Toxicology and Applied Pharmacology 258 (2012) 256–267

Contents lists available at SciVerse ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Cannabinoid receptor-2 (CB2) agonist ameliorates colitis in IL-10$^{-/-}$ mice by attenuating the activation of T cells and promoting their apoptosis

Udai P. Singh [a], Narendra P. Singh [a], Balwan Singh [b], Robert L. Price [c], Mitzi Nagarkatti [a], Prakash S. Nagarkatti [a,*]

[a] Pathology, Microbiology and Immunology, School of Medicine, University of South Carolina, Columbia, SC 29208, USA
[b] National Primate Research Center, Emory University, Atlanta GA 30329, USA
[c] Department of Cell and Developmental Biology, University of South Carolina, Columbia, SC 29208, USA

ARTICLE INFO

Article history:
Received 13 July 2011
Revised 30 October 2011
Accepted 9 November 2011
Available online 18 November 2011

Keywords:
Cannabinoid-2 receptors
JWH 133
Colitis
Inflammatory bowel disease

ABSTRACT

Inflammatory bowel disease (IBD) is a chronic intestinal inflammation caused by hyperactivated effector immune cells that produce pro-inflammatory cytokines. Recent studies have shown that the cannabinoid system may play a critical role in mediating protection against intestinal inflammation. However, the effect of cannabinoid receptor induction after chronic colitis progression has not been investigated. Here, we investigate the effect of cannabinoid receptor-2 (CB2) agonist, JWH-133, after chronic colitis in IL-10$^{-/-}$ mice. JWH-133 effectively attenuated the overall clinical score, and reversed colitis-associated pathogenesis and decrease in body weight in IL-10$^{-/-}$ mice. After JWH-133 treatment, the percentage of CD4^{+} T cells, neutrophils, mast cells, natural killer (NK1.1) cells, and activated T cells declined in the intestinal lamina propria (LP) and mesenteric lymph nodes (MLN) of mice with chronic colitis. JWH-133 was also effective in ameliorating dextran sodium sulfate (DSS)-induced colitis. In this model, JWH-133 reduced the number and percentage of macrophages and IFN-γ expressing cells that were induced during colitis progression. Treatment with aminoalkylindole 6-iodo-pravadoline (AM630), a CB2 receptor antagonist, reversed the colitis protection provided by JWH-133 treatment. Also, activated T cells were found to undergo apoptosis following JWH-133 treatment both in-vivo and in-vitro. These findings suggest that JWH-133 mediates its effect through CB2 receptors, and ameliorates chronic colitis by inducing apoptosis in activated T cells, reducing the numbers of activated T cells, and suppressing induction of mast cells, NK cells, and neutrophils at sites of inflammation in the LP. These results support the idea that the CB2 receptor agonists may serve as a therapeutic modality against IBD.

Published by Elsevier Inc.

Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are chronic intestinal inflammatory conditions that are collectively known as inflammatory bowel disease (IBD). IBD affects colon pathologies in over a million people in the United States alone and represents an important and wide spread health problem in modern society (Loftus, 2004; Mayer and Collins, 2002). The symptoms of IBD include abdominal pain, diarrhea and poor ability to digest food and in half of the severe cases require surgery to remove the affected bowl segment. IBD is also associated with infiltration in the intestinal lamina propria (LP) by immune cells including macrophages, neutrophils and lymphocytes that over-express NF-κB- regulated target genes, such as the proinflammatory cytokines TNF-α, IL-1β, and IL-6 (Podolsky, 2002). The occurrence of an enteric infection, trauma or inflammation has been suggested to relate to the initiation of these diseases (Mayer and Collins, 2002). Despite recent therapeutic advances and improved understanding on the underlying pathologies, patients with IBD are often resistant to treatment, justifying the continued search for new therapeutic approaches.

In IBD, it has been shown that neutrophil activation, migration, and degranulation are important effector mechanisms for intestinal damage (Verspaget et al., 1988). It has been reported that a correlation between activated neutrophils and pathological changes in affected colonic mucosa in UC patients exists (Kayo et al., 2006). There are reports showing that depletion of neutrophils decreases the severity of various experimental forms of colitis (Kuhl et al., 2007; Natsui et al., 1997; Palmen et al., 1995). The role of mast cells in colitis development has been less documented. However, there are several reports, which indicate that mast cells are also involved in the development of clinical symptoms of IBD (Rijnierse et al., 2006). It has been shown that mast cells are increased in human CD patients (Knutson et al., 1990).

* Corresponding author at: Dept. of Pathology, Microbiology and Immunology, School of Medicine, University of South Carolina, Columbia, SC 29208, USA. Fax: +1 803 733 1515.
E-mail address: Prakash.Nagarkatti@uscmed.sc.edu (P.S. Nagarkatti).

0041-008X/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.taap.2011.11.005

The major active constituents of the plant *Cannabis Sativa* have been shown to possess an anti-inflammatory activity (Zurier, 2003). Most of the biological action of these cannabinoids are mediated by the cannabinoid receptors-1 (CB1) and -2 (CB2), both coupled to G proteins (Howlett et al., 2002). CB1 is located pre-dominantly on the neurons (Herkenham et al., 1991) and CB2, primarily on the immune cells (Munro et al., 1993). The role of both CB receptors in colitis is getting increased recognition in recent years. For example, CB1 receptor-deficient mice were shown to be more sensitive to colitis induction by dextran sodium sulfate (DSS) than wild-type mice, and a CB1 antagonist worsened the colitis that was induced by 2,4-dinitrobenzene sulfonic acid (DNBS) (Massa et al., 2004). These results suggested that the endogenous cannabinoid system may regulate inflammation-associated colitis and that activation of CB receptors may help in the amelioration of colitis. In the past, studies have also been carried out, using primarily CB1 agonists, which demonstrated their ability to protect from various induced forms of experimental colitis (Engel et al., 2010; Kimball et al., 2006; Massa et al., 2004; Storr et al., 2008). Furthermore, some studies focusing on both CB1 and CB2 receptor agonists also showed protection against experimental colitis (Storr et al., 2009). In contrast, using SAB378, a peripherally restricted CB1/CB2 receptor agonist, a recent study, indicated that this compound did not affect the progression of experimental colitis (Cluny et al., 2010). It is noteworthy that the administration of CB1 receptor agonists as a therapeutic strategy is limited due to adverse psychotropic side effects. Instead, targeting CB2 receptors may constitute a novel approach because such a treatment would be restricted primarily to the immune cells, and would be devoid of psychotropic activity. Moreover, previous studies from our laboratory demonstrated that CB2 agonists can exhibit potent anti-inflammatory activity in-vivo (Lombard et al., 2007).

IBD is a major burden to both patients and society and novel therapeutic options are warranted. The side effects associated with current available treatments could result in adverse reactions or poor responses by the patients, thereby limiting their clinical use (Mouser and Hyams, 1999). Taking this into consideration, the cannabinoid system has emerged as a potential therapeutic target for IBD in recent years. In the current study, we demonstrate that the use of the CB2 selective agonist, JWH-133, reduces the chronic form of colitis by inducing apoptosis in activated T cells and inhibiting the infiltration of inflammatory cells in the colon.

Methods

Animals. Female IL-10$^{-/-}$ mice on C57BL/6 background and C57BL/6 wild-type mice 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 were 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 and the studies were repeated three times.

Spontaneous chronic colitis development in IL-10$^{-/-}$ mice. Under conventional housing conditions, IL-10$^{-/-}$ mice develop spontaneous colitis at 12 weeks of age. The histological feature of colitis presented by IL-10$^{-/-}$ mice shows similarities with IBD patients. Based on published study from our laboratory, we determined that the onset of chronic colitis occurred when serum amyloid A (SAA) and IL-6 levels reached 300 μg/ml and 5.0 ng/ml (Singh et al., 2003), respectively, with at least a 15–17% decrease from the original body weight of mice at an age of 18 weeks. Following the onset of spontaneous chronic colitis, after 18 weeks, mice received either 100 μl of vehicle or 1, 2.5, 5 mg/kg body weight dose of JWH 133, suspended in 70% alcohol and further diluted with distilled water, intraperitoneally (i.p) every second day until the week 25. In addition, 10 week-old mice termed "pre-colitis", were also used as a control for comparison with mice that had developed chronic colitis. The body weight of each mouse was monitored once a week. JWH-133 a purified compound with the linear formula $C_{22}H_{32}O$ was obtained from Sigma Chemical Co. (St. Louis, MO). The certificate of origin indicated that it was originally purified and found to be greater than 99% pure by both gas chromatography and thin-layer chromatography. Pilot studies demonstrated that the 2.5 mg/kg body weight dose was optimal in reducing colitis in IL-10$^{-/-}$ mice and therefore this dose was used throughout the study. At the end of the experiment on week 27, mice were sacrificed. The blood was collected for serum separation and colon samples were washed with phosphate-buffered saline (PBS), cut longitudinally, formalin fixed, and embedded in paraffin. The spleen, MLN and LP lymphocytes were isolated for flow cytometry staining.

Induction of DSS mediated acute colitis. To chemically induce experimental colitis, 8 to 10 week old BL/6 mice received either water or water containing 3% DSS (MP Biomedical, LLC Ohio) (ad libitum) for 7 days. This was followed by water alone for the next 7 days. The body weight of mice was recorded every day from day 0 at the start of both JWH-133 agonist and antagonist (AM630) treatment. AM630 select CB2 receptor antagonist was purchased from Tocris Biosciences, (Ellisville, Missouri). Mice received either 100 μl of both JWH-133 agonist (10 or 20 mg/kg body weight) or antagonist (AM630) at 20 mg/kg body weight or vehicle, every day till day 14 at the experimental end-point. We used a higher dose of agonist because DSS-induced colitis is an acute model unlike the colitis seen in IL-10$^{-/-}$ mice wherein we treated the mice for 7 weeks. At the end of the experiment, spleen, MLN and LP lymphocytes were collected for flow cytometry analysis of macrophages (CD11b^{+}) and IFN-γ^{+} expressing cells. Furthermore, the colon samples were washed with phosphate-buffered saline, cut longitudinally, formalin fixed, and paraffin embedded for histological analysis.

In-vitro proliferation assays. Lymphocytes from mesenteric lymph nodes (MLN ~75% T cells) were cultured either alone, or co-cultured in-vitro with (0, 5 μM or 50 μM/ml dose of JWH-133), stimulated with anti-CD3 (5 μg/ml) and CD28 (1 μg/ml) (BD PharMingen) Abs at 37 °C in 5% CO_2 for three days. Proliferation was measured by 5-Bromo-2′-deoxy uridine (BrdU) absorption and detection (Roche Diagnostics; Dusseldörf, Germany). In brief, after 72 h of culture, 10 μl of BrdU labeling solution (10 μM/ml final concentration per well) was added and incubated for 18 h at 37 °C with 5% CO_2. The cells were then fixed and incubated with 100 μl of nuclease in each well for 30 min at 37 °C. Next, cells were pulsed with BrdU labeling solution and incorporation was detected by ELISA assay (Roche Molecular Biochemical).

In-vitro apoptosis assays. Lymphocytes from MLN (~75% T cells) were cultured either with the vehicle, or with JWH (0, 1, 5,10, 20 and 50 μM) in the presence or absence of 20 or 50 μM concentration of AM630, stimulated with anti-CD3 (5 μg/ml) and CD28 (1 μg/ml) (BD PharMingen) Abs at 37 °C in 5% CO_2. After 3 days of culture, cells were washed and T cell apoptosis was measured as per company protocol by Vybrant apoptosis assay kit #2 (Molecular Probes™ Eugene, OR). The washed T cells were resuspended with 100 μl/ml of annexin binding buffer and added with 5 μl of Alexa Fluor® 488 annexin V and 1 μl of 100 μg/ml of PI working solution. The cells were incubated for 15 min at room temperature. After the incubation period, we added 400 μl annexin-binding buffer, mixed gently and kept the samples on ice and analyzed the stained cells by flow cytometry (FC 500, Beckman Coulter Fort Collins, CO).