REVIEW ARTICLE

The Important Role of the Endocannabinoid System and the Endocannabinoidome in Gut Health

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ABSTRACT

The endocannabinoid system is an endogenous pathway comprised of the cannabinoid receptors 1 and 2 (CB_1 and CB_2), their endogenous ligands known as endocannabinoids, and the enzymes responsible for their synthesis and degradation. The endocannabinoidome extends this system to include other receptors such as TRPV1, PPARα, GPR55 and 5-HT_{1A}. An extensive amount of research is now linking the endocannabinoidome to intestinal health through fascinating mechanisms that include endocannabinoid receptor expression in the gut and interplay with the intestinal microbiota. A dysregulated endocannabinoid system may lead to inflammatory bowel disease and colon cancer. (*Altern Ther Health Med.* 2019;25(S2):24-27.)

Since ancient times, *Cannabis sativa* has been used effectively by individuals with gastrointestinal disorders. However, in comparison, it is only relatively recently that the mechanism of action behind the beneficial gastrointestinal effect of cannabis and other non-psychoactive phytocannabinoids such as cannabidiol (CBD) and cannabigerol (CBG) have been investigated. These compounds act upon receptors that play a critical role in the endocannabinoid system expression in the gut. This commentary will discuss the endocannabinoid system's role in gut health including its influence on the microbiota and the potential benefits of its modulation in the intestines. The intestinal involvement of the endocannabinoid system in inflammatory bowel disease, colon cancer, and even atherosclerosis will be reviewed.

THE ENDOCANNABINOID SYSTEM IN THE GUT

Findings that the receptors involved in the endocannabinoid system are expressed in the gastrointestinal tract indicate that this system is intricately involved in gut health and intestinal disorders. The endocannabinoid system is comprised of the cannabinoid receptors 1 and 2 (CB_1 and CB_2), their endogenous ligands known as endocannabinoids, and the enzymes responsible for their synthesis and degradation.[1] The two primary endocannabinoids are anandamide and 2-arachidonoylglycerol (2-AG). In addition to the CB_1 and CB_2 receptors, other receptors such as transient receptor potential cation channel subfamily V member 1 (TRPV1), peroxisome proliferator-activated receptor alpha (PPARα), and G-protein coupled receptor 55 (GPR55) and others are involved in the endocannabinoid system, including its actions in the gut.[1]

CB_1 receptors are present throughout the GI tract of animals and healthy humans, especially in the enteric nervous system (ENS)[2] and the epithelial lining,[3] as well as smooth muscle cells of blood vessels in the colonic wall.[4] CB_2 receptors are expressed primarily on the epithelium of human colonic tissue associated with inflammatory bowel disease (IBD).[3,5] Activation of the CB_2 receptor in these patients may ameliorate colitis-related inflammation.[3,5] Furthermore, in rats exposed to lipopolysaccharides (LPS), increased endotoxin-related intestinal transit was inhibited by activation of CB_2 receptors.[6]

The non-CB receptors GPR55 and TRPV1, which are also present in the GI tract, may play an important role in non-CB_1/CB_2 receptor effects of cannabinoids in the gut. An abundance of evidence indicates that the endocannabinoid anandamide activates TRPV1 and GPR55 receptors.[7,8] The receptors PPARα and PPARγ, which are also present in the GI tract, are activated by phytocannabinoids, synthetic cannabinoids, and endogenous cannabinoids and may regulate many of the analgesic and anti-inflammatory effects of cannabinoid treatment.[9] For this reason, these non-CB receptors are considered to be a component of an expanded endocannabinoid system.[1] The term endocannabinoidome has been used to refer to the combination of the direct

components of the endocannabinoid system as well as the non-CB receptors involved in its function.[10]

Palmitoylethanolamide (PEA) is another indirect component of the endocannabinoid system. It is an endocannabinoid-like compound, but does not directly activate CB receptors. However, it can activate GPR55 and thus can regulate the signaling of anandamide.[11] PEA also activates PPARα and is found in high levels within the gut.[1]

Other support for the role of the endocannabinoid system in gut health is the many beneficial effects of the phytocannabinoid cannabidiol on mental health. Some of these effects are mediated by the serotonin receptor 5-HT_{1A}.[12] As 90% of the body's serotonin is synthesized in the gut, it is logical that the endocannabinoid system may interact with this receptor in the gastrointestinal tract and play a role in the gut-brain axis. Furthermore, systemic administration of serotonin in a murine model of colitis led to an exacerbation of abdominal pain through a mechanism that involved the endocannabinoid system.[13]

THE MICROBIOTA AND THE ENDOCANNABINOID SYSTEM

The endocannabinoid system interacts with the gut microbiota on a number of levels to influence numerous areas of health. These include obesity, atherosclerosis, and visceral hypersensitivity.

Obesity

The means by which the gut microbiota can influence obesity is mediated through the endocannabinoid system. Obesity is associated with altered gut microbiota, low-grade inflammation, impaired intestinal permeability, and upregulated endocannabinoid system tone (increased endocannabinoid plasma concentrations, changes in the expression of CB_1, and elevated endocannabinoid levels in adipose tissue).[14] Muccioli and colleagues found that the gut microbiota regulates the intestinal endocannabinoid system tone, which in turn influences gut permeability and plasma lipopolysaccharide (LPS) levels.[14] Elevated LPS levels are associated with inflammation and obesity. The study authors also found that altering the gut microbiota of obese mice decreased obesity and CB_1 mRNA expression as well as levels of anandamide in adipose tissue.[14] Conversely, activating the endocannabinoid system in lean mice caused obesity and elevated plasma LPS levels.[14]

The endocannabinoid 2-AG and endocannabinoid-like PEA trigger an increase in epithelial barrier function while anandamide acts as a "gate opener."[15] Alterations of the microbiota associated with obesity correlate with elevated concentrations of anandamide in the intestines, which increases gut permeability.[14] Consequently, the endocannabinoid system may regulate the systemic translocation of microbial products from the intestines and the ensuing pathogenesis of metabolic diseases.[15] Endocannabinoids can also alter the composition of the gut microbiota. For example, removing the endocannabinoid synthesizing enzyme, NAPE-PLD, from adipocytes leads to gut dysbiosis, resulting in increased body weight gain and fat mass.[16]

Atherosclerosis

Through activation of CB_1 and CB_2, endocannabinoids have cardioprotective properties, such as reduced neutrophil infiltration, inflammation, and oxidative stress and upregulation of signaling pathways beneficial to the cardiovascular system.[17] These properties may be the result of expression of the CB_1 and CB_2 receptors in the myocardium and endothelial and smooth muscle cells[18,19] and CB_2 receptors in coronary arteries.[19]

An in-depth discussion of the endocannabinoid system's role in cardiovascular health is outside the scope of this commentary. However, relevant to the topic of gastrointestinal health and the endocannabinoid system, it is worth noting that atherosclerosis is associated with a change in endocannabinoid system tone. As noted earlier, the gut microbiota can control endocannabinoid system tone.[14] Additionally, as previously mentioned, the endocannabinoid system can control gut permeability and influence obesity, two mechanisms involved in atherosclerosis, and modulating the gut microbiota through probiotic administration significantly affects these mechanisms.[14] This illustrates the wide impact the endocannabinoid system has on health and that it may be an integral player in a gut-cardiovascular axis.

Visceral Hypersensitivity

Visceral hypersensitivity also is correlated with the gut microbiota and the endocannabinoid system. The alteration in the composition of the gut microbiota known as dysbiosis is thought to play a critical role in the etiology of IBD, a disorder in which visceral hypersensitivity plays a prominent role. Administration of *Lactobacillus acidophilus* NCFM reduced pain in an animal model of chronic colonic hypersensitivity through a mechanism involving upregulation of CB_2 receptors.[20] This same study found that *Lactobacillus* strains yielded analgesic effects in the gut-similar to those achieved with morphine.[20] In another rodent study, mice treated with antibiotics experienced reduced pain along with a small elevation in CB_2 receptor activation in colon tissue and a decrease in CB1 receptor activity.[21] Furthermore, total luminal bacterial counts were associated with CB receptor expression,[21] supporting the concept that gut microbes interact with CB receptors. However, these results were not replicated in a clinical trial, where colonic mucosal biopsies from individuals given *Lactobacillus acidophilus* NCFM over 21 days did not exhibit upregulated CB_2 receptors.[22] This study did not investigate the effect of *L. acidophilus* on CB_1 receptors.[22]

Dysfunctions in the endocannabinoid system may also be responsible for the correlation between chronic psychological stress and abdominal pain.[23] In rodents, early-life stress alters the endocannabinoid system, which makes the animals more susceptible to IBS.[24] Anandamide

levels fall during chronic stress while 2-AG concentrations are elevated in the brain and CB_1 receptors are downregulated in sensory ganglia, which regulate visceral pain.[25]

THE ROLE OF THE ENDOCANNABINOID SYSTEM IN INTESTINAL INFLAMMATION

The endocannabinoid system is a regulator of intestinal inflammation. Cannabinoids assist with the recruitment of immune cells to the site of intestinal inflammation[26,27] and inhibit synthesis of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β).[28,29] Furthermore, research indicates that the endocannabinoid system is altered in IBD. Massa and colleagues found that the CB_1 receptor counteracts proinflammatory responses in the colons of mice.[30] Grill and associates noted that levels of anandamide, PEA, and oleoylethanolamide (OEA) were significantly increased in the plasma of patients with ulcerative colitis (UC) and Crohn's disease (CD), although PEA fell just short of significance in UC and CD.[10] The other primary endocannabinoid, 2-AG, was also increased in IBD patients compared with controls, but was only significant in CD and not UC.[10] These researchers also investigated whether any of the endocannabinoids would correlate with CD and UC severity and discovered that only OEA levels were associated with the Harvey Bradshaw Index (HBI) index in CD patients but not with the total Mayo score, which is an indicator of UC severity. Concentrations of 2-AG were negatively associated with the HBI of CD patients, but not with the total Mayo score of subjects with UC. Genes from endocannabinoid and endocannabinoid-like lipid synthesizing and degrading enzymes were also affected in IBD.[10] Differential patterns of expression of these genes was observed in intestinal mucosal biopsies from patients with UC and CD. These differences in expression were more notable in patients with CD compared with UC.10 Furthermore, the mechanism by which serotonin exacerbates abdominal pain in mice with induced colitis was found to be associated with reduced anandamide levels and downregulation of CB_1 receptors.[13] PEA has also been shown to suppress inflammation markers in a mouse model of inflammatory bowel disease (IBD).[31]

The evidence pointing to the endocannabinoid system as a key player in the gut-brain axis is clearly demonstrated in functional bowel disorders. The association between these disorders and psychological comorbidities such as depression may be mediated by the endocannabinoid system among other signaling pathways.[32] Anandamide and PEA are both thought to be involved in the function of the central nervous system and gut-brain axis.[22]

Targeting the Endocannabinoid System in Inflammatory Bowel Disease

A large number of preclinical studies and a few clinical trials suggest the modification of the endocannabinoid system may have a role to play in the treatment of IBD. Jamontt and associates observed that in rats with experimental colitis, treatment with delta 9-tetrahydrocannabinol (Δ9-THC), the psychoactive component of marijuana, reduced inflammation and the occurrence of gastrointestinal symptoms.[33] Using THC or marijuana to reduce the symptoms of IBD has limitations due to the psychoactive effects resulting from the activation of CB_1 receptors in the brain. Additionally, smoking marijuana can lead to inflammation in the lungs. Consequently, other non-psychoactive cannabinoids that have low or non-existent affinity for CB_1 receptors have also been studied in IBD. In the previously mentioned Jamontt study, although THC was the most effective, the non-psychoactive CBD markedly enhanced the effectiveness of an otherwise ineffective low-THC dose.[33] In other rodent studies, either CBD or CBG prevented or attenuated experimental colitis.[29,34]

Enteric glial cells (EGC) are known to regulate acute and chronic inflammation in the gut. De Filippis and colleagues observed that CBD significantly reduced EGC activation in mice with LPS-induced intestinal inflammation.[30] The researchers achieved similar results in biopsies from ulcerative colitis patients.30 Other components that play a role in the endocannabinoidome have also been shown to have benefits. PEA, which acts on CB_2 receptors, TRPV1, PPARα, and GRR55, had significant anti-inflammatory effects in a mouse model of colitis.[35]

There is a paucity of clinical trials demonstrating the effects of phytocannabinoids and their related compounds on IBD. The research in humans is really just in its infancy. In cultured human colonic biopsies extracted from UC patients, PEA led to a reduction in expression and release of inflammatory mediators through its effect on PPARα.[36] Naftali and associates found that twice daily cannabis, in the form of cigarettes containing 115 mg of THC, did not achieve the primary endpoint of Crohn's disease remission which occurred in only 5 of the 11 subjects using cannabis.[37] However, a clinical response occurred in 10 of 11 subjects in the cannabis group compared with only 4 of 10 in the placebo group. Three subjects using cannabis were able to stop using steroids. Participants in the cannabis group experienced an improvement in appetite and sleep with no significant side effects. In another study by Naftali, 10 mg of CBD twice per day was compared to a placebo in 20 patients with moderately active CD.[38] Although CBD was found to be safe, it was not effective in this group of patients. The study authors noted, "This could be due to lack of effect of CBD on Crohn's disease, but could also be due to the small dose of CBD, the small number of patients in the study, or the lack of the necessary synergism with other cannabinoids."

More recently, Irving and coworkers investigated the effects of 10 weeks' supplementation with a CBD-rich botanical extract in ulcerative colitis patients.[39] The CBD-rich extract contained smaller concentrations of other phytocannabinoids such as cannabigerol, terpenoids, flavonoids, sterols and 3.2% to 4.7% THC. The percentage of patients in remission after treatment served as the primary endpoint. The participants were less tolerant of the CBD

compared with the placebo and consumed on average a third fewer capsules. Due to these compliance issues, the researchers identified a per protocol (PP) analysis set. The primary endpoint was not achieved with remission rates similar in the CBD and placebo groups at the end of the intervention. However, according to the PP analysis, the total and partial Mayo scores were better in the CBD group compared with the placebo. Furthermore, PP analyses of physician's global assessment of illness severity, subject global impression of change, and patient-reported quality-of-life outcomes showed an improvement for patients in the CBD group. Adverse events were mainly mild or moderate in the CBD group, and possibly due to the THC content. The placebo group suffered from more gastrointestinal-related adverse events, suggesting the worsening of UC.

It should be emphasized that in targeting the endocannabinoid system with phytocannabinoids, sustaining or regaining homeostasis is the goal. Therefore, starting dosing low and increasing gradually is important due to the interplay of many components such as receptors, the gut brain connection, the gut flora, and many more pathways. There have been a large number of reports of cannabinoid hyperemesis syndrome, an acute illness characterized by severe nausea and vomiting after exposure to inhaled or edible psychoactive cannabis.[40] It is possible that people suffering from this syndrome may be overwhelming their endocannabinoid system.

THE ENDOCANNABINOID SYSTEM AND COLON CANCER

The endocannabinoid system has been found to be altered in colorectal cancer. Ligresti and colleagues found 3-fold higher anandamide levels and 2-fold higher 2-AG concentrations in colorectal cancer lesions compared to normal colonic mucosa.[41] The levels of these endocannabinoids were higher in adenomatous polyps compared with carcinomas. Jung and colleagues noted that distant metastasis was reduced in colon cancer patients with high CB_1 receptor expression.[42] They also found that in stage IV patients, high CB_1 immunoreactivity was associated with a worse survival rate. Similar results were achieved in a study of Swedish stage II colorectal cancer patients in which elevated CB_1 expression in tumors was associated with worse disease-specific survival.[43] Further supporting the role of the endocannabinoid system in colon health is the fact that cannabinoids can suppress carcinogenesis in animal models of colon cancer.[44] Cannabidiol inhibited the formation of aberrant crypt foci (ACF), polyps and tumors in the colon of mice.[44] In another colon cancer animal model, a CBD-rich *Cannabis sativa* extract suppressed ACF, polyp, and tumor formation through activation of CB_1 and CB2.[45]

CONCLUSION

Evidence indicates the endocannabinoid system plays an important role in gut health. It interacts with the gut microbiota and key receptors in this system are expressed throughout the GI tract. Despite a prolific amount of preclinical support, there is a surprising scarceness of human studies investigating the effects of phytocannabinoids on inflammatory bowel disease. Yet, the extensive preclinical support led Hasenoehrl and associates to conclude, "From a scientist's perspective and all the caveats in mind, it seems to be a matter of time when cannabinoid compounds will be used in the treatment of GI disease."

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