Agonists of cannabinoid receptor 1 and 2 inhibit experimental colitis induced by oil of mustard and by dextran sulfate sodium

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Kimball, Edward S., Craig R. Schneider, Nathaniel H. Wallace, and Pamela J. Hornby. 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 291: G364–G371, 2006. First published March 30, 2006; doi:10.1152/ajpgi.00407.2005.—Oil of mustard (OM) is a potent neuronal activator that is known to elicit visceral hyperalgesia when given intracolonomally, but the full extent to which OM is also proinflammatory in the gastrointestinal tract is not known. We have previously shown that male CD-1 mice given a single administration of 0.5% OM develop a severe colitis that is maximum at day 7 and that gradually lessens until essentially absent by day 14. OM-induced neuronal stimulation is reported to be reduced by cannabinoid agonists, and cannabinoid receptor 1 (CB1R)−/− mice have exacerbated experimental colitis. Therefore, we examined the role of cannabinoid in this OM-induced 3-day model of colitis in CD-1 mice and in a 7-day dextran sulfate sodium (DSS) colitis model in BALB/c mice. In OM colitis, the CB1R-selective agonist ACEA and the CB2R-selective agonist JWH-133 reduced (P < 0.05) colon weight gain (means ± SE; 82 ± 13% and 47 ± 15% inhibition, respectively), colon shrinkage (98 ± 24% and 42 ± 12%, respectively), colon inflammatory damage score (49 ± 11% and 40 ± 12%, respectively), and diarrhea (58 ± 12% and 43 ± 11%, respectively). Histological damage was similarly reduced by these treatments. Likewise, CB1R agonists attenuated DSS colitis, albeit at higher doses; ACEA at 10 mg/kg, twice daily, inhibited (P < 0.05) macroscopic and microscopic scores (46 ± 9% and 63 ± 7%, respectively); whereas 20 mg/kg, twice daily, of JWH-133 was required to diminish (P < 0.05) macroscopic and microscopic scores (29 ± 7% and 43 ± 5%, respectively). CB1R and CB2R immunostaining of colon sections revealed that CB1R in enteric neurons was more intense in colitic vs. control mice; however, CB1R was also increased in the endothelial layer in OM colitis only. CB2R immunostaining was more marked in infiltrated immune cells in OM colitis. These findings validate the OM colitis model with respect to the DSS model and provide strong support to the emerging idea that cannabinoid receptor activation mediates protective mechanisms in experimental colitis. The demonstration of CB1R agonist effects in colitis support the neurogenic nature of the OM-induced colitis model and reinforce the importance of neuronal activation in intestinal inflammation.

allyl isothiocyanate; experimental inflammatory bowel disease; CB1R; CB2R; oil of mustard colitis

INFLAMMATORY BOWEL DISEASES (IBD) such as ulcerative colitis and Crohn’s disease contain elements of classical patterns of inflammation that originate via innate and adaptive immune responses. These diseases may also partly be comprised of inflammatory pathways that originated via neuronal activation. Intrinsic and extrinsic afferents innervate the intestinal tract, and phenotypic changes among these neuronal pathways occur during clinical and experimental IBD (4, 20, 24, 29, 31). This has prompted interest in the role of neuronal control of colitis, especially with respect to pathways involved in nociception. Allyl isothiocyanate, whose common name is oil of mustard (OM), is one of the pungent components of mustard seed, horseradish, and wasabi (19). It is a direct stimulant of small nerve fibers (2) and has been used to study neuronal signaling in nociception (3, 28, 32). Independent of its neuronal actions, OM is a potent, acute inflammatory irritant (18) whose inflammatory mode of action has not been fully elucidated. We have previously reported (22) that its intracolonic application produced an acute colitis in mice that, over a 3-day period, was characterized by body weight loss, colon shrinkage, colon edema and thickening, severe diarrhea, and transmural inflammatory lesions that occasionally penetrated through the seromuscular layers.

The purpose of the present study was to compare this novel model of colitis with respect to a previously accepted model of colitis, dextran sulfate sodium (DSS)-induced colitis, by evaluating responses to cannabinoid agonists. Cannabinoid receptor (CBR) activation figures prominently in neuronal signaling induced by OM (3, 19). Both CB1R and CB2R are G protein-coupled receptors (8), with CB1R located predominantly on neurons (16) and CB2R on immune cells (5, 36). CBR activation via CB1R and CB2R agonists has been reported to prevent and ameliorate symptoms of inflammatory models such as experimental multiple sclerosis (1, 38) and experimental colitis. Specifically, CB1R−/− mice were more sensitive to colitis induction by DSS than wild-type mice, and a CB1R antagonist worsened the colitis that was induced by DNBS (33). Based on these various reports, we examined the role of CBR modulation in two experimental models of colitis, OM-induced colitis and DSS colitis. The goals of this research were twofold: to establish whether CBR stimulation by selective CBR agonists could have a beneficial effect on experimental colitis and to compare this in the recently described OM-induced colitis model to the DSS-induced colitis model so as to help establish the utility of the OM model and relate that to an established colitis model.

In this report we present data showing that OM-induced colitis was inhibited by intraperitoneal application of ACEA, a CB1R-selective agonist, and by JWH-133, a CB2R-selective agonist. These agonists inhibited OM-induced distal colon weight gain, colon shrinkage, inflammatory damage, diarrhea, and histological damage. Similarly, the CB1R agonist ACEA prevented colitis development in mice given 5% DSS, as evidenced by reversing colon shrinkage and diminishing inflammatory damage, diarrhea, and histological damage. This is...
the first direct comparison of OM colitis to an established colitis model, DSS colitis. This is also the first demonstration of a role for CB2R activation in experimental colitis. The demonstration that both CB1R and CB2R agonists are active in both colitis models lends additional support to the concept that signaling through CBRs may mediate protective mechanisms in colitis.

MATERIALS AND METHODS

**Mice.** Male CD-1 mice (Charles River Laboratories, Kingston, NC), 9- to 12-wk-old, were used for OM colitis studies, and female BALB/c mice (Taconic Farms, Germantown, NY) were used for DSS colitis studies. All treatments were carried out in accordance with the Federal Animal Welfare Act and with methods approved by the Institutional Animal Care and Use Committee of Johnson and Johnson Pharmaceutical Research and Development.

Fresh OM (allyl isothiocyanate of 95% or 98% purity; Sigma-Aldrich, St. Louis, MO) was used in each experiment. ACEA and JWH-133 were purchased from Tocris-Cookson (Ellesville, MO).

**OM colitis induction.** Colitis induction by OM has previously been described (22). Mice (n = 9 per treatment group) were briefly anesthetized with ketamine/xylazine (Sigma) and held in a vertical position so that 50 μl of a solution of 0.5% OM in 30% ethanol could be administered intracolonically. The OM administration occurred to a depth of 4 cm via a syringe equipped with a ball-tipped 22-gauge needle.

Body weights were measured daily. Three days after OM administration, the experiment was terminated; colons were resected, examined for signs of inflammation, and weighed after removing fecal contents (which were examined for signs of diarrhea); and the length from the aboral end of the cecum to the anus was determined. These data and observations were assigned a score as previously reported (22). The sum of the individual macroscopic indices, i.e., colon weight gain, colon shrinkage, stool consistency, and inflammatory damage scores, were combined into a macroscopic score for each colon, where 0 = normal and 15 = maximally affected.

**DSS-colitis induction.** Mice (n = 10 per treatment group) were provided with a solution of tap water containing 5% DSS (45 kDa; ICN Biomedicals, Irvine, CA) ad libitum over a 7-day period. The DSS solution was replenished daily, and the amount consumed was measured. At the end of this 7-day period, animals were euthanized, and their colons were examined for signs of inflammation and diarrhea. The colon length was measured from the oral end of the cecum to the anus. These measurements and observations were assigned a score as previously reported (22). The sum of these three individual macroscopic indices were combined into a macroscopic score for each colon, where 0 = normal and 11 = maximally affected.

**Histology evaluations.** Segments from the distal colon taken from the first to fourth centimeter from the anus of each animal were removed, rinsed in saline, fixed in 10% neutral buffered formalin, embedded longitudinally in paraffin, sectioned, and finally stained with hematoxylin and eosin (H&E). The tissues were examined for epithelial damage using light microscopy by an investigator who was blinded to the sample groups and were then scored for percent of colon length exhibiting epithelial damage, cellular infiltration, and damage or alteration of smooth muscle architecture by a second investigator, according to the scale previously reported (22). The sum of these individual histology scores comprised a microscopic score for each colon, where 0 = normal and 9 = maximally affected.

Immunohistochemical methods were applied to characterize CB1R and CB2R immunostaining in colitis tissues and to study the effect of CBRR agonists treatment on neutrophil infiltration. Tissues were formalin-fixed and paraffin-embedded prior to immunohistochemical treatments, as previously described (22). Briefly, 5-μm tissue sections were mounted on microscope slides and then routinely dewaxed and rehydrated. After a 5-min exposure in a microwave in target buffer (Dako, Carpinteria, CA), slides were treated in 3% H2O2 for 5 min, followed by treatment with avidin-biotin reagent (Serotec, Raleigh, NC) to eliminate endogenous peroxidase and biotin activity, respectively. The slides were then routinely processed for immunohistochemistry. All incubations were performed at room temperature for 30 min. After a 10-min blocking step with normal goat serum, the tissues were incubated with the primary antibodies. Rabbit anti-myeloperoxidase (Dako) was used to identify neutrophils. Rabbit anti-CB1R (1:25 dilution) and rabbit anti-CB2R (1:2 dilution) (Chemicon, Temecula, CA) polyclonal antibodies were used to identify CB1 immunoreactivity in tissue sections. Biotinylated goat anti-rabbit secondary antibody was purchased from Vector Labs (Burlingame, CA). Avidin-horseradish peroxidase was obtained from Serotec. 3,3’-Diaminobenzidine was purchased from Biomedia (Foster City, CA) and used to detect peroxidase activity.

**Dosing regimen.** For OM colitis studies, compounds were administered at 2.5 mg/kg intraperitoneally, once daily, 24 h prior to OM administration and then once daily thereafter. These doses were chosen on the basis of previously published data (1). In some experiments, compounds were administered 24 h after OM administration and then once daily thereafter. In DSS studies, these same compounds were administered at 10 mg/kg or 20 mg/kg intraperitoneally, twice daily, based on empirical data. Dosing began on the day of DSS administration and daily thereafter until the end of the experiment. In all experiments, compounds were dissolved in a vehicle consisting of 5% Tween 80, 5% ethanol, and 5% dextrose in water. Drug-treated groups were compared against an OM control, which was administered vehicle only.

**Statistics.** Experimental groups were analyzed for significance of differences between the means of treatment groups and control groups by ANOVA with a Dunnett or Bonferroni test using GraphPad Prism (version 3.03; GraphPad Software, San Diego, CA). P values <0.05 were considered significant.

RESULTS

**OM-induced colitis and inhibition by CBRR agonists.** Intra-colonic administration of 0.5% OM in 30% ethanol causes body weight loss, increased colon weight as a measure of edema, colon shrinkage and thickening, inflammatory lesions and often penetrating ulcers, and diarrhea. The data shown in Table 1 recapitulate previously reported results (22) and, for OM-treated controls, show increases in colon weight of 0.187 g, colon shrinkage of 1.7 cm, stool scores indicative of severe bowel dysmotility, moderate-to-severe inflammatory damage, and histology damage. In a previous report we had demonstrated that the 30% ethanol used to dissolve the OM, when administered intracolonically, did not produce the above-mentioned macroscopic changes, inflammation, or alterations in stool consistency; nor did it cause histological damage. Accordingly, we did not perform any additional tests with this vehicle.

Results from these experiments with a CB1R-selective agonist, ACEA, and a CB2R-selective agonist, JWH-133, are shown in Fig. 1. Doses for these compounds were chosen based on previously published results (1). At 2.5 mg/kg, once daily, ACEA significantly inhibited all colitis parameters. All colitis parameters were also significantly reduced as a result of treatment with the CB2R-selective agonist JWH-133, albeit to a lesser extent than seen with ACEA. Thus ACEA and JWH-133 reduced OM-induced colon weight gain (means ± SE; 82 ± 13% and 47 ± 15% inhibition, respectively), colon shrinkage (98 ± 24% and 42 ± 12%, respectively), inflammation score
Table 1. Colitis parameters 3 days post oil of mustard instillation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Colon Weight, g</th>
<th>Colon Length, cm</th>
<th>Stool Scores</th>
<th>Inflammation Scores</th>
<th>Macroscopic Scores</th>
<th>Microscopic Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.292 ± 0.018*</td>
<td>10.1 ± 0.2*</td>
<td>0*</td>
<td>0.7 ± 0.3*</td>
<td>0.2 ± 0.2*</td>
<td></td>
</tr>
<tr>
<td>OM + vehicle</td>
<td>0.479 ± 0.045</td>
<td>8.4 ± 0.2</td>
<td>1.9 ± 0.4</td>
<td>2.0 ± 0.3</td>
<td>8.8 ± 1.2</td>
<td>4.0 ± 0.9</td>
</tr>
</tbody>
</table>

Data are absolute measurements of colon weights and lengths, or determinations of stool consistency and apparent inflammation in colons taken from untreated CD-1 mice or from mice given intracolonic 0.5% oil of mustard, as described in MATERIALS AND METHODS. Vehicle, consisting of 5% Tween 80, 5% ethanol, and 5% dextrose in water, was used to dissolve experimental compounds used in subsequent studies, and was given ip in the OM + Vehicle group. *P < 0.01 vs. OM + vehicle.

(49 ± 11% and 40 ± 12%, respectively), and diarrhea (58 ± 12% and 43 ± 11%, respectively). Macroscopic scores (64 ± 11% and 41 ± 10% inhibition) and microscopic scores (57 ± 7% and 36 ± 15% inhibition) were significantly inhibited by ACEA and JWH-133, respectively (Fig. 2). These results indicate that activation of either CBR subtype can attenuate OM colitis development.

Histological evaluation of tissues from OM-treated mice. Tissues collected at day 3 for OM-treated mice (Fig. 3) revealed discontinuous lesions that exhibited cellular infiltration throughout the mucosa, submucosa, and smooth muscle, destruction of epithelial architecture, submucosal edema, and loss of smooth muscle architecture (Fig. 3B). We have previously demonstrated (22) that the cellular infiltrate was composed of neutrophils and lymphocytes in damaged epithelia, with a monocytic infiltrate in addition to neutrophils and lymphocytes in the muscularis and submucosa and that there was little or no microscopic damage observed in tissues treated only with the ethanol vehicle used to deliver OM. Tissues from mice treated with either ACEA (Fig. 3C) or JWH-133 (Fig. 3D) demonstrated nearly complete resolution of epithelial damage, a near absence of an inflammatory infiltrate, elimination of submucosal edema, and normal smooth muscle appearance. To further substantiate the inflammatory aspects of this model and of the anti-inflammatory activity of CBR agonists, we analyzed tissues for neutrophilic invasion by quantitating myeloperoxidase-positive cells in 2- to 3-cm-long segments of distal colon.

Normal control tissues contained no MPO-positive cells, whereas tissues from OM-inflamed mice exhibited an average of 199 ± 33 MPO-positive cells/cm (n = 7; P < 0.001 vs. normal controls). In contrast, tissues from OM-inflamed mice treated with ACEA contained only 5.7 ± 4.4 MPO-positive cells/cm (n = 9; P < 0.001 vs. OM). Thus CB1R and CB2R agonists preserved tissue architecture in the mucosa, submucosa, and muscularis, and prevented or resolved inflammatory responses.

Correction of DSS colitis by CBR agonists. Daily administration of 5% DSS caused colon shrinkage, mild diarrhea, and inflammation of the tissues of the distal colon (Table 2). When tested with either ACEA or JWH-133, we found that DSS colitis was less sensitive than OM colitis to resolution by CBR agonists, requiring higher doses and more frequent dosing. ACEA and JWH treatments were ineffective at 2.5 mg/kg, when administered either once daily or twice daily (data not shown). However, at 10 mg/kg, twice daily, ACEA was an effective treatment in this model (Table 2). Thus colon shrinkage, stool score, and inflammatory colon damage were significantly decreased following ACEA treatment. Similarly, significant improvements in macroscopic colitis damage parameters were obtained with JWH-133 when administered at 10 or 20 mg/kg, twice daily (Table 2 and Fig. 4). Macroscopic damage scores (Fig. 4) for ACEA-treated mice showed a 46 ± 9% inhibition (P < 0.001 vs. OM treatment), whereas treatments with JWH-133 at 10 and 20 mg/kg, twice daily, resulted in 20 ± 6% (P > 0.05) and 29 ± 7% inhibition (P < 0.01), respectively.
respectively, of this score. These data, therefore, demonstrate that experimental colitis induced by DSS and by OM share certain common features that render both models correctable by cannabinoid agonists.

**Histological evaluation of tissues from DSS-treated mice.** H&E staining of distal colon tissues taken at day 7 from mice given 5% DSS (Fig. 5) revealed extensive tissue damage that included damage to epithelium and smooth muscle architecture and invasion of these tissues by monocytic and granulocytic cells (Fig. 5B). Following treatment with either 10 mg/kg ACEA, twice daily (Fig. 5C) or 20 mg/kg JWH-133, twice daily (Fig. 5D), we observed that smooth muscle thickening had subsided to approximately normal appearance, the epithelium appeared intact, and the cellular infiltrate had significantly diminished. These improvements occurred to a greater extent and with higher frequency in ACEA-treated mice than in JWH-133-treated mice. The microscopic damage score for ACEA-treated mice was inhibited 63 ± 7% (P < 0.01 vs. OM treatment, Table 4) and was inhibited 43 ± 5% by JWH-133 (20 mg/kg, twice daily, P < 0.05).

**Immunohistochemical staining for CB1R and CB2R.** Immunohistochemical staining for CB1R and CB2R immunoreactive cells revealed differences between OM colitis and DSS colitis (Fig. 6). The CB2R-positive cells were evident as a dense, intensely stained infiltrate in OM colitis tissue. Nearly all of the infiltrating cells in the submucosa and a large proportion of those that infiltrated the epithelium were CB2R positive. In DSS tissue, CB2R immunoreactivity was evident but to a lesser extent than seen in OM colitis, both in the proportion of stained cells in the submucosal infiltrate and in the epithelial infiltrate.

CB1R was highly expressed in endothelium in OM-lesioned areas but was not evident in DSS. In contrast, myenteric ganglia exhibited strong upregulation of CB1R in both OM and DSS colitis. These results suggest that the increased sensitivity to CB1R and CB2R agonists by OM colitis may be due to the greater abundance of CB2R-positive cells in the inflammatory infiltrate and to the strong upregulation of CB1R expression in endothelium in OM colitis tissue compared with DSS colitis tissue.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Colon Length, cm</th>
<th>Stool Scores</th>
<th>Inflammation Scores</th>
<th>Macroscopic Scores</th>
<th>Microscopic Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>9.4±0.1**</td>
<td>0*</td>
<td>0**</td>
<td>0.1±0.1***</td>
<td>0.2±0.2**</td>
</tr>
<tr>
<td>OM + vehicle</td>
<td>7.2±0.2</td>
<td>1.3±0.15</td>
<td>1.8±0.2</td>
<td>5.5±0.4</td>
<td>7.4±0.5</td>
</tr>
<tr>
<td>OM + ACEA</td>
<td>8.3±0.2**</td>
<td>0.6±0.2</td>
<td>1.3±0.2</td>
<td>3.0±0.5***</td>
<td>3.3±0.4**</td>
</tr>
<tr>
<td>Untreated</td>
<td>10.3±0.3**</td>
<td>0**</td>
<td>0</td>
<td>0.2±0.1**</td>
<td>0.2±0.1**</td>
</tr>
<tr>
<td>OM + vehicle</td>
<td>8.6±0.4</td>
<td>2.2±0.2</td>
<td>1.0±0.0</td>
<td>5.1±0.2</td>
<td>5.7±0.8</td>
</tr>
<tr>
<td>OM + JWH-133</td>
<td>8.4±0.3</td>
<td>1.1±0.2**</td>
<td>0.8±0.1</td>
<td>3.9±0.3**</td>
<td>3.3±0.3**</td>
</tr>
</tbody>
</table>

Data are absolute measurements of colon lengths, determinations of stool consistency and apparent inflammation in colons taken from untreated BALB/C mice or from mice given 5% DSS in drinking water, as described in MATERIALS AND METHODS. ACEA was administered at 10 mg/kg, bid, twice daily, ip. JWH-133 was administered at 20 mg/kg intraperitoneally, twice daily. *P < 0.05 vs. OM + vehicle, **P < 0.01 vs. OM + vehicle, ***P < 0.001 vs. OM + vehicle.
Prophylactic vs. therapeutic dosing regimens in OM colitis.
Because OM colitis is an acute model, one of the questions related to evaluating its utility was to determine whether it could be used not only with prophylactic dosing as shown above, but also with a therapeutic dosing regimen. We therefore compared dosing at 24 h to dosing at 24 h relative to intracolonic OM administration and induction of colitis. In these studies, daily ACEA dosing was equally as effective when first administered at 24 h as at 24 h (Fig. 7), yielding 49 ± 9% (P < 0.05) and 44 ± 11% (P < 0.05) inhibition of macroscopic scores and 49 ± 9% (P < 0.001) and 56 ± 11% (P < 0.001) inhibition of microscopic scores. JWH-133 was appreciably more effective if first administered at 24 h than if started at 24 h, producing a mean inhibition of macroscopic scores of 78 ± 11% (P < 0.01) compared with 41 ± 10% (P < 0.05) and mean inhibition of microscopic scores of 64 ± 7% (P < 0.01) for doses starting at +24 h compared with 36 ± 15% inhibition (P < 0.05) for dosing that started at −24 h.

DISCUSSION
The results demonstrate that CBR activation is protective in two models of experimental colitis, OM-induced colitis (22) and the DSS model of colitis (9). We report for the first time that symptoms in OM-induced colitis were prevented by the CB1R agonist ACEA and by the CB2R agonist JWH-133. In addition, enteric neuronal CB1R immunostaining was markedly enhanced in OM and DSS colitis, and CB2R was more intensely stained in inflammatory infiltrates in OM colitis than in DSS colitis, possibly accounting for the higher sensitivity of OM colitis to attenuation by CBR agonists.

As previously reported, CB1R−/− mice are more susceptible to colitis induction by DSS, and administration of a CB1R antagonist to mice undergoing DNBS colitis worsens the disease (33). We extended these findings by demonstrating that a CB1R agonist similarly ameliorates the extent of DSS disease symptoms, confirming the role of CB1Rs in DSS colitis, and by demonstrating a protective role for CB2R activation in OM and DSS colitis. The data reveal that the cannabinoid-related reductions in overt inflammatory damage and bowel dysmotility are accompanied by preservation of tissue architecture in the mucosa, submucosa, and muscularis. These results, thereby, further demonstrate the importance of CBR modulation in colitis and validate the recently described model of OM-induced colitis via its comparison to results in the more well-known DSS colitis model and via its responses to CBR reference agonists.

Both CB1R and CB2R are G protein-coupled receptors (7, 8, 13, 17, 40). CB1Rs were originally described in rat cerebellar membrane preparations (35) but have since been located on neuronal and nonneuronal cells (3, 5, 6, 10–12, 14–16, 25, 37). CB1R agonists prevent hyperalgesia development in a model...
of neuropathic pain (3). Similar to the present studies are reports of CB1R expression in the intestinal nervous system (27, 39), both in myenteric and submucosal ganglia (6, 10). CB1Rs are also expressed on macrophage-like cerebellar glial cells (42) and, in addition, have been detected on myeloid and lymphoid cells, having been shown to reside on adherent and nonadherent spleen cells (14) that include B cells, T cells, NK cells, primed recruited macrophages, dendritic cells, and neutrophils (41). CB2Rs were originally thought essentially to be confined to immune system cells and to be absent from brain tissues (36). Thus CB2R has been demonstrated to reside on a wide variety of peripheral blood leukocytes and spleen cell populations (26) and to a greater extent than seen for CB1R in the same cell types that include B cells, T cells, NK cells, splenic macrophages, dendritic cells, and neutrophils (5, 25, 30). More recently, the existence of a CB2R on peripheral neurons (15) and perivascular microglial cells (37) in human brain has been reported.

Data presented in this paper show differences in CB1R and CB2R expression patterns after induction of OM and DSS colitis that may also explain the differences between the two models’ sensitivity to CBR agonists. Using immunohistochemical staining of distal colon sections, we observed a more abundant CB2R-positive cell population in the inflammatory infiltrate in OM colitis and a strong upregulation of CB1R expression in endothelium in OM colitis tissue, compared with DSS colitis tissue. The higher sensitivity of OM colitis argues in favor of greater CBR activation and/or presence induced by OM, consistent with the increased CBR expression we observed. The requirement for even higher doses of ACEA and JWH-133 in DSS colitis may reflect that DSS colitis may have a lower dependency on CBR mechanisms. The endothelial CB1R changes seen in OM colitis are consistent with changes in expression in human IBD tissues reported by Wright et al. (43), who reported elevated CB1R and CB2R in colon tissues from patients with IBD. CB1R was increased in myenteric

Fig. 6. Immunohistochemical staining for CB1R and CB2R expression reveals differences between OM colitis and DSS colitis. A: CB2R-positive infiltrate in OM tissue, showing intensely stained, dense infiltrate in the submucosa (red arrows) and in the epithelium (black arrows). B: CB2R-positive infiltrate in DSS tissue, showing CB2R immunoreactivity in the submucosal infiltrate (red arrows) and in the infiltrate found in the epithelium (black arrows). There are fewer CB2R-positive cells in the DSS infiltrate than in the OM infiltrate. C: CB1R-positive staining on endothelial cells, indicated by arrows, in lesioned epithelial areas of OM colitis. D: absence of CB1R-positive staining on endothelial cells, indicated by arrows, in epithelial areas of DSS colitis. E–G: CB1R immunoreactivity in myenteric plexus, indicated by arrows, in normal mice (E), OM mice (F), and DSS mice (G). H: CB1R staining in normal mice. I: CB2R staining in normal mice. A–G: ×40 objective. H and I: ×20 objective.
plexus neurons and endothelial cells, further supporting the OM colitis model as one that contains elements of clinical disease, whereas CB2R was increased in epithelial tissues. In addition, a CB1R agonist was shown to enhance epithelial restitution in vitro. Except for the data supplied herein, there are no reports for a role for CB2R agonist activity in IBD models, but Mathison et al. (34) demonstrate that a CB2R agonist corrected small bowel dysfunction in an inflammatory model of accelerated small intestinal transit. Both studies are consistent with our results and may help to explain the protective role for CB1R and CB2R agonists seen in our studies.

There may be additional explanations for the differences in sensitivity to colitis inhibition by CBR agonists in OM colitis vs. DSS colitis. This may reflect differences between the two mouse strains used or sex differences. Female BALB/c mice are one of the standard strains used for DSS colitis, and male CD-1 mice are the standard and, thus far, the only strain used in the OM model. Our aim was to simply compare responses to cannabinoid agonists. It is not clear yet whether these strain or sex differences contributed to the differences in sensitivity observed between the two models.

CB1R activation has been shown to predispose Th1/Th2 balance towards Th2 (44). The fact that agonists active at either CB1R demonstrated efficacy in averting tissue damage in OM and DSS colitis suggests that activation of either population of CBR might result in correction of a Th1/Th2 cytokine imbalance. CB2R agonist signaling on activated immune cells has been shown to result in apoptosis of those cells (45). OM colitis was attenuated both by prophylactic and therapeutic CB1R agonist dosing regimens, whereas it appeared to respond better to late administration of a CB2R agonist. This suggests that immune cell activation renders the CB2R-positive cell population more susceptible to CB2R activation and may be a prerequisite for subsequent downregulation or apoptosis by CB2R agonists. This difference in sensitivity to damage resolution by the CB1R and CB2R agonists has a number of implications. First, it demonstrates that OM-induced colitis can be corrected after colitis initiation and that CBR signaling is important at later time points, not only at inception of disease. Second, the apparent greater sensitivity to JWH-133 when first administered at +24 h, a time when the damage seen in OM colitis is nearly maximum (unpublished observations), might imply that CB2R signaling may be related to time-dependent recruitment of CB2R-expressing cells or their activation state. Alternatively, the effects of JWH-133 might also be explained not only by acting to prevent recruitment of CB2-expressing cells but by inhibiting the inflammation through acting on increased CB2R expression on resident cells during colonic inflammation. The result may also reflect pharmacodynamic properties of JWH-133.

A considerable body of literature supports the premise that OM is a neuronal stimulant, with published data showing that cannabinoid activation can moderate OM-induced nociceptive responses (3). Our results presented in this report are consistent with those data. The fact that predosing with CB1R-selective agonists substantially prevented OM-induced colitis development argues in favor of this model developing via early neuronal stimulation which continues at least out to 24 h after induction as seen by inhibition of colitis symptoms after initiation of colitis. The presence of CB1R on immune cells, as reported to occur on splenic macrophages (12, 26), cannot be discounted, but CB1R is considered to predominantly exhibit a neuronal expression pattern. Our experiments with CB2R modulators also demonstrate a protective function by this CBR and is consistent with anti-inflammatory CB2R agonist activity in a T. thierriae virus model of MS (1).

DSS colitis has also previously shown to have a neuronal component. For example, reduction of DSS-induced colitis has been reported to occur after treatment by vanilloid receptor 1 antagonists (21, 23). This demonstration of inhibition of DSS colitis by ACEA is consistent with a neuronal element in DSS colitis, as is the report by Massa et al. (33) implicating CB1R in DSS colitis.

The combined data for OM colitis and DSS colitis demonstrate the potential utility for OM colitis as an acute model of experimental colitis. More importantly, these findings support a model in which CBR activation is an important protective feature for preventing colitis and that agents active at either CBR are able to further stimulate or enhance CBR signaling as a protective mechanism.

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REFERENCES


