DSS + FAAH) or phosphate buffered saline (PBS; DSS + vehicle) beginning on the day after DSS induction and continuing every day until Day 14, the experimental end-point. The body weight of mice was monitored every day after DSS induction. During the 14 days of the experiment, we also monitored mice for clinical symptoms of colitis (diarrhea, stool consistency, and blood in fecal matter). At the end of the experiment, animals were sacrificed by ether vapor and their blood collected for measurement of cytokines and chemokines. Colon samples were isolated, washed with PBS, cut longitudinally, one part immediately kept in −80 °C to make tissue homogenate for cytokine measurement and other part fixed in 10% formalin, then embedded in paraffin for histological analysis.

2.3. Cell isolation

At the end of the experiment, single-cell suspensions were prepared from the spleens, MLNs, PPs and colon lamina propiria lymphocytes (LPL) from each group of mice. Cells were dissociated and RBCs lysed with lysis buffer (Sigma St. Louis, MO). After centrifugation, single-cell suspensions from spleens, MLNs, and PPs were passed through a sterile filter 70 μm (Sigma St. Louis, MO) to remove any cell debris. Cells were washed twice in RPMI 1640 (Sigma St. Louis, MO) and stored in media containing 5% fetal bovine serum (FBS) on ice until their use, which occurred within two hours for flow cytometry. The cells from colon LP were isolated as described previously (Singh et al., 2003). In brief, the colon was cut into 1-cm stripes and stirred in PBS containing 1 mM EDTA at 37 °C for 30 min. Next, LP was isolated by digesting intestinal tissue with collagenase type IV (Sigma St. Louis, MO) in RPMI 1640 (collagenase solution) for 45 min at 37 °C with moderate stirring. After each 45 min interval, the released cells were centrifuged, stored in complete media and mucosal pieces were replaced with fresh collagenase solution for at least three times. LP cells were further purified using a discontinuous Percoll (Pharmacia, Uppsala, Sweden) gradient collecting at the 40–75% interface. The cells were maintained in complete medium consisting of RPMI 1640 supplemented with 10 ml/L of nonessential amino acids (Mediatech, Washington, DC), 1 mM sodium pyruvate (Sigma), 10 mM HEPES

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