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Activation of the Cannabinoid 2 Receptor (CB_2) Protects Against Experimental Colitis

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Abstract

Background—Activation of cannabinoid $(CB)_1$ receptors results in attenuation of experimental colitis. Our aim was to examine the role of CB_2 receptors in experimental colitis using agonists (JWH133, AM1241) and an antagonist (AM630) in trinitrobenzene sulfonic acid (TNBS)-induced colitis in wildtype and CB_2 receptor-deficient ($CB_2^{-/-}$) mice.

Methods—Mice were treated with TNBS to induce colitis and then given intraperitoneal injections of the CB_2 receptor agonists JWH133, AM1241, or the CB_2 receptor antagonist AM630. Additionally, $CB_2^{-/-}$ mice were treated with TNBS and injected with JWH133 or AM1241. Animals were examined 3 days after the induction of colitis. The colons were removed for macroscopic and microscopic evaluation, as well as the determination of myeloperoxidase activity. Quantitative reverse-transcriptase polymerase chain reaction (RT-PCR) for CB_2 receptor was also performed in animals with TNBS and dextran sodium sulfate colitis.

Results—Intracolonic installation of TNBS caused severe colitis. CB_2 mRNA expression was significantly increased during the course of experimental colitis. Three-day treatment with JWH133 or AM1241 significantly reduced colitis; AM630 exacerbated colitis. The effect of JWH133 was abolished when animals were pre-treated with AM630. Neither JWH133 nor AM1241 had effects in CB_2 mice.

Conclusions—We show that activation of the CB_2 receptor protects against experimental colitis in mice. Increased expression of CB_2 receptor mRNA and aggravation of colitis by AM630 suggests a role for this receptor in normally limiting the development of colitis. These results support the idea that the CB_2 receptor may be a possible novel therapeutic target in inflammatory bowel disease.

Keywords

cannabinoid 2 receptor; TNBS; colitis; JWH133; AM1241; AM630; inflammatory bowel disease

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Two types of cannabinoid receptor have been cloned and characterized: the CB_1 receptor in 1990 and the CB_2 receptor in 1993.[1] They are 7 transmembrane, G-protein-coupled receptors with a limited homology (44%).[1,2] These 2 receptors along with the endogenous ligands (endocannabinoids) and their biosynthetic and degradative enzymes constitute the endocannabinoid system (ECS). The ECS is an important regulatory system in the gastrointestinal tract that is involved in the control of motility, sensation, and intestinal inflammation.[3–7] Strong evidence suggests the involvement of the CB_1 receptor in the regulation of colitis.[8,9] This is supported by studies demonstrating a role of endocannabinoids in protection against experimental colitis induced by either dinitrobenzene sulfonic acid (DNBS), dextran sodium sulfate (DSS), or oil of mustard.[9,10] These studies are complemented by observations in samples from patients with inflammatory bowel disease (IBD). Here it was shown that mucosal levels of the endogenous cannabinoid anandamide, but not 2-arach-idonoylglycerol (2-AG), were increased in colonic biopsies from patients with ulcerative colitis.[8]

Another approach to harnessing endocannabinoids for the treatment of intestinal inflammation is to use drugs that reduce the degradation of endogenously released endocannabinoids. This results in the buildup of local endocannabi-noid levels at sites of synthesis. This approach has been successfully used in mice treated with DNBS or trinitrobenzene sulfonic acid (TNBS) and rats treated with TNBS.[8,11] Interestingly, using CB receptor-deficient mice, endogenous cannabinoids were shown to exert their protective effect by CB_1 and CB_2 receptors.[11] This observation led us to examine the role of CB_2 receptors in colitis, because while there is good evidence to support a role for CB_1 receptors in the resolution of colitis, the role of CB_2 receptors is not well understood. Since CB_2 receptor activation is not associated with the central side effects of CB_1 receptor activation, this receptor has the potential for development as a therapeutic target in the treatment of IBD.

Recently, CB_2 receptors have been characterized on enteric neurons, where they are involved in the control of intestinal motility in inflammation.[12] Whether it is the CB_2 receptors on enteric neurons involved in the mechanisms limiting intestinal inflammation is unknown. Taken together, these observations suggest that CB_2 receptors may be activated in conditions of inflammation and may, like CB_1 receptors, regulate the extent of colitis. We tested this hypothesis using well-established models of colonic inflammation in mice.

In the present study we investigated whether CB_2 receptor mRNA expression is altered during acute inflammation induced by either TNBS or DSS. We tested whether 2 structurally different selective CB_2 receptor agonists, JWH133 and AM1241, could protect from or reduce the degree of inflammation in the well-characterized TNBS model of colitis in wildtype mice and whether the effects are reversed by the CB_2 antagonist AM630. To further characterize the involvement of the CB_2 receptor we employed CB_2 receptor gene-deficient $(CB_2^{-/-})$ mice.

Materials and Methods

Wildtype C57BL/6N mice (7–9 weeks, 20–26 g, male) were obtained from Charles River (Saint-Constant, Quebec, CA), housed at constant temperature (22°C) and 12:12-h light-dark cycle in plastic sawdust floor cages with free access to standard laboratory chow and tap water. Two breeding pairs of heterozygous $CB_2^{+/-C57BL/6N}$ mice were obtained from Dr. N. Buckley (California State Polytechnic University, Pomona, CA) and bred in our facility to obtain $CB_2^{-/-C57BL/6N}$ mice.[13] Animals used in these studies were backcrossed to C57BL/6N for 6 generations and were used at the same age (7–9 weeks) and maintained under the same conditions as the wildtype mice. All $CB_2^{-/-}$ mice were genotyped using an established protocol[13] and were confirmed as homozygous gene-deficient animals $(CB_2^{-/-C57BL/6N})$ prior to inclusion in the study. These studies were approved by the University of Calgary Animal Care Committee. Experiments were conducted in accordance with guidelines established by the Canadian Council on Animal Care.

Induction of Colitis

Colitis was induced by intracolonic administration of TNBS using a modification[9] of the method first described in rats.[14] Briefly, animals were lightly anesthetized and TNBS (4 mg in 100 μL of 30% ethanol) was infused into the colon through a catheter (outside diameter 1 mm) inserted 3 cm proximally to the anus in mice. Solvent alone (100 μL of 30% ethanol) was administered in control experiments. In pilot experiments, this dose of TNBS was found to induce reproducible colitis with mortality rates in the published range (0%–25%). An additional model of colitis was employed for real-time polymerase chain reaction (PCR) analysis of the CB_2 receptor. DSS (4%) was given in drinking water for 5 days. Real-time PCR was performed on day 1 and day 3 for TNBS treatment and on day 5 and day 7 for DSS treatment (n = 4–6 for each timepoint).

Pharmacological Treatments

Drugs were injected intraperitoneally. JWH133 (20 mg/kg body weight once or twice daily), AM1241 (10 or 20 mg/kg body weight twice daily), and AM630 (10 mg/kg body weight once daily) were dissolved in a vehicle solution (2% DMSO and 1% Tween 80 in saline) and injected either 30 minutes before the induction of colitis and then once or twice daily for 3 days following the induction of colitis (n = 6-8 each group). Vehicle (4 mL/ kg body weight) alone was injected in TNBS treated control animals (n = 6–8). The doses of JWH133, AM1241, and AM630 were chosen based on previously published studies or empirically determined in preliminary studies.[10]

Evaluation of Colonic Damage

All animals were killed by cervical dislocation 3 days after TNBS treatment unless otherwise specified. The colon was removed, rinsed gently with saline solution, opened longitudinally, and immediately examined. Colonic damage was assessed by a semiquantitative scoring system[15] adapted to mice for the present study. Macroscopic damage was scored according to the following scale, adding individual scores for ulcer,

adhesion, colonic shortening, wall thickness, and presence of hemorrhage, fecal blood, or diarrhea. Ulcer: 0.5 points for each 0.5 cm; adhesion: 0 points = absent, 1 point = 1 adhesion, 2 points = 2 or more adhesions or adhesions to organs; shortening of the colon: 1 point = >15%, 2 points = >25% (based on a mean length of the untreated colon of 6.99 ± 0.28; $n = 8$); wall thickness measured in mm. The presence of hemorrhage, fecal blood, or diarrhea increased the score by 1 point for each additional feature. We illustrate the total damage score, but also illustrate a component of the score, adhesions, that were sensitive to our treatments and may be of significance if these compounds were to be tested clinically.

Determination of Tissue Myeloperoxidase Activity

Samples of colon were weighed, snap-frozen in liquid nitrogen, and stored at −80°C prior to further processing for the determination of myeloperoxidase (MPO) activity. MPO activity represents an index of neutrophil accumulation.[16,17] Tissue was homogenized in hexadecyltrimethyl-ammonium-bromide (HTAB) buffer (0.5% HTAB; Sigma-Aldrich, Oakville, ON, Canada) in 50 mM potassium phosphate buffer, pH 6.0; 50 mg of tissue/mL. HTAB is a detergent that releases MPO from the primary granules of neutrophils. The homogenate was centrifuged (10 min, 14,000g, 4°C) and 7 μL of supernatant was added to 200 μL of 50 mM potassium phosphate buffer (pH 6.0), containing 0.167 mg/mL of O-dianisidine hydrochloride and 0.0005 H_2O_2. Absorbance was measured at 460 nm (Thermo Fischer Labsystems Multiskan, Thermo Scientific, Ottawa, ON, Canada). MPO was expressed in milliunits per gram of wet tissue, 1 unit being the quantity of enzyme able to convert 1 μmol of H_2O_2 to water in 1 minute at room temperature. Units of MPO activity per minute were calculated from a standard curve using purified peroxidase enzyme (Sigma-Aldrich).

Histology

Following macroscopic scoring, segments of distal colon were stapled flat, mucosal side up, onto cardboard and fixed overnight in Zamboni's fixative (2% paraformaldehyde, 15% picric acid; pH 7.4) at 4°C. Tissues were then rinsed (3 × 10 min) in phosphate-buffered saline (PBS) and cross- and sagittal-sections of the specimens cryoprotected in PBS containing 20% sucrose for several hours or overnight. Specimens were embedded in optimum cutting temperature (OCT; Tissue-Tek, Sakura Finetechnical, Tokyo, Japan) compound and cryostat-sectioned at 12 μm prior to thaw mounting onto poly-D-lysine-coated slides. Colonic wall full thickness sections were stained with hematoxylin and eosin and examined using a Zeiss Axioplan microscope (Carl Zeiss, Toronto, ON, Canada). Photographs were taken using a digital imaging system consisting of a digital camera (Sensys; Photometrics, Tucson, AZ) and image analysis software (V for Windows; Digital Optics, Auckland, New Zealand).

RNA Extraction and cDNA Generation—Total RNA was extracted from mouse proximal colon, distal colon and ileum using the QIAGEN RNeasy Mini Kit (Qiagen, Mississauga, ON, Canada). DNase I treatment was performed according to the manufacturer's instruction. Total nucleic acid concentration was determined by UV-spectrophotometry at 260 nm. cDNA was generated from 1.8 μg of total RNA using Superscript II according to the manufacturer's instructions. In some experiments, Superscript II enzyme was withheld from the mix reaction to determine if there was any contamination

with genomic DNA. Due to the extensive tissue damage in the TNBS model, RNA was extracted and investigated separately in distal (site of TNBS administration) and proximal colon. The proximal is inflamed as well, but due to less direct contact to TNBS the tissue damage (ulceration, etc.) is less pronounced.

Real-time PCR—TaqMan Gene Expression assay kits for the CB_2 target gene (Mm0438286_m1) were purchased from Applied Biosystems (Foster City, CA) for this study. The rodent GAPDH probe (VIC) from Applied Biosystems was used as internal control.

Duplicate samples of 5 μL of each cDNA (1:5 diluted) were amplified by real-time PCR in the ABI Prism 7000 Sequence Detection System (Applied Biosystems). GAPDH was coamplified as an internal control to normalize for variable amounts of cDNA in each sample. The thermocycler parameters were as follows: 50°C for 2 minutes, 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds, and 60°C for 1 minute. Results were collected and analyzed using ABI Prism 7000 SDS software (Applied Biosystems).

Drugs

TNBS was purchased from Sigma-Aldrich and DSS (MW 40,000) was purchased from MP Biomedicals (Solon, OH). (6aR,10aR)-3-(1,1-dimethylbutyl)-6a,7,10,10a-tertahydro-6,6,9-trimethyl-6H-dibenzo[b,d]pyran (JWH133) and 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl-1H-indol-3-yl](4-methoxyphenyl)methanone (AM630) were from Tocris Cookson (Bristol, UK) and (R,S)-(+)-(2-iodo-5-nitrobenzoyl)-[1-(1-methyl-piperidin-2-ylmethyl)-1H-indole-3-yl] me-thanone (AM1241) was synthesized by Dr. A. Makriyannis (Center for Drug Discovery, Northeastern University Boston, MA).

Statistical Analysis

For the animal experiments the results are expressed as mean ± SEM and were compared using an analysis of variance (ANOVA) followed by a Bonferroni correction where appropriate. P-values < 0.05 were considered statistically significant.

Results

CB_2 Receptor Gene Expression in Experimental Colitis

We first assessed whether CB_2 receptor expression was altered in TNBS colitis. One day after TNBS treatment there was a marked upregulation of CB_2 expression in the proximal colon, but little change at the site of TNBS administration in the distal colon, possibly due to the extensive damage at that site. By 3 days, when inflammation has peaked in this model, there was upregulated CB_2 receptor expression in the distal colon and a tendency for enhanced expression in the proximal colon (Fig. 1). To determine whether this effect was specific to this model, we also examined animals treated with DSS, to induce a pancolitis, without the need for ethanol treatment. In the early stages of DSS-induced colitis, CB_2 receptor was upregulated in the proximal colon, the first site to be inflamed, and this continued throughout the colon as the extent and degree of colitis was increased at 7 days

(Fig. 1). Based on these data we then conducted a pharmacological assessment of the action of CB_2 receptor agonists in TNBS colitis.

CB_2 Receptor Agonist JWH133 Attenuates TNBS Colitis

In order to examine the role of the CB_2 receptor on the development of TNBS colitis, we used the well-characterized CB_2 receptor agonist JWH133.[1,18,19] JWH133 (20 mg/kg i.p.) was given 30 minutes prior and then once or twice daily following TNBS treatment for 3 days. Intra-colonic administration of ethanol alone resulted in minor and somewhat variable degree of inflammation which was significantly different from administration of TNBS (macroscopic damage score: 1.8 ± 1.9, colonic length, 6.1 ± 0.8 cm, MPO activity 24.4 ± 20.1% of activity in TNBS-treated mice; $n = 6$). The vehicle used to dissolve the inhibitors did not significantly alter the degree of inflammation induced by TNBS (data not shown). Both once or twice daily treatment with JWH133 significantly attenuated all the parameters of inflammation measured in this study (Fig. 2). Macroscopic damage score and MPO activity were significantly attenuated with both treatments (Fig. 2a,b) and twice-daily treatment with JWH133 was somewhat more effective at reducing MPO activity when compared to the single daily treatment. In JWH133-treated mice the reduction in macroscopic damage was due to a reduced extent of ulceration, as well as a reduction in colonic adhesions (Fig. 2c). Finally, we also noted that compared to vehicle-treated inflamed mice, colonic shortening was significantly reduced in JWH133-treated mice treated once daily. Qualitative histological evaluation supports the protective effect of JWH133 after 3 days of daily treatment (Fig. 3).

CB_2 Receptor Agonist AM1241 Attenuates TNBS Colitis

In order to examine whether this effect could be reproduced with a different class of CB_2 receptor agonist we examined the effect of AM1241.[20] The CB_2 receptor agonist AM1241 was given to mice twice daily either 10 or 20 mg/kg i.p. Both doses of AM1241 significantly attenuated the parameters of inflammation observed to levels comparable to those seen with JWH133 treatment. The macroscopic damage score and MPO activity were both attenuated (Fig. 4a,b), and the higher concentration of AM1241 was somewhat more effective at reducing MPO activity when compared to the lower concentration. The reduction in macroscopic damage was due to a decreased extent of ulceration as well as a reduction in colonic adhesions (Fig. 4c). Colonic shortening in the AM1241-treated mice was significantly reduced to control values (Fig. 4d). Qualitative histological evaluation also supports the protective effect of AM1241 after 3 days of treatment (Fig. 3).

CB_2 Receptor Antagonist AM630 Exacerbates Colitis and Reverses JWH133 Effects

We next assessed whether the CB_2 receptor antagonist AM630 alone altered the degree of colitis and whether it would reverse the actions of JWH133 (20 mg/kg, twice daily). AM630 (10 mg/kg i.p.) was given to the mice once daily and was found to aggravate TNBS-induced colitis. Macroscopic colitis and adhesions were enhanced (Fig. 5a,c) and there was some tendency for elevated levels of MPO activity, but no changes were observed in the length of colon compared to the TNBS-treated mice. When AM630 and JWH133 (20 mg/kg i.p., twice daily) were coinjected, the protective effects of JWH133 were completely abolished (Fig. 5), suggesting that JWH133 exerts its effect via CB_2 receptors. Qualitative histological

evaluation shows that in the presence of AM630, colitis is aggravated and that the effect of JWH133 in the presence of AM630 is abolished (Fig. 3).

CB_2 Agonists JWH133 and AM1241 Are Ineffective in Inamed $CB_2^{-/-}$ Mice

We finally examined the effects of the CB_2 receptor agonists in CB_2 receptor gene-deficient mice $(CB_2^{-/-})$. $CB_2^{-/-}$ mice did not differ compared to wildtype mice in the extent or quality of TNBS colitis. Neither JWH133 (20 mg/kg i.p., twice daily) nor AM1241 (20 mg/kg i.p., twice daily) exerted protective effects in $CB_2^{-/-}$ mice when given in doses effective in wildtype mice (Fig. 6). This lack of effect can be seen in the macroscopic damage score, MPO activity, adhesion score, and colon length (Fig. 6a–d) and demonstrates that the CB_2 receptor is the receptor that mediates the protective effects of the CB_2 agonists JWH133 and AM1241.

Discussion

Ulcerative colitis and Crohn's disease are a major burden to both patients and society. Novel therapeutic options are warranted because conventional therapies are neither uniformly effective nor without significant side effects. The endocannabinoid system (ECS) has emerged as a potential therapeutic target in IBD. The ECS was shown to participate in protective mechanisms in experimental colitis.[9] Specifically, the involvement of CB_1 receptor-dependent pathways was characterized.[9,10,21] Pharmacological activation of CB_1 receptors results in reduced inflammation and blockade of CB_1 receptors, either by antagonists or through genetic ablation of the receptor, and results in aggravation of experimental colitis.[9,21] The role of CB_2 receptors in colitis is less clear, although suggested by studies in which the CB_2 receptor antagonist AM630 reversed the effects of endogenously produced endocannabinoids in murine TNBS colitis[11] and the CB_2 receptor agonist JWH133 reduced damage in DSS and oil of mustard colitis.[10] Due to the relatively low receptor selectivity of the available CB_2 receptor ligands,[1] studies using a combination of agonists, antagonists, and receptor gene-deficient animals are required to confirm the receptor involved. In the present study, using 2 different CB_2 receptor agonists, a selective antagonist and CB_2 receptor gene-deficient mice, we show that activation of CB_2 receptors protects against TNBS colitis, a pharmacological effect that might be used as the basis for the development of future treatments for IBD. Furthermore, we have now shown that the CB_2 receptor-mediated signaling is upregulated in colitis, and our data using the CB_2 receptor antagonist alone suggests that CB_2 receptors are involved in maintaining defense mechanisms in the colon, since colitis was aggravated by this treatment. To our surprise, however, aggravated colitis was not observed in $CB_2^{-/-}$ mice and we suggest that compensatory mechanisms account for this observation. This is in contrast to $CB_1^{-/-}$ mice in which colitis is exacerbated; apparently the CB_1 receptor is both necessary and sufficient with regard to protection in colitis, whereas the CB_2 receptor, though capable of reducing damage, is not sufficient to confer protection alone.

Messenger RNA for the CB_2 receptor has been isolated from the gastrointestinal tract.[22,23] Storr et al[23] were the first to identify CB_2 receptor expression in dissected preparations of

the rat ileum containing only longitudinal muscle with the adherent myenteric plexus, suggesting that CB_2 receptors may be in the enteric nervous system. This was confirmed and extended by Duncan et al,[12] who localized CB_2 receptor on enteric neurons of the myenteric plexus and in preliminary studies on enteric neurons of the human ileum.[24] In humans, CB_2 receptors are either absent or weakly expressed in colonic epithelium, but are evident in the apical membranes at ulcerative margins in IBD.[25] For human colitis, it was also shown that there is an increase in receptor expression in the submucosal infiltrate, but not a dramatic increase in epithelial CB_2 receptor expression.[25] In the present study we examined CB_2 receptor-mediated signaling in experimental colitis. We found that in general there was an increase in CB_2 receptor mRNA expression, once again emphasizing the pathophysiological importance of this receptor. The consequences of the increased CB_2 receptor mRNA on protein expression levels and the specific cell types that have inducible receptor expression will be addressed in future studies.

A previous study used CB_2 receptor agonists to treat colitis, although the receptor specificity of the compounds was not confirmed. The CB_2 receptor agonist JWH133 improved microscopic and macroscopic scores of inflammation when administered prophylactically in DSS colitis, although it required relatively high doses of the compound[10] and was less effective than treatment with the CB_1 receptor agonist ACEA. Oil of mustard-induced colitis is an acute model of colitis which has an extensive neurogenic component that provides support for a neurogenic contribution to IBD.[10,26] Oil of mustard-induced colitis is sensitive to prophylactic administration of a CB_2 receptor agonist, as with DSS-colitis, but again JWH133 was less effective than treatment with a CB1 agonist.[10] JWH133 was also tested therapeutically, after oil of mustard-induced colitis was established and, interestingly, it was more effective in treating colitis compared to when given in advance of the development of colitis.

CB_2 receptor activation in the TNBS and DSS models limited immune cell recruitment, decreased cytokine and chemokine production, and improved macroscopic and histological scores, as reported in a preliminary study.[27] Both acute (DSS colitis) and an immune colitis model ($G\alpha i2^{-/-}$ T-cell transfer model of colitis) were compared for the ability of the CB_2 agonist, AM1241, to protect against the development of colitis.[28] In this preliminary study it was found that AM1241 was unable to protect mice from acute DSS colitis, but was able to protect animals from the immune-mediated colitis. In the present study we used 2 structurally different agonists for the CB_2 receptor and showed that both reduced TNBS-induced colitis, suggesting the CB_2 receptor as the receptor involved. Both drugs were not effective in $CB_2^{-/-}$ mice, demonstrating that it clearly is the CB_2 receptor mediating these effects.

The psychotropic effect of CB_1 receptor activation limits potential treatments using compounds targeting this receptor. Ways to avoid these side effects such as using blockers of endocannabinoid reuptake or degradation, employing peripherally restricted compounds, or selectively targeting the CB_2 receptors have all been suggested. Blocking endocannabinoid reuptake and degradation protects against experimental colitis[8,11] by elevating locally produced endocannabinoids, which in turn activate CB_1 and CB_2 receptors. Since the

endocannabinoid system is regarded as being an on-demand system, blockade of endocannabinoid reuptake and degradation is not associated with psychotropic side effects since the endocannabinoids are produced locally.[29] Targeting the ECS with peripherally restricted drugs might be a future option.[30] Another way to avoid psychotropic side effects is to completely avoid CB_1 receptor activation and to selectively target CB_2 receptors. Our study indicates that targeting the CB_2 receptor reduces inflammation and thus an antiinflammatory cannabinoid-receptor-mediated action can be achieved without involving central CB_1 receptors.

In summary, this study shows that activation of the CB_2 receptor may be an option in the treatment of IBD. Recently, activation of the CB_1 receptor and targeting endocannabinoid degradation were suggested as useful options to reduce intestinal inflammation.[9,11] We now add that agonists at the CB_2 receptor reduce intestinal inflammation in the TNBS model of inflammation. Using antagonists at the CB_2 receptor and additionally genetically deficient mice, we show that the CB_2 receptor is involved in protective mechanisms against intestinal inflammation. The benefit of using these CB_2 active compounds is that they are devoid of the unwanted psychotropic side effects that accompany the administration of CB_1 receptor agonists. Our results suggest that targeting the CB_2 receptor might be a promising therapeutic tool for the treatment of diseases characterized by inflammation of the colon.

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References

1. Howlett AC, Barth F, Bonner TI, et al. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev. 2002; 54:161–202. [PubMed: 12037135]
2. Raitio KH, Salo OM, Nevalainen T, et al. Targeting the cannabinoid CB2 receptor: mutations, modeling and development of CB2 selective ligands. Curr Med Chem. 2005; 12:1217–1237. [PubMed: 15892633]
3. Izzo AA, Mascolo N, Capasso F. The gastrointestinal pharmacology of cannabinoids. Curr Opin Pharmacol. 2001; 1:597–603. [PubMed: 11757815]
4. Massa F, Storr M, Lutz B. The endocannabinoid system in the physiology and pathophysiology of the gastrointestinal tract. J Mol Med. 2005; 83:944–954. [PubMed: 16133420]
5. Pertwee RG. Cannabinoids and the gastrointestinal tract. Gut. 2001; 48:859–867. [PubMed: 11358910]
6. Storr M, Sharkey KA. The endocannabinoid system and gut-brain signalling. Curr Opin Pharmacol. 2007; 7:575–582. [PubMed: 17904903]
7. Storr M, Yuce B, Goeke B. Perspectives of cannabinoids in gastroenterology. Z Gastroenterol. 2006; 44:185–191. [PubMed: 16456761]
8. D'Argenio G, Valenti M, Scaglione G, et al. Up-regulation of anandamide levels as an endogenous mechanism and a pharmacological strategy to limit colon inflammation. FASEB J. 2006; 20:568–570. [PubMed: 16403786]

9. Massa F, Marsicano G, Hermann H, et al. The endogenous cannabinoid system protects against colonic inflammation. J Clin Invest. 2004; 113:1202–1209. [PubMed: 15085199]
10. Kimball ES, Schneider CR, Wallace NH, et al. Agonists of cannabinoid receptor 1 and 2 inhibit experimental colitis induced by oil of mustard and by dextran sulfate sodium. Am J Physiol Gastrointest Liver Physiol. 2006; 291:G364–G371. [PubMed: 16574988]
11. Storr M, Keenan CM, Emmerdinger D, et al. Targeting endocannabinoid degradation protects against experimental colitis in mice: involvement of CB1 and CB_2 receptors. J Mol Med. 2008; 86:925–936. [PubMed: 18493729]
12. Duncan M, Mouihate A, Mackie K, et al. Cannabinoid CB2 receptors in the enteric nervous system modulate gastrointestinal contractility in lipopolysaccharide-treated rats. Am J Physiol Gastrointest Liver Physiol. 2008; 295:G78–G87. [PubMed: 18483180]
13. Buckley NE, McCoy KL, Mezey E, et al. Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoid CB(2) receptor. Eur J Pharmacol. 2000; 396:141–149. [PubMed: 10822068]
14. Morris GP, Beck PL, Herridge MS, et al. Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology. 1989; 96:795–803. [PubMed: 2914642]
15. Wallace JL, Keenan CM. An orally active inhibitor of leukotriene synthesis accelerates healing in a rat model of colitis. Am J Physiol. 1990; 258:G527–G534. [PubMed: 1970708]
16. Bradley PP, Christensen RD, Rothstein G. Cellular and extracellular myeloperoxidase in pyogenic inflammation. Blood. 1982; 60:618–622. [PubMed: 6286012]
17. Krawisz JE, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models. Gastroenterology. 1984; 87:1344–1350. [PubMed: 6092199]
18. Baker D, Pryce G, Croxford JL, et al. Cannabinoids control spasticity and tremor in a multiple sclerosis model. Nature. 2000; 404:84–87. [PubMed: 10716447]
19. Huffman JW, Liddle J, Yu S, et al. 3-(1′,1′-Dimethylbutyl)-1-deoxy-delta8-THC and related compounds: synthesis of selective ligands for the CB2 receptor. Bioorg Med Chem. 1999; 7:2905–2914. [PubMed: 10658595]
20. Ibrahim MM, Deng H, Zvonok A, et al. Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. Proc Natl Acad Sci USA. 2003; 100:10529–10533. [PubMed: 12917492]
21. Sibaev A, Massa F, Yuce B, et al. CB1 and TRPV1 receptors mediate protective effects on colonic electrophysiological properties in mice. J Mol Med. 2006; 84:513–520. [PubMed: 16501934]
22. Griffin G, Fernando SR, Ross RA, et al. Evidence for the presence of CB_2 like cannabinoid receptors on peripheral nerve terminals. Eur J Pharmacol. 1997; 339:53–61. [PubMed: 9450616]
23. Storr M, Gaffal E, Saur D, et al. Effect of cannabinoids on neural transmission in rat gastric fundus. Can J Physiol Pharmacol. 2002; 80:67–76. [PubMed: 11911227]
24. Wright KL, Duncan M, Sharkey KA. Cannabinoid CB2 receptors in the gastrointestinal tract: a regulatory system in states of inflammation. Br J Pharmacol. 2008; 153:263–270. [PubMed: 17906675]
25. Wright K, Rooney N, Feeney M, et al. Differential expression of cannabinoid receptors in the human colon: cannabinoids promote epithelial wound healing. Gastroenterology. 2005; 129:437–453. [PubMed: 16083701]
26. Kimball ES, Palmer JM, D'Andrea MR, et al. Acute colitis induction by oil of mustard results in later development of an IBS-like accelerated upper GI transit in mice. Am J Physiol Gastrointest Liver Physiol. 2005; 288:G1266–G1273. [PubMed: 15691868]
27. Thuru X, Chamaillard M, Karsak M, et al. Cannabinoid receptor 2 is required for homeostatic control of intestinal inflammation. Gastroenterology. 2007; 132(suppl 2):A228.
28. Ziring DA, Braun J. AM1241, a CB2-specific agonist protects against immune but not acute colitis. Gastroenterology. 2007; 132(suppl 2):A232.
29. Cravatt BF, Lichtman AH. Fatty acid amide hydrolase: an emerging therapeutic target in the endocannabinoid system. Curr Opin Chem Biol. 2003; 7:469–475. [PubMed: 12941421]
30. Dziadulewicz EK, Bevan SJ, Brain CT, et al. Naphthalen-1-yl-(4-pentyloxynaphthalen-1-yl)methanone: a potent, orally bioavailable human CB(1)/CB(2) dual agonist with antihyperalgesic

properties and restricted central nervous system penetration. J Med Chem. 2007; 50:3851–3856. [PubMed: 17630726]

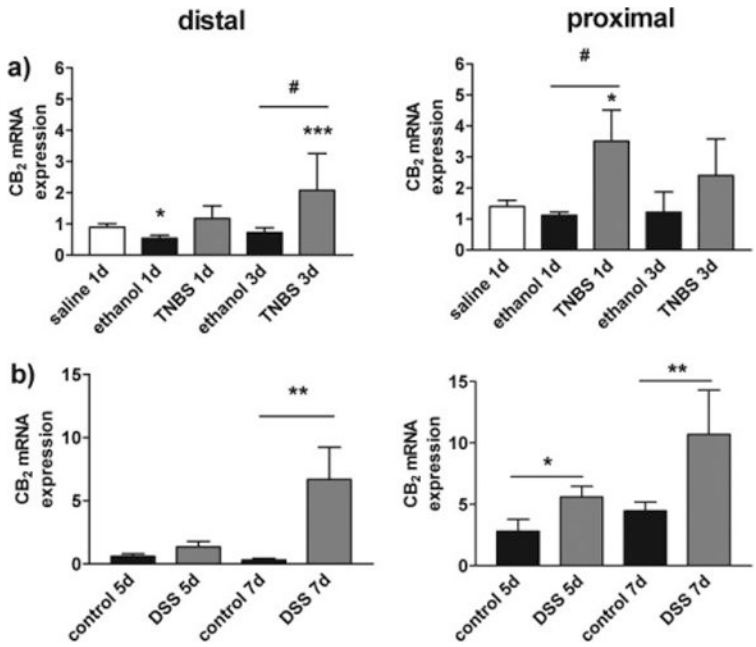

FIGURE 1.

Quantitative PCR of CB_2 mRNA expression in proximal (right) and distal (left) colon of mice treated with (a) TNBS at 1 and 3 days posttreatment, and (b) DSS at 5 and 7 days posttreatment. Respective ethanol controls are included. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; #P < 0.05 TNBS versus ethanol; $n = 4$--6 each.

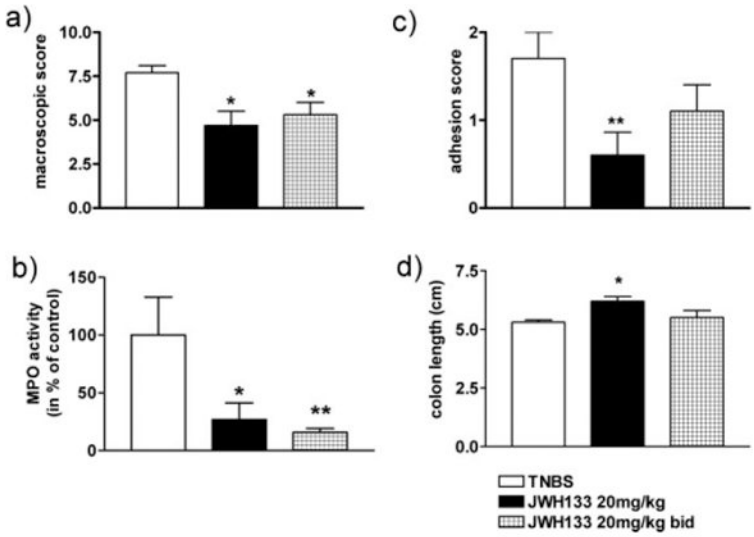

FIGURE 2.

The CB_2 receptor agonist JWH133 (20 mg/kg; i.p.) injected once or twice daily over 3 days attenuates TNBS-induced colitis. This figure shows data for (a) macroscopic score, (b) MPO activity, (c) adhesion score, and (d) colon length. $*P < 0.05$; $**P < 0.01$; n = 8--10 each.

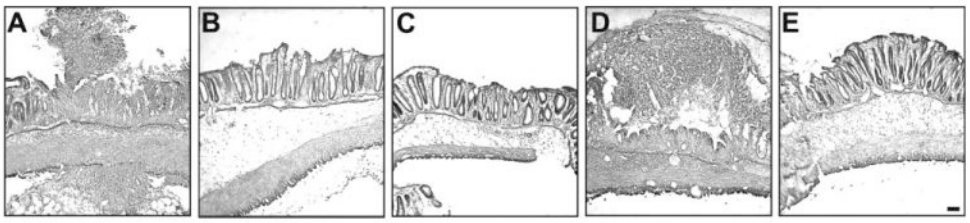

FIGURE 3.

Representative micrographs of hematoxylin and eosin-stained sections of distal colon from TNBS-treated mice. Control (A), 3 days JWH133 (20 mg/kg, twice daily) treated (B), 3 days AM 1241 (20 mg/kg, twice daily) treated (C), 3 days AM 630 (10 mg/kg once daily) treated (D), and 3 days AM 630 (10 mg/kg once daily)/JWH133 (20 mg/kg, twice daily) treated (E) mice. Panel A shows a representative section from a TNBS-treated mouse that received vehicle treatment only: note the massive inflammatory infiltrate with mucosal destruction and ulceration which is largely ameliorated in the CB_2 agonist (JWH 133 and AM 1241)-treated animals but not in the CB_2 antagonist (AM 630)-treated animals. Scale bar = 100 μm.

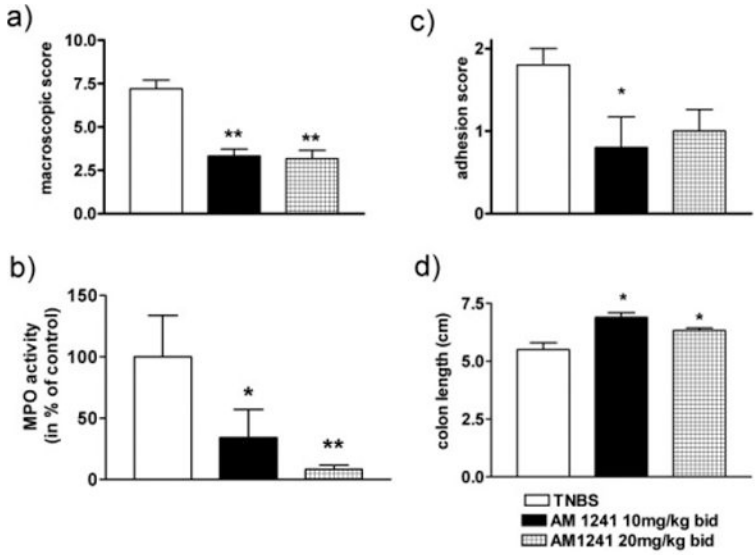

FIGURE 4.

The CB_2 receptor agonist AM1241 (10 or 20 mg/ kg; i.p.) injected twice daily over 3 days attenuates TNBS-induced colitis. This figure shows data for (a) macroscopic score, (b) MPO activity, (c) adhesion score, and (d) colon length. *$P < 0.05$; **$P < 0.01$; n = 5--6 each.

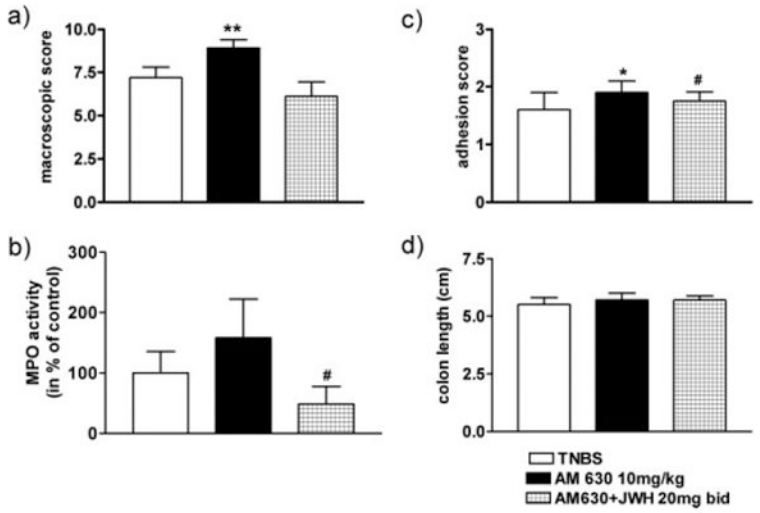

FIGURE 5.

The CB_2 receptor antagonist AM630 (10 mg/kg; i.p.) once daily over 3 days increases TNBS-induced colitis. The attenuation of colitis by JWH133 (20 mg/kg; twice daily) is reversed in the presence of AM630. This figure shows data for (a) macroscopic score, (b) MPO activity, (c) adhesion score, and (d) colon length. $*P < 0.05$; $**P < 0.01$; $\#P < 0.05$ for JWH133 versus AM630+JWH; n = 8--10 each.

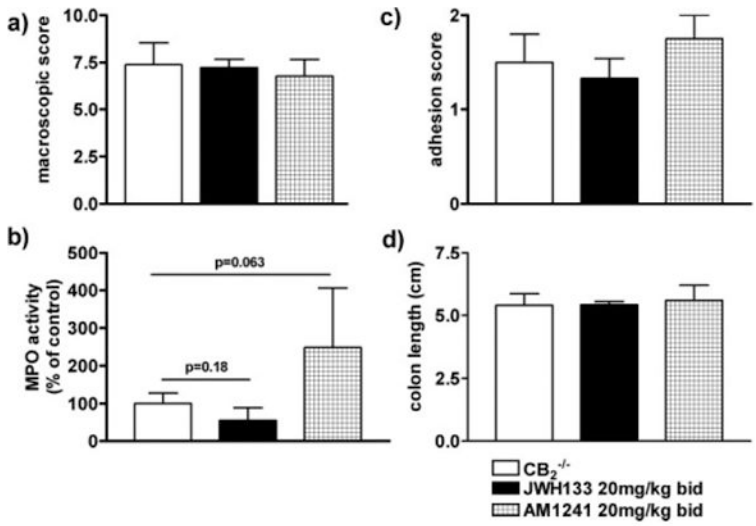

FIGURE 6.

JWH133 (20 mg/kg, twice daily) and AM1241 (20 mg/kg, twice daily) did not alter TNBS-induced colitis in $\mathrm{CB}_2^{-/-}$ mice. This figure shows data for (a) macroscopic score, (b) MPO activity, (c) adhesion score, and (d) colon length (n = 5--7 each).