

Medical Cannabis and Cannabinoids: An Option for the Treatment of Inflammatory Bowel Disease and Cancer of the Colon?

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Abstract

In the past few years, we have witnessed a surge of new reports dealing with the role of cannabinoids, synthetic as well as herbal, in the mechanisms of inflammation and carcinogenesis. However, despite the wealth of in vitro data and anecdotal reports, evidence that cannabinoids could act as beneficial drugs in inflammatory bowel disease (IBD) or in neoplastic development of the human gastrointestinal tract is lacking. Some insight into the effects of medical *Cannabis* (usually meaning dried flowers) and cannabinoids in IBD has been gained through questionnaires and small pilot studies. As to colorectal cancer, only preclinical data are available. Currently, Δ^9 -tetrahydrocannabinol (THC) and its synthetic forms, dronabinol and nabilone, are used as an add-on treatment to alleviate chronic pain and spasticity in multiple sclerosis patients as well as chemotherapy-induced nausea. The use of medical *Cannabis* is authorized only in a limited number of countries. None of the mentioned substances are currently indicated for IBD. This review is an update of our

knowledge on the role of cannabinoids in intestinal inflammation and carcinogenesis and a discussion on their potential therapeutic use.

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Introduction

The beneficial properties of cannabinoids on the gastrointestinal (GI) tract are built on the fact that the intestines are endowed with the endocannabinoid system (ECS), a regulatory network of cannabinoid receptors, enzymes, and ligands that play key roles in physiological as well as pathophysiological processes [1–4]. Thus, the gut expresses the classical cannabinoid receptors 1 and 2 (CB₁, CB₂) and cannabinoid-responsive non-CB₁/CB₂ receptors (G protein-coupled receptor 55 [GPR55], transient receptor potential cation channel subfamily V member 1 [TRPV1], and peroxisome proliferator-activated receptor gamma and alpha [PPAR γ and PPAR α]). Further,

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endocannabinoid (EC)-producing enzymes, such as N-acyl phosphatidylethanolamine-specific phospholipase D and diacylglycerol lipase, and EC-degrading enzymes, such as fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL), are found in the gut. CB₁ and CB₂ localize mainly to enteric nerves, the intestinal epithelium, and immune cells with variable expression [5–7]. While CB₁ is expressed at high levels on cholinergic enteric neurons [8], CB₂ is largely expressed on immune cells [9].

Anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are the best described ECs which activate CB₁, CB₂, and the abovementioned non-CB₁/CB₂ receptors [10–12]. In addition to these ECS components, EC-like lipids, mostly N-acylethanolamines like palmitoylethanolamide (PEA) and oleoylethanolamide, were found in the GI tract. There, they act on non-CB₁/CB₂ receptors like GPR55 and GPR119 and influence the signaling of AEA, also called “entourage effect” [13]. ECs are most likely involved in numerous regulatory mechanisms, e.g., keeping the epithelial barrier intact [14, 15] and maintaining immune tolerance through controlling the expansion of the regulatory T cell subset Tr1 and the presence of immunosuppressive CX3CR1^{hi} macrophages [16]. Not unexpectedly, changes in the levels of ECs and EC-like lipids have been reported in patients with inflammatory bowel disease (IBD) [17] and colorectal cancer (CRC) [18]. However, it is unknown whether these changes actually correlate with the disease progress. With regard to CRC, *in vitro* data from colon cancer cell lines convincingly show antiproliferative effects of cannabinoids [19] and, in fact, models of CRC in knockout mice suggest an anti-oncogenic role for at least CB₁ [20, 21]. With regard to CB₂, however, studies in human CRC patients show that its expression correlates with a decreased survival [22]. In a similar way, CB₁ expression was shown to correlate with a poorer survival rate in stage II microsatellite stable CRC [23]. The reports from human and experimental studies, therefore, are controversial concerning a beneficial role of the ECS in CRC. The following chapters briefly summarize the latest results on the role of the ECS and the action of cannabinoids in IBD and CRC, followed by a discussion of a potential therapy with cannabinoids.

Cannabinoids and IBD

IBD, of which ulcerative colitis and Crohn’s disease (CD) are major manifestations, is characterized by chronic and relapsing inflammatory attacks of the GI tract [24].

Although the detailed mechanisms are still unknown, an uncontrolled and misdirected immune response against microbial antigens combined with genetic predisposition and environmental factors contribute to the multifactorial appearance of the disease [24, 25].

Insights from Animal Models

Functional data to study the role of the cannabinoid receptors and the ECS in IBD are mainly received from animal models, and they have shown that ECS components are altered during experimental intestinal inflammation. Thus, the reports on increased levels of CB₁, CB₂, and AEA suggest an enhanced cannabinoid signaling under inflammatory conditions [26–28]. Pharmacological activation of CB₁ and CB₂ attenuates experimental colitis [28–30], while the use of antagonists or genetic ablation of the cannabinoid receptors aggravates inflammation [26, 31]. Furthermore, genetic deficiency in FAAH or inhibition of FAAH or MGL (and consequently an increase in AEA or 2-AG) proved to be protective against colitis induced by dextran sulfate sodium (DSS) or trinitrobenzene sulfonic acid (TNBS) [31–36].

Several phytocannabinoids as well as synthetic analogs and EC-like compounds have revealed beneficial effects in animal models of intestinal inflammation. The psychotropic Δ^9 -tetrahydrocannabinol (THC) and the nonpsychotropic cannabidiol (CBD) reduced TNBS-induced colitis in the rat colon [37]. Contrary to THC, CBD has extremely low affinity for CB₁ and CB₂, but was shown to have antagonistic effects on GPR55 [11]; CBD was described to act on PPAR γ [38] and TRPV1 and to inhibit FAAH activity, thereby altering EC levels [39, 40]. It was also shown to exert protective effects in the dinitrobenzene sulfonic acid (DNBS)-induced colitis model in mice [41]. More recent work demonstrated that a *Cannabis sativa* extract with high content in CBD rather than CBD alone exerts protection [34]. The CBD analog O-1602, which also lacks affinity to CB₁ and CB₂, but which has agonistic properties on GPR55 [11], was shown to have anti-inflammatory effects in colitis; however, these were not mediated by GPR55 [42]. O-1602 seems to mediate reduction of colonic motility via GPR55 [43]. Work in our own laboratory revealed that GPR55 may be a pro-inflammatory actor since a GPR55 antagonist and the genetic deletion of the GPR55 gene ameliorated DSS colitis in mice [44]. Cannabigerol, a nonpsychotropic phytocannabinoid, was shown to improve murine colitis and to reduce nitric oxide production in macrophages and reactive oxygen species formation in intestinal epithelial cells [45]. Another anti-inflammatory and nonpsychotropic

phytocannabinoid is cannabichromene, which was shown to inhibit EC inactivation [46].

The synthetic, nonselective CB₁/CB₂ agonist WIN 55,212-2 was recently tested in DSS-induced colitis and showed protective and anti-inflammatory properties that seem to be at least partially mediated via inhibition of p38 mitogen-activated protein kinase [47]. Another nonselective cannabinoid receptor agonist recently tested in DSS-induced colitis is HU210 [48]. Previously shown to be protective in the DNBS-induced colitis model [26], this substance maintained the integrity of intestinal barrier function independent of Toll-like receptor 4, but produced extraintestinal anti-inflammatory effects that were dependent on Toll-like receptor 4-mediated p38 mitogen-activated protein kinase activation [48].

The EC-like compound PEA is endogenously produced upon an inflammatory insult and has been described to act through CB₁, CB₂, GPR55, PPAR α , and TRPV1 [49]. In several chemically induced colitis models in mice, PEA has been shown to reduce inflammation [49, 50] and to protect intestinal permeability via PPAR α , CB₂, and GPR55 [49]. In line with these results, inhibition of the PEA-degrading enzyme N-acyl ethanolamine hydrolyzing acid amidase increased PEA levels and also protected against colitis [51]. Furthermore, Sarnelli et al. [52] reported that inhibition of inflammation-associated angiogenesis by PEA in a DSS model was dependent on PPAR α . Additionally, they showed that the release of vascular endothelial growth factor and the formation of vessels were decreased, leading to a reduction in mucosal damage [52]. Recently, the PEA analog adelmidrol was found to exert anti-inflammatory effects that were partly mediated via PPAR γ [53].

In summary, although detailed downstream mechanisms of actions of all these compounds are still missing, preclinical data are promising and suggest that synthetic and herbal cannabinoids, FAAH and MGL inhibitors (and consequently increased levels of ECs and EC-like lipids) may be useful to treat IBD. Nevertheless, it will be challenging to find a targeted treatment especially for long-term use with little side effects, considering that the ECS is present in various tissues throughout the body and is involved in many physiological processes.

Cannabinoids and CRC

Observations of altered expression levels of ECS components in tumor biopsies point at a crucial role of the ECS in the development of CRC. Levels of AEA and 2-AG

as well as of the enzymes responsible for the synthesis and degradation of ECs were increased in CRC lesions, indicating increased EC metabolism [18, 54, 55]. However, compared to adjacent nonneoplastic colonic mucosa, CB₁ expression was found to be downregulated in CRC samples as a consequence of DNA hypermethylation of CpG islands in the promoter region of *CNR1* (the gene coding for CB₁) [20, 21, 56]. Over the last years, a plethora of preclinical studies has been published, reporting possible antitumorigenic properties of (endo-)cannabinoids including antiproliferative, pro-apoptotic, anti-angiogenic, antimigratory, and anti-invasive properties. The molecular mechanisms by which cannabinoids exert their anticarcinogenic effects have been summarized in great detail elsewhere [57–60]. While data from cell-based assays appear very promising, data obtained from in vivo models are scarcer and paint a rather complicated picture. For instance, treatment with the nonselective CB₁/CB₂ agonist HU210 or CBD reduced the development of precancerous lesions in mouse models of chemically induced CRC [61, 62]. Similar results were obtained by increasing the levels of ECs by way of inhibiting the degrading enzymes FAAH and MGL [61, 63]. However, another study reported that blockade of ECS signaling by application of the CB₁ antagonist SR141716 also reduced preneoplastic lesion formation in a mouse model of CRC [64].

Recently, it has also been brought to light that GPR55, an atypical cannabinoid receptor responsive to certain (endo-)cannabinoids, exerts tumor-promoting effects by increasing tumor burden and metastasis in mice [21, 65]. This highlights the notion that the ECS and its related components exert diverse and often opposing functions and that a better understanding of the underlying mechanisms and interactions of the receptors is essential. For instance, it will be necessary to obtain more information on their effects on the immune cell population associated with tumors, i.e., the tumor microenvironment, before cannabinoids can be transferred to the clinic. With cancer immunotherapy now being introduced even for solid tumors [66], it is rather distressing that only a handful of studies have looked at the effects of cannabinoids on the tumor microenvironment as of now. WIN 55,212-2 (a nonselective CB₁/CB₂ agonist) and JWH133 (a CB₂-selective agonist) were reported to have more pronounced antitumor effects on human melanoma xenografts in immunocompetent mice than on those engrafted into SCID mice [67], indicating that antitumorigenic effects by (endo-)cannabinoids are mediated, partly, via immune cells. Another

study, however, showed that THC suppressed host immune reactivity against lung cancer in mice, thereby promoting tumor growth [68]. This finding was corroborated by Hegde et al. [69], who reported that THC administration caused a massive expansion of myeloid-derived suppressor cells, a heterogeneous cell population with potent immunosuppressive properties. Additionally, we have recently shown that the composition of the immune cells present in colorectal tumors of GPR55-deficient mice was altered compared to that in wild-type mice [21].

In conclusion, it seems that the effects of (endo-)cannabinoids in cancer are much more diverse than currently estimated from the promising *in vitro* data. Therefore, we may need more thorough basic research on the interactions of the ECS with the tumor immune compartment before embarking on clinical trials with cannabinoids or medical *Cannabis* as an anticancer treatment.

Are Cannabinoids a Therapeutic Option for Inflammation and Neoplasm of the GI Tract?

Cannabis/Cannabinoids in IBD

The inflamed gut is highly amenable to treatment with cannabinoids, which is documented for human IBD not only by anecdotal reports but, importantly, also by several questionnaire studies [70–73] as well as observational studies and prospective clinical trials [74–77]. Many of the questionnaires have revealed that patients with IBD frequently self-medicate with *Cannabis* to alleviate abdominal pain and diarrhea. However, long-term use of *Cannabis* may not always be beneficial. A survey by Storr et al. [72] showed that *Cannabis* use for more than 6 months was a strong predictor for surgery in patients with CD. Prospective studies also reported mixed results on the efficacy of cannabinoid treatment in patients with IBD. While a small trial in 21 subjects with CD revealed that short-term application (8 weeks) of THC-rich *Cannabis* caused a decrease in Crohn's Disease Activity Index scores in almost all patients [76], another trial showed that a 2-month treatment in moderate CD with the non-psychoactive CBD was not beneficial [78]. Additionally, experiments on human colonic tissues were performed that provide information on whether cannabinoid-based therapy for IBD would be helpful. By using tissue samples of colon from IBD patients and of appendices, Couch et al. [79] demonstrated that incubation with CBD and PEA prevented inflammatory cytokine production via CB₂ and TRPV1 pathways and via PPAR α , respectively.

Another study using explants from IBD patients showed that *Cannabis* extract suppressed cyclooxygenase-2 and metalloproteinase-9 expression [80]. The authors described Δ^9 -tetrahydrocannabinolic acid as an active non-psychoactive anti-inflammatory ingredient of the extract that could be useful for the treatment of IBD instead of CBD.

Taken together, these studies suggest that treatment with *Cannabis* and cannabinoids indeed may alleviate inflammation in IBD, but most likely this is dependent on dosage, mode of application, and consequently the tissue concentration that can be achieved with cannabinoids. As a downside, however, treatment with cannabinoids that activate the CB₁ receptor come with psychotropic side effects. THC and its derivatives may cause dizziness, somnolence, euphoria, and hallucinations as a result of CB₁ activation in the brain [81]. This problem might be circumvented by using peripherally acting cannabinoids, a strategy that seems highly plausible considering the prominent presence of the ECS in the gut. However, GI motility is strongly influenced by central CB₁ [82], and peripherally restricted agonists to CB₁/CB₂ that were applied intraperitoneally failed to improve inflammation in experimental models of intestinal inflammation [83, 84]. Instead, they were protective when applied intracerebroventricularly [84]. In addition, CB₁ deficiency in the vagal nerve slowed GI motility [85]. These observations indicate that peripheral activation of cannabinoid receptors may not be sufficient for improvement of GI inflammation and that central activation of cannabinoid receptors is necessary for a full healing process. The gut-brain axis may play an important role in this process as well.

Another option to minimize psychotropic adverse effects when treating GI inflammation may be by choosing a lower dose of cannabinoids. From preclinical studies it is known that low-dose application of THC has improving effects on, e.g., atherosclerosis [86] and cognitive function [87]. In patients with chronic upper motor neuron syndrome, treatment with 1 mg/day of nabilone significantly decreased pain [88]. Importantly, nabilone was well tolerated in this study. A clinical trial in elderly patients with dementia also revealed that low-dose THC (0.75 mg twice per day) was safe and well tolerated; there were no differences in feeling "high" between the drug and placebo groups [89]. Because of the prominent expression of CB₁ in the brain, enteric nervous system, and intestinal epithelium, low-dose treatment with cannabinoids in IBD may activate cannabinoid receptors both centrally and peripherally, thus contributing to wound

healing, restoration of barrier function, and relaxation of the gut.

Finally, inhibitors of EC-degrading enzymes raise EC levels and are suitable for the treatment of GI inflammation. Based on preclinical results, MGL inhibitors may be promising candidates for the treatment of IBD [32], but also FAAH inhibitors have proven effective in alleviating experimental intestinal inflammation [35]. However, translation of these effects into human GI disease is lagging behind, especially for FAAH inhibitors, as clinical trials were put on halt since the incident with BIA 10-2474 [90]. In addition, the possibility of cardiovascular side effects of FAAH inhibitors might also limit their use in humans [91–93].

Cannabis/Cannabinoids in CRC

Beneficial effects of cannabinoids in CRC are only known from experiments in mice. Knockout of CB₁ in *Apc^{Min/+}* and C57BL/6 mice [20, 21] causes, depending on the model used, increased tumor burden in the small intestines and the colon, indicating a tumor suppressor role of CB₁ in CRC. In contrast, CB₂ has been implicated in the progression of CRC [22, 94], making it difficult to establish a clear rationale for cannabinoid treatment in human CRC. More preclinical data on the role of CB₂ in experimental CRC are therefore necessary to create a basis for the translation of experimental results into the clinic. Since cannabinoids have been approved for the treatment of emesis and nausea in cancer patients undergoing chemotherapy, we may find out from observations in these patients whether anti-emetic cannabinoid treatment influences cancer progression during chemotherapy. In this context, it should be mentioned that a combination of THC with CBD effectively increased cell death and migration of multiple myeloma cell lines in synergy with carfilzomib, a proteasome inhibitor, used for the treatment of multiple myeloma [95]. The topic of cannabinoid therapy in CRC has also been addressed in a recent editorial [63].

References

- 1 Schicho R, Storr M: Targeting the endocannabinoid system for gastrointestinal diseases: future therapeutic strategies. *Expert Rev Clin Pharmacol* 2010;3:193–207.
- 2 Hasenoehrl C, Taschler U, Storr M, Schicho R: The gastrointestinal tract – a central organ of cannabinoid signaling in health and disease. *Neurogastroenterol Motil* 2016;28:1765–1780.
- 3 Alhouayek M, Muccioli GG: The endocannabinoid system in inflammatory bowel diseases: from pathophysiology to therapeutic opportunity. *Trends Mol Med* 2012;18:615–625.
- 4 Sharkey KA, Wiley JW: The role of the endocannabinoid system in the brain-gut axis. *Gastroenterology* 2016;151:252–266.
- 5 Duncan M, Davison JS, Sharkey KA: Review article: endocannabinoids and their receptors in the enteric nervous system. *Aliment Pharmacol Ther* 2005;22:667–683.
- 6 Wright K, Rooney N, Feeney M, Tate J, Robertson D, Welham M, Ward S: Differential expression of cannabinoid receptors in the human colon: cannabinoids promote epithelial wound healing. *Gastroenterology* 2005;129:437–453.

Conclusion

Experimental models of intestinal inflammation demonstrate that treatment with cannabinoids may be helpful. Also in experimental models of CRC, CB₁ was shown to be protective. As to the mechanisms of action, however, it is known that cannabinoids can signal through multiple pathways, most likely in a ligand-biased fashion [96]. Despite the overwhelming amount of data that cannabinoids have anticarcinogenic properties in vitro, in vivo data to support their actions are scarce. Some cannabinoid-responsive receptors, such as GPR55, even have shown pro-inflammatory and procarcinogenic properties. Thus, because of the complexity of mechanisms within the ECS, a beneficial effect of phytocannabinoids and synthetic cannabinoids in humans is not always certain. Additionally, the psychotropic effects of THC and synthetic CB₁ agonists still hamper their broader use, which could be avoided by strategies such as using low-dose cannabinoids with minimal central effects or using peripherally acting cannabinoid receptor ligands. Each approach comes with drawbacks and many questions. Certainly, large clinical studies are needed to create robust guidelines on the safety, effectiveness, dosage, route, form of application, and side effects of cannabinoids. Hopefully, in the near future, clinical data will provide a basis whether *Cannabis* or cannabinoids may become valuable drugs at least for IBD. For CRC, this will certainly take more time.

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Disclosure Statement

The authors have no conflict of interest to disclose.

- 7 Marquez L, Suarez J, Iglesias M, Bermudez-Silva FJ, Rodriguez de Fonseca F, Andreu M: Ulcerative colitis induces changes on the expression of the endocannabinoid system in the human colonic tissue. *PLoS One* 2009;4:e6893.
- 8 Sibaev A, Yuce B, Kemmer M, Van Nassauw L, Broedl U, Allescher HD, Goke B, Timmermans JP, Storr M: Cannabinoid-1 (CB1) receptors regulate colonic propulsion by acting at motor neurons within the ascending motor pathways in mouse colon. *Am J Physiol Gastrointest Liver Physiol* 2009;296:G119–G128.
- 9 Schmole AC, Lundt R, Gennequin B, Schrage H, Beins E, Kramer A, Zimmer T, Limmer A, Zimmer A, Otte DM: Expression analysis of CB2-GFP BAC transgenic mice. *PLoS One* 2015;10:e0138986.
- 10 Ross RA: Anandamide and vanilloid TRPV1 receptors. *Br J Pharmacol* 2003;140:790–801.
- 11 Ryberg E, Larsson N, Sjogren S, Hjorth S, Hermansson NO, Leonova J, Elebring T, Nilsson K, Drmota T, Greasley PJ: The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* 2007;152:1092–1101.
- 12 O'Sullivan SE: An update on PPAR activation by cannabinoids. *Br J Pharmacol* 2016;173:1899–1910.
- 13 Sharir H, Console-Bram L, Mundy C, Popoff SN, Kapur A, Abood ME: The endocannabinoids anandamide and virodhamine modulate the activity of the candidate cannabinoid receptor GPR55. *J Neuroimmune Pharmacol* 2012;7:856–865.
- 14 Cani PD, Plovier H, Van Hul M, Geurts L, Delzenne NM, Druart C, Everard A: Endocannabinoids – at the crossroads between the gut microbiota and host metabolism. *Nat Rev Endocrinol* 2016;12:133–143.
- 15 Karwad MA, Couch DG, Theophilidou E, Sarmad S, Barrett DA, Larvin M, Wright KL, Lund JN, O'Sullivan SE: The role of CB1 in intestinal permeability and inflammation. *FASEB J* 2017;31:3267–3277.
- 16 Acharya N, Penukonda S, Shcheglova T, Hagymasi AT, Basu S, Srivastava PK: Endocannabinoid system acts as a regulator of immune homeostasis in the gut. *Proc Natl Acad Sci USA* 2017;114:5005–5010.
- 17 Di Sabatino A, Battista N, Biancheri P, Rapino C, Rovedatti L, Astarita G, Vanoli A, Dainese E, Guerri M, Piomelli D, Pender SL, MacDonald TT, Maccarrone M, Corazza GR: The endogenous cannabinoid system in the gut of patients with inflammatory bowel disease. *Mucosal Immunol* 2011;4:574–583.
- 18 Chen L, Chen H, Li Y, Li L, Qiu Y, Ren J: Endocannabinoid and ceramide levels are altered in patients with colorectal cancer. *Oncol Rep* 2015;34:447–454.
- 19 Izzo AA, Muccioli GG, Ruggieri MR, Schicho R: Endocannabinoids and the digestive tract and bladder in health and disease. *Handb Exp Pharmacol* 2015;231:423–447.
- 20 Wang D, Wang H, Ning W, Backlund MG, Dey SK, DuBois RN: Loss of cannabinoid receptor 1 accelerates intestinal tumor growth. *Cancer Res* 2008;68:6468–6476.
- 21 Hasenoehrl C, Feuersinger D, Sturm EM, Barnthaler T, Heitzer E, Graf R, Grill M, Pichler M, Beck S, Butcher L, Thomas D, Ferreiros N, Schuligoi R, Schweiger C, Haybaeck J, Schicho R: G protein-coupled receptor GPR55 promotes colorectal cancer and has opposing effects to cannabinoid receptor 1. *Int J Cancer* 2018;142:121–132.
- 22 Martinez-Martinez E, Gomez I, Martin P, Sanchez A, Roman L, Tejerina E, Bonilla F, Merino AG, de Herrerros AG, Provencio M, Garcia JM: Cannabinoids receptor type 2, CB2, expression correlates with human colon cancer progression and predicts patient survival. *Oncoscience* 2015;2:131–141.
- 23 Gustafsson SB, Palmqvist R, Henriksson ML, Dahlin AM, Edin S, Jacobsson SO, Oberg A, Fowler CJ: High tumour cannabinoid CB1 receptor immunoreactivity negatively impacts disease-specific survival in stage II microsatellite stable colorectal cancer. *PLoS One* 2011;6:e23003.
- 24 Strober W, Fuss I, Mannon P: The fundamental basis of inflammatory bowel disease. *J Clin Invest* 2007;117:514–521.
- 25 Engel MA, Neurath MF: New pathophysiological insights and modern treatment of IBD. *J Gastroenterol* 2010;45:571–583.
- 26 Massa F, Marsicano G, Hermann H, Cannich A, Monory K, Cravatt BF, Ferri GL, Sibaev A, Storr M, Lutz B: The endogenous cannabinoid system protects against colonic inflammation. *J Clin Invest* 2004;113:1202–1209.
- 27 D'Argenio G, Valenti M, Scaglione G, Cosenza V, Sorrentini I, Di Marzo V: Up-regulation of anandamide levels as an endogenous mechanism and a pharmacological strategy to limit colon inflammation. *FASEB J* 2006;20:568–570.
- 28 Kimball ES, Schneider CR, Wallace NH, Hornby PJ: 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.
- 29 Storr MA, Keenan CM, Zhang H, Patel KD, Makriyannis A, Sharkey KA: Activation of the cannabinoid 2 receptor (CB2) protects against experimental colitis. *Inflamm Bowel Dis* 2009;15:1678–1685.
- 30 Singh UP, Singh NP, Singh B, Price RL, Nagarkatti M, Nagarkatti PS: Cannabinoid receptor-2 (CB2) agonist ameliorates colitis in IL-10(–/–) mice by attenuating the activation of T cells and promoting their apoptosis. *Toxicol Appl Pharmacol* 2012;258:256–267.
- 31 Storr MA, Keenan CM, Emmerding D, Zhang H, Yuce B, Sibaev A, Massa F, Buckley NE, Lutz B, Goke B, Brand S, Patel KD, Sharkey KA: Targeting endocannabinoid degradation protects against experimental colitis in mice: involvement of CB1 and CB2 receptors. *J Mol Med (Berl)* 2008;86:925–936.
- 32 Alhouayek M, Lambert DM, Delzenne NM, Cani PD, Muccioli GG: Increasing endogenous 2-arachidonoylglycerol levels counteracts colitis and related systemic inflammation. *FASEB J* 2011;25:2711–2721.
- 33 Salaga M, Mokrowiecka A, Zakrzewski PK, Cygankiewicz A, Leishman E, Sobczak M, Zatorski H, Malecka-Panas E, Kordek R, Storr M, Krajewska WM, Bradshaw HB, Fichna J: Experimental colitis in mice is attenuated by changes in the levels of endocannabinoid metabolites induced by selective inhibition of fatty acid amide hydrolase (FAAH). *J Crohns Colitis* 2014;8:998–1009.
- 34 Pagano E, Capasso R, Piscitelli F, Romano B, Parisi OA, Finizio S, Lauritano A, Marzo VD, Izzo AA, Borrelli F: An orally active *Cannabis* extract with high content in cannabidiol attenuates chemically-induced intestinal inflammation and hypermotility in the mouse. *Front Pharmacol* 2016;7:341.
- 35 Shamran H, Singh NP, Zumbun EE, Murphy A, Taub DD, Mishra MK, Price RL, Chatterjee S, Nagarkatti M, Nagarkatti PS, Singh UP: Fatty acid amide hydrolase (FAAH) blockade ameliorates experimental colitis by altering microRNA expression and suppressing inflammation. *Brain Behav Immun* 2017;59:10–20.
- 36 Zhao X, Liang P, Liu J, Jiang H, Fan X, Chen G, Zhou C: Elevation of arachidonoyl ethanolamide levels by activation of the endocannabinoid system protects against colitis and ameliorates remote organ lesions in mice. *Exp Ther Med* 2017;14:5664–5670.
- 37 Jamontt JM, Molleman A, Pertwee RG, Parsons ME: The effects of delta-tetrahydrocannabinol and cannabidiol alone and in combination on damage, inflammation and in vitro motility disturbances in rat colitis. *Br J Pharmacol* 2010;160:712–723.
- 38 De Filippis D, Esposito G, Cirillo C, Cipriano M, De Winter BY, Scuderi C, Sarnelli G, Cuomo R, Steardo L, De Man JG, Iuvone T: Cannabidiol reduces intestinal inflammation through the control of neuroimmune axis. *PLoS One* 2011;6:e28159.
- 39 Bisogno T, Hanus L, De Petrocellis L, Tchilibon S, Ponde DE, Brandi I, Moriello AS, Davis JB, Mechoulam R, Di Marzo V: Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *Br J Pharmacol* 2001;134:845–852.
- 40 De Petrocellis L, Ligresti A, Moriello AS, Allara M, Bisogno T, Petrosino S, Stott CG, Di Marzo V: Effects of cannabinoids and cannabinoid-enriched *Cannabis* extracts on TRP channels and endocannabinoid metabolic enzymes. *Br J Pharmacol* 2011;163:1479–1494.
- 41 Borrelli F, Aviello G, Romano B, Orlando P, Capasso R, Maiello F, Guadagno F, Petrosino S, Capasso F, Di Marzo V, Izzo AA: Cannabidiol, a safe and non-psychoactive ingredient of the marijuana plant *Cannabis sativa*, is protective in a murine model of colitis. *J Mol Med (Berl)* 2009;87:1111–1121.

- 42 Schicho R, Bashashati M, Bawa M, McHugh D, Saur D, Hu HM, Zimmer A, Lutz B, Mackie K, Bradshaw HB, McCafferty DM, Sharkey KA, Storr M: The atypical cannabinoid O-1602 protects against experimental colitis and inhibits neutrophil recruitment. *Inflamm Bowel Dis* 2011;17:1651–1664.
- 43 Li K, Fichna J, Schicho R, Saur D, Bashashati M, Mackie K, Li Y, Zimmer A, Goke B, Sharkey KA, Storr M: A role for O-1602 and G protein-coupled receptor GPR55 in the control of colonic motility in mice. *Neuropharmacology* 2013;71:255–263.
- 44 Stancic A, Jandl K, Hasenohrl C, Reichmann F, Marsche G, Schuligoi R, Heinemann A, Storr M, Schicho R: The GPR55 antagonist CID16020046 protects against intestinal inflammation. *Neurogastroenterol Motil* 2015;27:1432–1445.
- 45 Borrelli F, Fasolino I, Romano B, Capasso R, Maiello F, Coppola D, Orlando P, Battista G, Pagano E, Di Marzo V, Izzo AA: Beneficial effect of the non-psychotropic plant cannabinoid cannabigerol on experimental inflammatory bowel disease. *Biochem Pharmacol* 2013;85:1306–1316.
- 46 Romano B, Borrelli F, Fasolino I, Capasso R, Piscitelli F, Cascio M, Pertwee R, Coppola D, Vassallo L, Orlando P, Di Marzo V, Izzo A: The cannabinoid TRPA1 agonist cannabichromene inhibits nitric oxide production in macrophages and ameliorates murine colitis. *Br J Pharmacol* 2013;169:213–229.
- 47 Feng YJ, Li YY, Lin XH, Li K, Cao MH: Anti-inflammatory effect of cannabinoid agonist WIN55, 212 on mouse experimental colitis is related to inhibition of p38MAPK. *World J Gastroenterol* 2016;22:9515–9524.
- 48 Lin S, Li Y, Shen L, Zhang R, Yang L, Li M, Li K, Fichna J: The anti-inflammatory effect and intestinal barrier protection of HU210 differentially depend on TLR4 signaling in dextran sulfate sodium-induced murine colitis. *Dig Dis Sci* 2017;62:372–386.
- 49 Borrelli F, Romano B, Petrosino S, Pagano E, Capasso R, Coppola D, Battista G, Orlando P, Di Marzo V, Izzo AA: Palmitoylethanolamide, a naturally occurring lipid, is an orally effective intestinal anti-inflammatory agent. *Br J Pharmacol* 2015;172:142–158.
- 50 Esposito G, Capocchia E, Turco F, Palumbo I, Lu J, Steardo A, Cuomo R, Sarnelli G, Steardo L: Palmitoylethanolamide improves colon inflammation through an enteric glia/toll like receptor 4-dependent PPAR- α activation. *Gut* 2014;63:1300–1312.
- 51 Alhouayek M, Botteman P, Subramanian KV, Lambert DM, Makriyannis A, Cani PD, Muccioli GG: N-Acylethanolamine-hydrolyzing acid amidase inhibition increases colon N-palmitoylethanolamine levels and counteracts murine colitis. *FASEB J* 2015;29:650–661.
- 52 Sarnelli G, D'Alessandro A, Iuvone T, Capocchia E, Gigli S, Pesce M, Seguela L, Nobile N, Aprea G, Maione F, de Palma GD, Cuomo R, Steardo L, Esposito G: Palmitoylethanolamide Modulates inflammation-associated vascular endothelial growth factor (VEGF) signaling via the Akt/mTOR pathway in a selective peroxisome proliferator-activated receptor alpha (PPAR- α)-dependent manner. *PLoS One* 2016;11:e0156198.
- 53 Cordaro M, Impellizzeri D, Gugliandolo E, Siracusa R, Crupi R, Esposito E, Cuzzocrea S: Adelmidrol, a palmitoylethanolamide analogue, as a new pharmacological treatment for the management of inflammatory bowel disease. *Mol Pharmacol* 2016;90:549–561.
- 54 Ligresti A, Bisogno T, Matias I, De Petrocellis L, Cascio MG, Cosenza V, D'Argenio G, Scaglione G, Bifulco M, Sorrentini I, Di Marzo V: Possible endocannabinoid control of colorectal cancer growth. *Gastroenterology* 2003;125:677–687.
- 55 Ye L, Zhang B, Seviour EG, Tao KX, Liu XH, Ling Y, Chen JY, Wang GB: Monoacylglycerol lipase (MAGL) knockdown inhibits tumor cells growth in colorectal cancer. *Cancer Lett* 2011;307:6–17.
- 56 Cianchi F, Papucci L, Schiavone N, Lulli M, Magnelli L, Vinci MC, Messerini L, Manera C, Ronconi E, Romagnani P, Donnini M, Perigli G, Trallori G, Tanganelli E, Capaccioli S, Masini E: Cannabinoid receptor activation induces apoptosis through tumor necrosis factor alpha-mediated ceramide de novo synthesis in colon cancer cells. *Clin Cancer Res* 2008;14:7691–7700.
- 57 Velasco G, Sanchez C, Guzman M: Towards the use of cannabinoids as antitumor agents. *Nat Rev Cancer* 2012;12:436–444.
- 58 Pisanti S, Picardi P, D'Alessandro A, Laezza C, Bifulco M: The endocannabinoid signaling system in cancer. *Trends Pharmacol Sci* 2013;34:273–282.
- 59 Velasco G, Sanchez C, Guzman M: Anticancer mechanisms of cannabinoids. *Curr Oncol* 2016;23:S23–S32.
- 60 Schwarz R, Ramer R, Hinz B: Targeting the endocannabinoid system as a potential anticancer approach. *Drug Metab Rev* 2018;50:26–53.
- 61 Izzo AA, Aviello G, Petrosino S, Orlando P, Marsicano G, Lutz B, Borrelli F, Capasso R, Nigam S, Capasso F, Di Marzo V; Endocannabinoid Research Group: Increased endocannabinoid levels reduce the development of precancerous lesions in the mouse colon. *J Mol Med (Berl)* 2008;86:89–98.
- 62 Aviello G, Romano B, Borrelli F, Capasso R, Gallo L, Piscitelli F, Di Marzo V, Izzo AA: Chemopreventive effect of the non-psychotropic phytocannabinoid cannabidiol on experimental colon cancer. *J Mol Med (Berl)* 2012;90:925–934.
- 63 Pagano E, Borrelli F: Targeting cannabinoid receptors in gastrointestinal cancers for therapeutic uses: current status and future perspectives. *Expert Rev Gastroenterol Hepatol* 2017;11:871–873.
- 64 Santoro A, Pisanti S, Grimaldi C, Izzo AA, Borrelli F, Proto MC, Malfitano AM, Gazerro P, Laezza C, Bifulco M: Rimonabant inhibits human colon cancer cell growth and reduces the formation of precancerous lesions in the mouse colon. *Int J Cancer* 2009;125:996–1003.
- 65 Kargl J, Andersen L, Hasenohrl C, Feuersinger D, Stancic A, Fauland A, Magnes C, El-Heliebi A, Lax S, Uranitsch S, Haybaeck J, Heinemann A, Schicho R: GPR55 promotes migration and adhesion of colon cancer cells indicating a role in metastasis. *Br J Pharmacol* 2016;173:142–154.
- 66 Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Lubner BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huelbner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B, Diaz LA Jr: PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med* 2015;372:2509–2520.
- 67 Blazquez C, Carracedo A, Barrado L, Real PJ, Fernandez-Luna JL, Velasco G, Malumbres M, Guzman M: Cannabinoid receptors as novel targets for the treatment of melanoma. *FASEB J* 2006;20:2633–2635.
- 68 Zhu LX, Sharma S, Stolina M, Gardner B, Roth MD, Tashkin DP, Dubinett SM: Delta-9-tetrahydrocannabinol inhibits antitumor immunity by a CB2 receptor-mediated, cytokine-dependent pathway. *J Immunol* 2000;165:373–380.
- 69 Hegde VL, Nagarkatti M, Nagarkatti PS: Cannabinoid receptor activation leads to massive mobilization of myeloid-derived suppressor cells with potent immunosuppressive properties. *Eur J Immunol* 2010;40:3358–3371.
- 70 Lal S, Prasad N, Ryan M, Tangri S, Silverberg MS, Gordon A, Steinhart H: Cannabis use amongst patients with inflammatory bowel disease. *Eur J Gastroenterol Hepatol* 2011;23:891–896.
- 71 Ravikoff Allegretti J, Courtwright A, Lucci M, Korzenik JR, Levine J: Marijuana use patterns among patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2013;19:2809–2814.
- 72 Storr M, Devlin S, Kaplan GG, Panaccione R, Andrews CN: Cannabis use provides symptom relief in patients with inflammatory bowel disease but is associated with worse disease prognosis in patients with Crohn's disease. *Inflamm Bowel Dis* 2014;20:472–480.
- 73 Phatak UP, Rojas-Velasquez D, Porto A, Pashankar DS: Prevalence and patterns of marijuana use in young adults with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2017;64:261–264.
- 74 Lahat A, Lang A, Ben-Horin S: Impact of cannabis treatment on the quality of life, weight and clinical disease activity in inflammatory bowel disease patients: a pilot prospective study. *Digestion* 2012;85:1–8.
- 75 Naftali T, Lev LB, Yablecovitch D, Half E, Konikoff FM: Treatment of Crohn's disease with cannabis: an observational study. *Isr Med Assoc J* 2011;13:455–458.

- 76 Naftali T, Bar-Lev Schleider L, Dotan I, Lansky EP, Sklerovsky Benjaminov F, Konikoff FM: Cannabis induces a clinical response in patients with Crohn's disease: a prospective placebo-controlled study. *Clin Gastroenterol Hepatol* 2013;11:1276–1280.e1.
- 77 Weiss A, Friedenberg F: Patterns of cannabis use in patients with inflammatory bowel disease: a population based analysis. *Drug Alcohol Depend* 2015;156:84–89.
- 78 Naftali T, Mechulam R, Marii A, Gabay G, Stein A, Bronshtain M, Laish I, Benjaminov F, Konikoff FM: Low-dose cannabidiol is safe but not effective in the treatment for Crohn's disease, a randomized controlled trial. *Dig Dis Sci* 2017;62:1615–1620.
- 79 Couch DG, Tasker C, Theophilidou E, Lund JN, O'Sullivan SE: Cannabidiol and palmitoylethanolamide are anti-inflammatory in the acutely inflamed human colon. *Clin Sci (Lond)* 2017;131:2611–2626.
- 80 Nallathambi R, Mazuz M, Ion A, Selvaraj G, Weininger S, Fridlender M, Nasser A, Sagee O, Kumari P, Nemichenizer D, Mendelovitz M, Firstein N, Hanin O, Konikoff F, Kapulnik Y, Naftali T, Koltai H: Anti-inflammatory activity in colon models is derived from Δ^9 -tetrahydrocannabinolic acid that interacts with additional compounds in *Cannabis* extracts. *Cannabis Cannabinoid Res* 2017;2:167–182.
- 81 Whiting PF, Wolff RF, Deshpande S, Di Nisio M, Duffy S, Hernandez AV, Keurentjes JC, Lang S, Misso K, Ryder S, Schmidtkofer S, Westwood M, Kleijnen J: Cannabinoids for medical use: a systematic review and meta-analysis. *JAMA* 2015;313:2456–2473.
- 82 Izzo AA, Pinto L, Borrelli F, Capasso R, Mascolo N, Capasso F: Central and peripheral cannabinoid modulation of gastrointestinal transit in physiological states or during the diarrhoea induced by croton oil. *Br J Pharmacol* 2000;129:1627–1632.
- 83 Cluny NL, Keenan CM, Duncan M, Fox A, Lutz B, Sharkey KA: Naphthalen-1-yl-(4-pentylxynaphthalen-1-yl)methanone (SAB378), a peripherally restricted cannabinoid CB1/CB2 receptor agonist, inhibits gastrointestinal motility but has no effect on experimental colitis in mice. *J Pharmacol Exp Ther* 2010;334:973–980.
- 84 Fichna J, Bawa M, Thakur GA, Tichkule R, Makriyannis A, McCafferty DM, Sharkey KA, Storr M: Cannabinoids alleviate experimentally induced intestinal inflammation by acting at central and peripheral receptors. *PLoS One* 2014;9:e109115.
- 85 Vianna CR, Donato J Jr, Rossi J, Scott M, Economides K, Gautron L, Pierpont S, Elias CF, Elmquist JK: Cannabinoid receptor 1 in the vagus nerve is dispensable for body weight homeostasis but required for normal gastrointestinal motility. *J Neurosci* 2012;32:10331–10337.
- 86 Steffens S, Veillard NR, Arnaud C, Pelli G, Burger F, Staub C, Karsak M, Zimmer A, Frossard JL, Mach F: Low dose oral cannabinoid therapy reduces progression of atherosclerosis in mice. *Nature* 2005;434:782–786.
- 87 Bilkei-Gorzo A, Albayram O, Draffehn A, Michel K, Piyanova A, Oppenheimer H, Dvir-Ginzberg M, Racz I, Ulas T, Imbeault S, Bab I, Schultze JL, Zimmer A: A chronic low dose of Δ^9 -tetrahydrocannabinol (THC) restores cognitive function in old mice. *Nat Med* 2017;23:782–787.
- 88 Wissel J, Haydn T, Muller J, Brenneis C, Berger T, Poewe W, Schelosky LD: Low dose treatment with the synthetic cannabinoid nabilone significantly reduces spasticity-related pain: a double-blind placebo-controlled cross-over trial. *J Neurol* 2006;253:1337–1341.
- 89 Ahmed AI, van den Elsen GA, Colbers A, Kramers C, Burger DM, van der Marck MA, Olde Rikkert MG: Safety, pharmacodynamics, and pharmacokinetics of multiple oral doses of delta-9-tetrahydrocannabinol in older persons with dementia. *Psychopharmacology (Berl)* 2015;232:2587–2595.
- 90 Butler D, Callaway E: Scientists in the dark after French clinical trial proves fatal. *Nature* 2016;529:263–264.
- 91 Mukhopadhyay P, Horvath B, Rajesh M, Matsumoto S, Saito K, Batkai S, Patel V, Tanchian G, Gao RY, Cravatt BF, Hasko G, Pacher P: Fatty acid amide hydrolase is a key regulator of endocannabinoid-induced myocardial tissue injury. *Free Radic Biol Med* 2011;50:179–195.
- 92 Lenglet S, Thomas A, Soehnlein O, Montecucco F, Burger F, Pelli G, Galan K, Cravatt B, Staub C, Steffens S: Fatty acid amide hydrolase deficiency enhances intraplaque neutrophil recruitment in atherosclerotic mice. *Arterioscler Thromb Vasc Biol* 2013;33:215–223.
- 93 Hoyer FF, Khoury M, Slomka H, Kebschull M, Lerner R, Lutz B, Schott H, Lutjohann D, Wojtalla A, Becker A, Zimmer A, Nickenig G: Inhibition of endocannabinoid-degrading enzyme fatty acid amide hydrolase increases atherosclerotic plaque vulnerability in mice. *J Mol Cell Cardiol* 2014;66:126–132.
- 94 Martinez-Martinez E, Martin-Ruiz A, Martin P, Calvo V, Provencio M, Garcia JM: CB2 cannabinoid receptor activation promotes colon cancer progression via AKT/GSK3 β signaling pathway. *Oncotarget* 2016;7:68781–68791.
- 95 Nabissi M, Morelli MB, Offidani M, Amanitini C, Gentili S, Soriani A, Cardinali C, Leoni P, Santoni G: Cannabinoids synergize with carfilzomib, reducing multiple myeloma cells viability and migration. *Oncotarget* 2016;7:77543–77557.
- 96 Ibsen MS, Connor M, Glass M: Cannabinoid CB1 and CB2 receptor signaling and bias. *Cannabis Cannabinoid Res* 2017;2:48–60.