CANNABINOID PHARMACOLOGY AND THERAPY IN GUT DISORDERS

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ABSTRACT

Cannabis sp and their products (marijuana, hashish…), in addition to their recreational, industrial and other uses, have a long history for their use as a remedy for symptoms related with gastrointestinal diseases. After many reports suggesting these beneficial effects it was not surprising to discover that the gastrointestinal tract expresses endogenous cannabinoids, their receptors, and enzymes for their synthesis and degradation, comprising the so-called endocannabinoid system. This system participates in the control of tissue homeostasis and important intestinal functions like motor and sensory activity, nausea, emesis, the maintenance of the epithelial barrier integrity, and the correct cellular microenvironment. Thus, different cannabinoid-related pharmacological agents may be useful to treat the main digestive pathologies. To name a few examples, in irritable bowel syndrome they may normalize dysmotility and reduce pain, in inflammatory bowel disease they may decrease inflammation, and in colorectal cancer, apart from alleviating some symptoms, they may play a role in the regulation of the cell niche.

This review summarizes the main recent findings on the role of cannabinoid receptors, their synthetic or natural ligands and their metabolizing enzymes in normal gastrointestinal function and in disorders including irritable bowel syndrome, inflammatory bowel disease, colon cancer and gastrointestinal chemotherapy-induced adverse effects (nausea/vomiting, constipation, diarrhea).

KEYWORDS: cannabinoid, chemotherapy-induced adverse effects, colorectal cancer, inflammatory bowel disease, irritable bowel syndrome.

1. Cannabinoids and the endocannabinoid system in the gut

Historically, many different herbal and plant-based remedies have been used for the treatment of gastrointestinal (GI) disorders. Among them, those derived from the marijuana plant *Cannabis sp*. have a controversial history since its introduction in Western medicine in the XIX century [1, 2]. *Cannabis* has been used to treat a variety of GI disorders, from dysmotility, emesis, abdominal pain and functional pathologies like irritable bowel syndrome (IBS) or functional dyspepsia to enteric infections and inflammatory conditions, including inflammatory bowel disease (IBD) and even cancer [3-6]. The active compound behind these applications has been considered to be Δ^9-tetrahydrocannabinol (THC), the main psychoactive molecule in *Cannabis*. However, there are a number of cannabinoid compounds like cannabidiol (CBD), tetrahydrocannabivarin, cannabidivarin, cannabichromene, cannabigerol and others whose effects might be similarly important [7, 8].

The first cannabinoid receptors cloned were the G-protein-coupled cannabinoid receptors 1 and 2 (CB1 and CB2) [9, 10]. These are the classical receptors for all kinds of cannabinoids. Since then, new molecules have been added to the list of cannabinoid receptors. Thus, the orphan G-protein coupled receptors 55 and 119 (GPR55 and GPR119), the transient receptor potential cation channel subfamily 5 member 1 (TRPV1) and the peroxisome proliferator-activated receptor family receptors (PPAR) have also been found to be responsible for some of the effects observed after cannabinoid administration [8, 11].

Endogenous ligands (endocannabinoids) are short-lived lipids, arachidonoyl ethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) being the best characterized. They can bind to any of the CB receptors although at low concentrations 2-AG is more specific for CB1 [12]. AEA is synthesized by N-acyl

phosphatidylethanolamine phospholipase D (NAPE-PLD) and 2-AG by diacylglycerol lipases (DAGL). After their release, endocannabinoids induce the biological response and are then inactivated through reuptake and enzymatic hydrolysis. They are degraded by fatty acid amide hydrolase (FAAH), a membrane-bound hydrolase, and monoacylglycerol lipase (MAGL), respectively [6]. Other acylethanolamides, chemically related to anandamide, like oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) are considered endocannabinoid-like compounds since they do not activate the canonical CB receptors [5, 6]. PEA and OEA are also degraded by FAAH and other hydrolases like N-acylethanolamine-hydrolyzing acid amidase (NAAA) [13].

All these endogenous ligands, their receptors and their synthesizing and degrading enzymes constitute the so-called endocannabinoid system (ECS) [14], which is broadly distributed in the gut.

The relationship between cannabinoids and digestive function emerged long before the identification of any receptor. For example, Gill et al [15], and then Roth [16], showed that cannabinoid ligands, like THC, inhibit cholinergic transmission in the myenteric plexus of the guinea pig ileum. Moreover, endogenous ligands are synthesized postsynaptically and act in the synaptic cleft as a kind of retrograde messengers binding to presynaptic receptors that indirectly modulate neurotransmitter release. After that, endocannabinoids are reuptake and hydrolyzed by their respective enzymes [7]. These observations were later confirmed by means of *in vitro* experiments using isolated intestinal tubes and *in vivo* studies, and helped to explain the effect of cannabinoids on GI motility. Other aspects like anti-inflammatory, anti-emetic and anti-secretory properties or anti-proliferative effects

were later described and increased attention was directed to explain the precise way of action of the endocannabinoid system within the GI tract.

CB1 and CB2 receptors are present throughout the enteric nervous system (ENS) of the GI tract [for more extensive reviews see refs 4, 5 and 8]. Immunostaining has shown that CB receptors are expressed on excitatory motor neurons, interneurons and afferent neurons, especially in the enteric ganglia. Both receptor types are located on cholinergic neurons but not on nitrergic inhibitory neurons. Additionally, CB1 and CB2 receptors are expressed in the mucosa cells with certain differences between humans and animal models. Thus, CB1 receptors are present in colonic epithelial and plasma cells. On the contrary, CB2 are expressed in murine epithelial cells and, in the case of human, in macrophages and, in a weaker manner, in plasma cells. CB1 receptor is also present in the vascular smooth muscle cells of the colon [6, 8, 17]. Both receptors are expressed in the *lamina propria* by macrophages and plasma cells [8].

Regarding the other kind of receptors, TRPV1 binds to AEA and, to a lesser extent, to OEA with a lower affinity than CB1 receptors. These receptors are involved in visceral hypersensitivity signaling, and are found on extrinsic afferent fibers, mainly within the innervation of muscle layers (myenteric plexus) and in immune cells adjacent to blood vessels. It has been observed that under inflammatory conditions activation of TRPV1 receptors may involve an increase in intestinal contractility [18]. Similarly to CB receptors, PPAR-α are also expressed throughout the whole GI tract. However, they bind a different set of ligands including AEA, 2-AG, OEA, PEA and others. They may be found in enterocytes of the small intestine, in enteric neurons of the myenteric and submucosal plexuses and in vagal afferent fibers [8]. PPAR-α receptors are also expressed on enteric glial cells, where they may be indirectly

activated by PEA through TLR4-dependent pathways [19]. Other PPAR-family receptors, like PPAR-γ, bind to THC, CBD, 2-AG and AEA [11]. Finally, GPR55 receptors have been found in the GI tract, mainly in the small intestine, both in epithelial cells and enteric neurons where they are activated by PEA [20]. On the contrary, GPR119 display a narrower expression pattern, and is found predominantly within the villi where it is expressed on enteroendocrine L cells regulating the release of the anti-diabetic peptide glucagon-like peptide-1 (GLP-1). GPR119 binds OEA and PEA, as well as, more weakly, AEA [20].

There is limited data about the cellular sources of endocannabinoids in the GI tract. FAAH (the degrading enzyme of AEA, PEA and OEA) is located in cells of the myenteric plexus, both in stomach and intestine [5, 8]. MAGL (which breaks down 2-AG) is present in the nerve cells and fibers throughout the muscle and mucosal layers of the duodenum, ileum, and colon. Interestingly, the activity of MAGL varies throughout the GI tract: the highest activity is observed in the upper GI tract (duodenum), and it decreases, reaching the lowest level in the distal colon. According to this, the presence of 2-AG is higher in the ileum than in the colon, and AEA is higher in the colon than in the ileum [8]. The specific location and concentration of these elements vary between human and animal samples and under pathological conditions. Thus, human mucosal biopsies of patients with IBD showed high levels of AEA but in rat the increase was observed in the submucosa and muscular layers, although the results depend also on the method used to induce colitis [8]. This could reflect methodological or interspecies differences but it is an important aspect to consider when comparing human with animal models. Other pathologies, like coeliac disease or diverticulitis, involve increases in AEA synthesis

but not in other ligands like 2-AG. On the contrary, both ligands are significantly increased in colorectal cancer patients [8].

In summary, the GI tract is able to locally produce and metabolize, according to its physiological needs, its own endocannabinoid receptors and ligands that influence gut homeostasis.

2. Cannabinoids and gastrointestinal motor function

Cannabinoid effects on GI motility have been reviewed extensively by other authors [6, 7, 8] and by us [4, 21]. As mentioned above, cannabinoids affect gut motility mainly by activating CB1 and CB2 receptors present on enteric neurons [6,21]. The activation of these receptors attenuates large and small bowel muscle tone, as shown *in vitro* using different preparations from different species [4,15,21]. Both receptors inhibit GI muscle contraction via the presynaptic reduction of excitatory neurotransmitter release (mainly acetylcholine and substance P) from the myenteric neurons [4,14,21]. As previously mentioned, the first experiments investigating the effects of cannabininoids on intestinal motility were those performed by Gill during the seventies using guinea-pig ileum [15]. In this model, *Cannabis sativa* tincture elicited a reduction in electrically evoked contractions suggesting that the effect of Δ^9-THC in the GI tract is related to the inhibition of acetylcholine release [15, 16]. This effect was confirmed to occur in other GI preparations too, and other cannabinoids (both natural and synthetic) were shown to reduce electrically evoked contractions in the mouse or rat stomach, guinea pig and human ileum, as well as human colon [reviewed in 4, 8 and 21].

GPR55, another potential cannabinoid receptor, seems to be also implicated in gut motility. Its selective agonist, O-1602, reduced elicited contractions in colonic and ileal muscle strips from mice and this effect was reversed by CBD, but not by CB1 or CB2 receptor antagonists [22]. In addition, the pharmacological inhibition of FAAH or MAGL decreased gut motility through mechanisms that involved a rise in AEA or 2-AG levels, respectively, and the activation of CB1 receptors [4]. *In vitro*, AEA and 2-AG are able to suppress cholinergic contractility via a non-cannabinoid receptor-mediated pathway in humans. Thus, endocannabinoids and/or other products of arachidonate metabolism [23] may tonically modulate GI motility. In contrast, cannabinoid antagonists or inverse agonists such as rimonabant (SR141716A) increased intestinal motility *in vitro* [22, 24]. However, 1,2,3-triazole derivatives, which have similar chemical structure to rimonabant, have demonstrated a multidirectional action in the mouse GI tract. Some compounds decreased ileal and colonic contractility, whereas others, depending on the concentration, increased or decreased ileal contractility [24].

In vivo, synthetic and natural CB1 receptor agonists decrease intragastric pressure and inhibit gastric emptying, pyloric contraction, and intestinal transit and colonic propulsion [for review see 4]. In humans, Δ^9-THC significantly reduced gastric emptying of solid food [25] and dronabinol decreased postprandial colonic tone and increased compliance [26], but did not affect colonic transit [27].

In addition to CB1 receptors, other “classical” and “nonclassical” cannabinoid receptors have been proposed to be implicated in GI motility. CB2 receptors are suggested to play an important role in the regulation of gut motility under pathological conditions [28]. In this sense, a CB2 agonist, JWH-133, attenuated accelerated gut transit in lipopolysaccharide-treated rats [29]. As previously mentioned, the GPR55

receptor is involved in GI motility. In fact, O-1602 slowed whole gut transit and colonic bead expulsion. Interestingly, activation of GPR55 was not associated with central effects [22]. Endocannabinoids can also stimulate neurons of the ENS via TRPV1, resulting in enteritis and enhanced motility [20]. In fact, exogenous and endogenous cannabinoids have a crucial role in states of gut inflammation [18], as discussed below.

Although cannabinoids have been proposed for the treatment of chronic pathologies, the effects of repeated administration of cannabinoids have been less studied in rodents. In our laboratory, the effect of different patterns of chronic administration of the non-selective cannabinoid WIN 55,212-2 (WIN) on gastrointestinal motility was radiographically studied in the rat. Upon daily administration, tolerance developed to the effect of the drug in the intestine but not in the stomach [30]. However, intermittent (weekly) WIN administration enhanced the effect of WIN in the stomach [31]. CB1 receptors were involved in both cases, but an additional, not yet identified receptor could also be implicated in the effect of WIN. The effect of cannabinoids on GI motility might not be long-lasting, even after repeated administration, because one week after WIN treatment cessation GI motility was normal again.

Cannabinoid agonists at low doses (lacking psychoactive effects), cannabinoid ligands that do not induce central effects, like CB13, a CB2 receptor selective agonist, or even peripherally-restricted agonists that act on CB1 receptors, like AM841 or [32,33], might be particularly useful in the treatment of GI motility disorders. Figure 1 illustrates the effects of WIN (at low and high doses) and AM841 on GI motility and the central nervous system. Doses of these two drugs equally effective to depress GI motility induced central effects in the case of WIN but not in

the case of AM841 [32]. As sown in the figure, the effects of these drugs on GI motility were completely blocked by previous administration of a CB1 receptor selective antagonist.

In summary, endocannabinoids and cannabinoids exogenously administered (either natural or synthetic) are able to regulate GI motility in both physiological and pathological situations. Their involvement in GI diseases will be described more deeply in the next section.

3. Cannabinoids and GI diseases

Apart from the traditional use of *Cannabis* for the treatment of GI diseases, the manipulation of the ECS could be useful for the treatment of GI motility alterations, nausea and emesis, gastroesophageal reflux, paralytic ileus, or diarrhea**.**

3.1 Gastroesophageal Reflux Disease (GERD) and alterations of gastric secretion

The main symptoms of GERD are heartburn and regurgitation. Acid-suppressive or mucosal-protective agents reduce heartburn, and they are the main treatment for GERD. Transient lower esophageal sphincter relaxations (TLESR) have been proposed as alternative therapeutic targets because they are the main mechanism underlying gastroesophageal reflux [4, 34]. CB1 receptors have been located in brain areas related to the triggering of TLESRs in the ferret [35] and the expression of CB1 mRNA in patients with non-erosive esophageal reflux disease (NERD) was increased compared with erosive esophagitis [36]. Also, cannabinoid agonists reduced the occurrence of TLESRs in dogs and healthy volunteers [37, 38]. Interestingly, the use

of a CB1 receptor antagonist (rimonabant) in healthy human subjects decreased TLESRs. On the other hand, rimonabant enhanced in dogs the rate of TLESRs and reflux events. This discrepancy could be due to interspecies differences, but also to the fact that rimonabant could exert its effect through other receptors, not necessarily CB1 [39-41].

Finally, GERD and esophageal motility disorders are more common in obese patients. This has been related to reduced endocannabinoids and CB receptor expression and to a loss of neurons containing neuronal nitric oxide synthase (nNOS) [42].

Direct activation of CB1 receptors by cannabinoid agonists reduces both gastric acid secretion and gastric motor activity, as well as the formation of gastric mucosal lesions induced by stress, pylorus ligation, nonsteroidal anti-inflammatory drugs (NSAIDs) or alcohol [for review see, ref. 43]. In addition, the elevation of EC levels using inhibitors of their metabolizing enzymes (FAAH, MAGL) reduces the gastric mucosal lesions induced by NSAIDs in a CB1 receptor-dependent fashion. Preliminary clinical studies are convincing, and the ECS represents a promising target in the treatment of gastric mucosal lesions and other pathologies related to inflammation and motility [43].

3.2 Nausea, emesis and gastric dysmotility

Nausea and vomiting are defense mechanisms against toxin ingestion, but they are also distressing side effects associated with some medications like chemotherapeutics. Since the introduction of antiemetics like 5-HT_3 antagonists, together with the corticoid dexamethasone and aprepitant (a neurokinin 1 receptor

antagonist), chemotherapy-induced nausea and vomiting (CINV) has been better controlled. However, these drugs are not as effective in the treatment of nausea as in that of emesis [for review see 21]. Traditionally, cannabinoids have been used for the treatment of nausea and vomiting and they are specially indicated in case of failure in response to other treatments [44]. Cannabinoids have also been effective in animal models subjected to emetic stimuli: drugs, radiation and motion [4,6,21].

The development of new antiemetic therapies has the problem that rodents, the most commonly used laboratory animals, lack the reflex of vomiting. In these animals, it is necessary to use other markers of nausea and emesis. There are two possibilities to overcome this technical problem. First, conditioned taste avoidance and conditioned gaping can be assessed [45]: thus, after pairing a novel flavored solution with the emetic stimuli (which induce malaise), rats not only avoid consumption of the flavored solution, they also display conditioned gaping reactions (the wide opening of the mouth) [46]. Changes in facial expression have also been proposed recently as a marker of nausea because the time-course of changes in facial expression was similar to clinical evidence of cisplatin-induced nausea in humans [47]. Second, pica, which is the consumption of non-nutritive substances (e.g., kaolin clay) in response to nausea-inducing agents, and gastric distension, temporally related with pica in rodents, can be used [48, 49].

Cannabinoids reduced taste avoidance and gaping in rats, but not pica or delayed gastric emptying [review in 21]. Although cannabinoids are used in humans to prevent chemotherapy-induced nausea and vomiting (CINV), the synthetic cannabinoid WIN was not able to reduce pica, anorexia or delayed gastric emptying induced by cisplatin in rats [50,51]. Moreover, small intestinal transit was further delayed [50]. The effect of WIN on gastric motor dysfunction induced by cisplatin (the

most emetogenic antitumoral drug) in the rat is illustrated in figure 2A. On the other hand, the alterations induced by vincristine on gastric emptying were at least partially prevented by the CB1 antagonist, AM251. However, AM251 was more effective to block vincristine-induced constipation and partalytic ileus. Thus, constipation and paralytic ileus and, to a lesser extent, gastric dysmotility induced by this antineoplastic drug may be, at least partly, associated to an activation of the ECS [52].

Cannabinoids have been approved by the Food and Drug Administration (FDA) as treatment for CINV since 1985 [53]. Nabilone (Cesamet®) and the synthetic THC dronabinol have been approved for use as antiemetics and dronabinol as an appetite stimulant too [for review, see 4]. The main drawback for their use in the clinic is their psychoactive effects. Non-psychoactive compounds, such as CBD, could be used. In fact, Sativex®, a mixture of CBD and THC, was effective in preventing delayed CINV in a phase II trial. Unfortunately, one patient withdrew due to neuropsychiatric side effects [54]. However, cannabinoids represent a valuable option for treating CINV, despite the adverse events related to treatment shown in some recent studies and metaanalysis [55-58].

We cannot forget that cannabinoids may also induce paroxysmal vomiting or cannabinoid hyperemesis syndrome (CHS), characterized by cyclic nausea and vomiting and abdominal pain among long-term, heavy marijuana users, which can be relieved by compulsive hot water bathing. This phenomenon was first described by Allen et al. [59]. CHS resolves with cannabis cessation, but recurs when patients resume the use of cannabinoids after hospital discharge [60]. It has been suggested that CHS could be due to a dysregulation of peripheral enteric nerves causing delayed gastric emptying and abdominal pain [60, 6]. This could be related to our

preclinical findings in rats using WIN at high doses: gastric dysmotility was resistant to the development of tolerance when WIN was given daily [30] and increased when given weekly [31]. Clearly, the role of cannabinoids in controlling gastric motility warrants further investigation.

3.3 Irritable Bowel Syndrome and related pathologies

Irritable bowel syndrome (IBS) is the most frequent gastrointestinal disorder, with a prevalence ranging between 10 and 20% in the developed world and, in addition to the economic cost, it diminishes the quality of life of patients who suffer it [61]. Rome IV Criteria define IBS as periodic pain present at least 3 days per month over 3 months together with at least two of the following: (1) improvement with defecation, (2) episodes associated with a change in stool frequency, and (3) episodes associated with a change in stool consistency [62]. The syndrome has four main subtypes including diarrhea predominant (IBS-D), constipation predominant (IBS-C), and mixed (IBS-M) IBS. However, there are patients who cannot be included in these groups and are considered to have an unclassified IBS (IBS-U) [62]. IBS is difficult to diagnose, and current treatments are not always effective and usually treat the symptomatology but do not cure the disease [63]. These patients suffer from alterations in GI motility, abnormal visceral hypersensitivity, disruptions of brain-gut interactions, and abnormalities in processing of visceral afferent inputs [64]. Modulation of the ECS may allow for correction in several of these abnormalities. Due to their effects on motility and secretion, CB1 agonists may be useful to treat IBS-D, whereas CB1 antagonists could be useful to treat IBS-C. Activation of CB2 receptors, which are overexpressed in the gut under inflammatory conditions, may also be used to treat IBS-D [21].

The ECS has mainly an inhibitory role in the GI tract: it reduces motility and secretion in physiological and pathophysiological states [21] and also regulates the sensation of pain. Activation of the CB1 receptor (with nabilone, THC, or AEA) slows GI motility. This effect could be blocked with the CB1 receptor antagonist, SR141716A (rimonabant) [65, 66]. Other compounds like AM841 could be used in the treatment of IBS-D. As illustrated in figure 1, we and others have demonstrated that this compound reduces motility in a CB1 receptor-dependent manner, in both rats and mice [32, 67]. Remarkably, the dose of AM841 used to inhibit GI motility did not produce the central side effects typical of other cannabinoid agonists (figure 1), and thus, this drug might be a milestone in the field of therapeutic application [32].

In the crotone oil model that triggers ileitis, the CB1 receptor is overexpressed, and CB1 agonists reduce GI transit [5, 68, 69]. In a mouse IBS-C model, the inverse agonist of the CB1 receptor taranabant improved the symptoms related to the decrease in GI motility and abdominal pain [5, 70]. In humans, the increase in colonic transit that occurs in IBS-D has been related to genetic variations in endocannabinoid metabolism [71]. The expression of FAAH (the enzyme that degrades CBs) is decreased in patients with IBS-C, which would explain why there is a delay in GI motility in these patients [72]. As CB1 receptor activation slows motility, CB1 antagonists could be used to treat opioid-induced constipation and gastroparesis. Motility was found to be increased with the application of rimonabant, insinuating that the ECS provides a basal suppressive tone to motility [73, 74].

The CB2 receptor may also affect motility. In lipopolysaccharide-induced inflammation, which decreased transit time, JWH-133 returned transit times to control values and this effect was blocked by the CB2 receptor antagonist, AM630 [28]. Interestingly, JWH-133 had no effect on basal transit times. Thus, during

inflammation, the CB2 receptor provides a mechanism for the re-establishment of normal GI transit [29].

In addition, in patients with IBS-D, the development of the symptomatology and alteration of colonic transit has been related to the CB1 and FAAH receptors [75]. Finally, in patients with IBS-C there is an expression of the CB1 receptor higher than in patients with IBS-D or IBS-M [76].

In patients with slow transit constipation (STC), the expression and enzymatic activity of FAAH were decreased and levels of AEA and 2-AG were higher than controls [77]. In animal models of mice genetically preconditioned to constipation, the inhibition of DAGL, the enzyme that produces 2-AG, reduced the levels of 2-AG and normalized fecal output [78]. Interestingly, 2-AG alone did not affect gut transit time but, when it was administered with an agent to prevent its degradation, JZL184, motility was slowed [78].

In conclusion, activation of the CB1 receptor could be useful in IBS-D while its inhibition decreases GI transit time and could be useful for the treatment of IBS-C.

4. Visceral sensitivity and pain

Many GI disorders are related to visceral pain. Pain or nociception can be triggered by inflammation, ischemia, or distension. Visceral pain is frequently diffuse and many patients with abnormal visceral sensitivity fall into the category of functional dyspepsia and IBS [61, 79].

Previous studies have demonstrated an analgesic effect of cannabinoids in animal models of visceral pain through both CB1 and CB2 receptor activation [for review see 3, 61, 79]. Also, the inhibition of AEA degradation led to an attenuated behavioral

response to noxious stimuli in rodents [80]. This suggests a central role of CB1 receptors in mitigating pain-related inputs to the central nervous system.

However, some findings related to the involvement of cannabinoids and visceral pain are somehow controversial. The activation of both CB1 and CB2 receptors inhibits the abdominal sensitivity produced by colorectal distention in rats under basal conditions [81]. In a rat colitis model, a CB1, but not a CB2 receptor antagonist, produced an increase in visceral hyperalgesia [82]. Similarly, the non-selective cannabinoid agonist dronabinol, at relatively low doses, increased the colonic sensation due to distention in humans [26].

5. Inflammatory Bowel Disease and cannabinoids

The term Inflammatory Bowel Disease (IBD) comprises two chronic disorders of the GI system: Crohn´s disease (CD) and ulcerative colitis (UC). These are chronic inflammatory conditions that may occur in all parts of the GI tract in the case of CD while UC is located specifically in the colon. IBD is diagnosed in 1-2% of population, with increasing incidence in western countries [83, 84]. The etiology of IBD is unknown although deregulation of the steady state between the immune system and the gut microbiota after damage in epithelial barrier function is a major factor (83). The major symptoms of IBD include abdominal pain, fecal bleeding, diarrhea, and weight loss. Taking this into account, many studies have been performed to elucidate the role of ECS in IBD due to its role in gut homeostasis and its effects in relieving some of the symptoms [3, 6].

Experimental colitis may be induced in animal models with a series of methods [86]. In this way it has been shown an enhanced ECS signaling during intestinal inflammation, with an increased expression of receptors, altered endocannabinoid

levels, and decreased expression of endocannabinoid degrading enzymes. Thus, increased expression of CB1 [87, 88] and CB2 receptors [88, 89], and of AEA [90] have been described. The activation of CB receptors by their ligands produces a protective effect in animal models [29, 88, 89, 91]. On the contrary, mice lacking functional CB receptors are less resistant to colonic inflammation than wild type animals [87, 89, 92] and FAAH mRNA levels, reduced at the beginning of colitis after 2,4,6-trinitrobenzene sulfonic acid (TNBS) administration, increased when damage was maximal [93]. According to this, several strategies to enhance endocannabinoid levels have been assayed, either by inhibition of endocannabinoid degradation [93, 94] or increasing the transport across plasma membrane, resulting in an ameliorated inflammation. In particular, inhibition of FAAH genetically or by means of PF-3845, ARN2508 or FAAH-II improved colitis by reducing the number of activated T cells, macrophages, neutrophils, and NK/NKT cells, as well as inflammatory miRNAs and cytokines at effector sites in the colon [87, 94-96]. At the same time, these authors observed raised levels of anandamide, PEA and OEA that most likely contributed to the beneficial effect [96]. In this way, it has been shown that inhibition of PEA degradation significantly improves the effects of experimental colitis [97]. In accordance, oral administration of THC and PEA resulted in anti-inflammatory effects in the gut [98]. Regarding membrane trafficking, the inhibition of AEA reuptake increased its concentration and abolished inflammation [90]. Similarly, the blockade of FAAH and EMT (with URB597 and VDM-11, respectively) protected against TNBS-induced colitis in wild type, but not in CB1- and CB2-KO mice [93]. Furthermore, the blockade of FAAH may even alter the levels of other CB receptor ligands, such as 2-AG, PGE2, and glycerol-derived lipids [94] (Table 1).

One concern about the translational use of *cannabis* is the psychoactive central effects of Δ^9-THC. For this reason, other different non-psychotropic cannabinoids have been assayed in IBD models. For example, cannabigerol acts reducing inflammatory cytokines production, reactive oxygen species (ROS) formation and the number of macrophages and mast cells after binding to CB2 receptors after DNBS induction of experimental colitis [99]. CBD exerted similar effects when administered intraperitoneally or orally [100]. In the same way, it elicited anti-inflammatory effects on models of lipopolysaccharide-induced colitis and in biopsies from UC patients where it reduced TNF-α and iNOS expression in a way mediated by the PPAR-γ receptor pathway [101]. In CD, the action of CBD was additive to that of THC in a dose-response manner, with a bell-shaped pattern [102]. Similarly, it has been shown that the synthetic analogue of CBD, O-1602, agonist of the putative cannabinoid receptor GPR55, reduces the severity of dextran sulfate sodium (DSS) and TNBS-induced colitis by inhibiting neutrophil recruitment [103]. The pro-inflammatory role of GPR55 has been also demonstrated when treatment with its antagonist CID16020046 alleviated intestinal inflammation [104].

In humans, several papers based on questionnaires have revealed varied results regarding the use of *cannabis* as a self-medication to relieve IBD-related symptoms. They show that it appears as an important option for patients, although some concerns about its long-term effects in CD patients have been reported [105-107]. Unfortunately, there are scarce retrospective placebo-controlled studies in IBD patients although a beneficial response has been reported with up to 45% of clinical improvement after treatment with Δ^9-THC [108]. Similarly, a prospective study with 13 IBD patients reported an improvement in the quality of life and weight gain after three-month treatment with inhaled *Cannabis* [109]. These effects of herbal

cannabinoids could be caused mainly by THC since a recent randomized placebo-controlled trial with 20 CD patients who were treated with CBD for 8 weeks did not show any beneficial effect [110]. However, other *Cannabis*-derived compounds, apart from THC, might also contribute to these effects. Thus, Nallathambi et al [111] have shown that the anti-inflammatory activity of *Cannabis* could be attributed to the action of Δ^9-tetrahydrocannabinolic acid (THCA) via GPR55 receptors since it suppresses cyclooxygenase-2 (COX2) and metalloproteinase-9 (MMP9) gene expression both in cell culture and colon tissues from IBD patients. In contrast to Naftali et al [110] these authors found that CBD had dose dependent cytotoxic activity, with anti-inflammatory activity only found at low concentrations. Clinical trials testing THCA instead of other cannabinoid non-psychoactive treatments for IBD are lacking. Similarly, it is worth considering that the limited number of participants in studies performed so far do not allow for statistical conclusions to be made. A detailed summary of these clinical studies are available in ref. 112.

Regarding human endocannabinoids, colonic biopsies derived from UC patients have also been analyzed by liquid chromatography-mass spectrometry showing high concentrations of anandamide but not of 2-AG [100]. However, other reports using immunohistochemistry with acute untreated active UC and treated quiescent patients in comparison with healthy human colonic tissue obtained contradictory outcomes since the expression of CB2 receptor and the enzymes DAGL and MAGL was increased, mainly in mild and moderate colitis patients. In contrast, NAPE-PLD expression decreased in moderate and severe colitis patients. During quiescent colitis, CB1, CB2 and DAGL expression dropped, while NAPE-PLD expression rose [113]. Similarly, immunostaining for CB receptors in tissues from IBD patients revealed that CB2 receptor was significantly increased in colonic mucosal samples

[17]. This activation of CB2 receptors might be an attempt to restore balance in damaged intestinal barrier function, at least at the early stages of colitis. Regarding this, the CB2-selective agonist JWH-015 attenuated inflammatory cytokine-elicited mucosal damage in human colonic explants. This anti-inflammatory role had been previously described in the HT29 colonic cell line where it was found that a number of cannabinoid receptor agonists and antagonists were able to inhibit tumor necrosis factor alpha (TNF-α)-induced interleukin-8 (IL-8) release through activation of CB2 receptors [114]. Similarly, AEA was also protective while CB1 receptor agonism with ACEA was without effect [115]. Considering all these data, the role of CB2 receptors could be limited to colitis when its concentration is increased since studies with CB1 receptors and its agonists have demonstrated that wound closure is likely to be mediated by this receptor [17]. Importantly, the method used to induce mucosal inflammation should be considered when working with human samples. Cell culture of Caco-2 monolayers treated over 48 hours with cytokines to induce damage did not respond to CB2 or CB1 receptor activation [115]. However, when the same cell type was exposed to EDTA-induced increased permeability, both THC and CBD enhanced the speed of recovery. In this case all cannabinoids tested increased the mRNA levels of the tight junction proteins although endocannabinoids also decreased the mRNA levels of claudin-1, suggesting that they play a role in the homeostasis of intestinal permeability [116].

These findings point out to the function of the ECS in regulating gut homeostasis and its therapeutic potential in inflammatory GI disorders. However, treatment should be carefully considered. Clinical trials are urgently needed to determine the efficacy of cannabinoids and gain a better insight into the exact mechanism underlying herbal/endogenous cannabinoids effects [112]. Finally, the relationship between gut

microorganisms and the ECS is of special interest since microbiota is a main factor of inflammatory pathologies and plays a central role in digestive physiology [117].

6. Cannabinoids and colorectal cancer

There is a long history of cannabinoid use to alleviate cancer symptoms such as pain, emesis, cachexia or dysgeusia. Many of these studies may be considered anecdotal, with important methodological drawbacks like scarce number of patients or adequate controls. The negative connotations of marijuana have not been of help in these regards, although dronabinol (Marinol®), a synthetic form of THC, and nabilone (Cesamet®), a synthetic cannabinoid receptor agonist, were approved in 1985 for chemotherapy-induced nausea and vomiting [see refs 1, 56, 57, 118-120 for cancer-related reviews]. The more important epidemiological study so far has been recently published [121]. Authors recruited 2970 cancer patients for two years. After 6 months of follow up 1211 of then responded to the questionnaires with a 95.9% reporting an improvement either significant or moderate in their medical condition and almost 70% in their quality of life. Moreover, since the beginning of the XXI century numerous experimental data indicate that the activation of the ECS might represent a potential strategy for the development of treatment for other side effects of chemotherapy like diarrhea or constipation [52, 122]. Figure 2 illustrates the effects of cannabinoid ligands on chemotherapy-induced dysmotility in rats: cisplatin-induced gastric dysmotility; vincristine-induced constipation; 5-fluorouracil- (5-FU) induced diarrhea.

Moreover, new properties of endocannabinoids are arising that make them candidates to be considered as potential anticancer drugs [123]. According to recent

estimations, colorectal cancer (CRC) is the third most common cancer in men and the second in women with a variable incidence worldwide. In Western countries, it is the second leading cause of cancer death. Only a minor fraction of cases may be considered of genetic origin and in fact, chronic inflammation is one of the main causes of CRC [124].

The expression of ECS components like AEA and 2-AG, and some of their synthesizing enzymes (NAPE-PLD), has been found to be higher in CRC than in normal mucosa, although some results are controversial since the highest concentrations were found at the beginning of the carcinomatous process in one report, whereas in another paper, the highest concentrations were found when lymphatic metastasis had already occurred [125, 126]. Levels of FAAH as well as MAGL were also increased [126]. Intriguingly when MAGL was knocked down, tumor growth was inhibited by down-regulating cyclin D1 and Bcl-2 [127].

Contrary to ligands, CB1 receptor expression has shown to be decreased in CRC patients compared to adjacent non-neoplastic mucosa [128-130]. This down-regulation of expression may be due to epigenetic silencing by CpG islands methylation around the transcription site of CB1 receptor [129]. However, some inter-studies differences are apparent regarding this receptor. Thus, when samples of Korean CRC patients were analyzed using microarrays, low CB1 receptor expression was more frequently identified at stage IV than at stage I/II or III tumors, although there were no differences in lymph node metastasis, tumor invasion, or tumor size. However, at stage IV patients, high CB1 immunoreactivity was correlated with a statistically significant poorer overall survival [128]. Similarly, an increase in CB1 expression has been cited in Chinese patients [126]. When European patients were studied, a significant positive association of the tumor grade with CB1 receptor

intensity was observed in microsatellite stable tumors, the type that comprises most colon cancers [131]. Finally, studies on CB2 receptor also showed conflicting results with either an intense immunoreactivity in CRC samples [130] or only in a 28.6% of cases correlating with poor prognostic markers of cancer progression [132]. No differences in CB2 expression have also been published [125, 126].

In conclusion, the human studies performed so far indicate that an increase in endocannabinoids does exist although a clear description of the role of their receptors in CRC in lacking (Table 2).

More detailed studies can be performed with CRC cell lines and animal models where CRC can be induced by a series of methods such as germline mutations of pivotal genes related to colon carcinogenesis, like the adenomatous *polyposis coli* (Apc) gene or by administration of azoxymethane (AOM). Using these experimental approaches, cannabinoids have been shown to exert anti-proliferative effects on tumor cells through the activation of anti-inflammatory and pro-apoptotic pathways (see ref 133 for a detailed description of the mechanism). When *ApcMin/+* mice had their CB1 receptors silenced with the CB1 antagonist AM251 or were additionally knocked out for the CB1 gene the number of intestinal polyps were increased, while activation of CB1 induced tumor cell death by means of down-regulating the anti-apoptotic factor survivin. On the contrary, deletion of the gene encoding CB2 receptor had no effect on polyp growth [130]. However, the CB2 receptor agonist CB13 has been able to inhibit the growth of tumors derived from xenografts of the CRC cell line DLD-1. In this case, CB2 receptor activation induced apoptosis through TNFα-mediated ceramide synthesis [130]. In the same way, Greenhough et al [134] reported that THC induces apoptosis in CRC cells after activation of CB1 receptors that resulted in the inhibition of both RAS-MAPK/ERK and PI3K-AKT survival

signaling pathways. In mice treated with AOM, AEA and 2-AG concentrations were found to be increased in aberrant crypt foci (ACF, the earliest preneoplastic lesions), with no changes in FAAH. However, inhibition of FAAH with N-arachidonoylserotonin not only increased colon endocannabinoid concentrations but reduced ACF formation and contributed to normalize caspase-3 expression [135]. Similar results were obtained in the same model with the non-psychotropic CBD [136, 137]. In the same way, GPR55 blockade with CBD elicited a decrease in adhesion to endothelial cells and migration of the CRC cell line HTC116 [138]. An important contribution to the antiproliferative mechanism of endocannabinoids has been recently made using rimonabant, a CB1 receptor inverse agonist. It had been previously reported that this compound was able to reduce the formation of ACF [139]. More recently, Proto et al [140] have shown that rimonabant inhibited, in cell lines and xenografts, the Wnt/β-catenin canonical pathway, one of the main routes over-expressed in epithelial transformation in CRC. This effect partially depended on histone acetyltransferase, an epigenetic coactivator of β-catenin gene regulation. Wnt/β-catenin pathway plays a central role in colon homeostasis, so it is of great importance not to alter its normal values. Interestingly, rimonabant may inhibit cancer cells development without affecting normal cells as it has been demonstrated using colon organoids [141]. Antitumorigenic properties were also observed with the synthetic analogue of CBD, O-1602, using cell lines and a model of colitis-associated colon cancer induced by administration of a combination of AOM and DSS. In this case, O-1602 induced apoptosis in colon cancer cells and tumor incidence *in vivo* by 30%. It also reduced tumor area by 50%, decreasing proliferating cell nuclear antigen (PCNA) and STAT3 levels, and proinflammatory pathways mediated by NFκB and TNFα while pro-apoptotic factors were increased [142]. Other synthetic agonists like WIN induced

apoptosis in colon cancer cell lines after reduction of PPAR-γ levels, which blocked the pro-survival autophagic response of cancer cells [143]. Besides apoptosis, cannabinoids have been shown to theoretically prevent metastasis since treatment of CB1 receptor with its agonist docosatetraenoylethanolamide (DEA) inhibited the norepinephrine-induced migration of CRC cells [144]. Finally, cannabinoid compounds have been shown to inhibit angiogenesis in human cancer xenografts and CRC cell lines. For instance, the cannabinoid-like compound LYR-8 significantly reduced the expression of the transcription factor responsible for induction of angiogenesis (HIF-1a), and also of the vascular endothelial growth factor (VEGF), cyclooxygenase-2 (COX-2) and the Akt signalling pathway [145].

In conclusion, so far data indicate that cannabinoid ligands, their receptors and metabolizing enzymes play a role in the maintenance of colon homeostasis. Preclinical investigations show an implication of the ECS in the regulation of the cell niche, migration ability and induction of apoptosis that should be further investigated.

7. Conclusions

Nowadays, the presence of the different components of the endocannabinoid system in the gut is well recognized, as it is their involvement in the development of different disorders of the gastrointestinal tract. Thus, many drugs aimed at modulating their expression and action in this organ have been tested in different animal models and some of them also in humans.

The complexity of this system as well as the important side effects that may be encountered, particularly those affecting the central nervous system has delayed research in this field and incorporation of new drugs to the market. However, the

huge amount of information collected in recent years opens up the possibility that additional novel strategies are tested.

Time will tell if these strategies will aid to reduce the impact of the prevalent, costly annoying and/or dangerous gut disorders reviewed here, like gastroesophageal reflux disease, irritable bowel syndrome, inflammatory bowel disease, colorectal cancer or disorders induced by chemotherapy.

COMPETING INTERESTS

The authors declare that they have no competing interests.

CONTRIBUTIONS

All authors contributed to manuscript writing, read and accepted its final version.

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Figure legends

Figure 1: Effects of synthetic cannabinoid agonists on gastrointestinal (GI) motility in the rat. Rats received vehicle (Tocrisolve® in saline, 30 µl/kg), WIN 55, 212-2 (WIN, 0.5 or 5 mg/kg) or AM841 (0.1 mg/kg) by intraperitoneal (ip) route. GI motility was evaluated using radiographic methods. Barium sulfate (2.5 ml, 2 g/ml in water) was intragastrically administered immediately after drug and plain facial images of the GI tract were obtained using a Digital X-Ray apparatus (60 kV, 7 mA) and captured with NPG Real DVD Studio II software. Exposure time was adjusted to 0.02-0.06 s. Rats were briefly immobilized in the prone position by placing them inside adjustable hand-made transparent plastic tubes. No anesthesia was applied to avoid GI motility alterations. Representative X-rays obtained 4 h after contrast are shown for the different treatments (scale bar: 3 cm). S = stomach; SI = small intestine; C = caecum; CR = colorectum – notice the faecal pellets within this region. In panel A, the cannabinoid tetrad was used to test for the occurrence of the central effects typically induced by cannabinoids in rodents. WIN at a low dose (0.5 mg/kg) reduced small intestinal transit (barium did not reach the caecum) and only produced analgesia, whereas at a higher dose (5 mg/kg), it intensely decreased gastric emptying and small intestinal transit and produced the four signs of the cannabinoid tetrad. In Panel B, the selective CB1 receptor antagonist AM251 (1 mg/kg, ip) was injected 20 minutes prior to WIN (5 mg/kg) or AM841 (0.1 mg/kg). AM251 was able to block the effect of both WIN and AM841 on GI motor function.

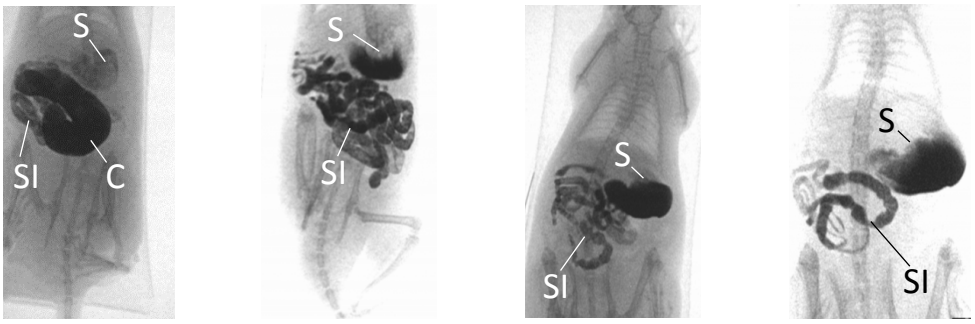
Figure 2: Effect of cannabinoid drugs on chemotherapy-induced gastrointestinal (GI) dysmotility in the rat. Three antineoplastic drugs were intraperitoneally (ip) administered to induce gastric dysmotility (cisplatin, panel A), constipation (vincristine, panel B) or diarrhea (5-fluorouracil, panel C). GI motility was evaluated

using radiographic methods. Barium sulfate (2.5 ml, 2 g/ml in water) was intragastrically administered and plain facial images of the GI tract were obtained using a digital X-Ray apparatus (60 kV, 7 mA) and captured with NPG Real DVD Studio II software (B) or recorded on X-ray film housed in a cassette provided with regular intensifying screen (A and C). Exposure time was adjusted to 0.02-0.06 s. Rats were briefly immobilized in the prone position by placing them inside adjustable hand-made transparent plastic tubes. No anesthesia was applied to avoid GI motility alterations. Representative X-rays obtained 4 h after contrast are shown for the different treatments (scale bar: 3 cm). S = stomach; SI = small intestine; C = caecum; CR = colorectum – notice the faecal pellets within this region. In panel A, cisplatin (CISPT) was administered at 2 mg/kg/week for 4 weeks, and the non-selective cannabinoid agonist WIN 55,212-2 (WIN, 0.5 mg/kg, ip) or vehicle (Tocrisolve® in saline, 30 µl/kg, ip) was administered 30 min before each cisplatin injection. The radiographic study was performed after the last drug administration. Cisplatin produced gastric dysmotility and WIN was not able to prevent it. In panel B, vincristine (VC) was administered at 0.5 mg/kg and the selective CB1 receptor antagonist AM251 (1 mg/kg, ip) or vehicle (Tocrisolve® in saline, 30 µl/kg, ip) was administered twice (30 min before VC and 24 h after). Barium sulfate was administered immediately after the last AM251/vehicle administration and X-rays were obtained afterwards. VC reduced intestinal transit and production of faecal pellets, and the cannabinoid antagonist was able to block these effects. In panel C, 5-fluorouracil (5-FU) was administered at 150 mg/kg for two consecutive days, and WIN (0.5 mg/kg, ip) or vehicle (Tocrisolve® in saline, 30 µl/kg, ip) was administered once daily for 4 days starting 20 min before the first 5-FU administration. Contrast was administered immediately after the last WIN injection and X-rays were obtained

afterwards. 5-FU reduced gastric emptying and increased water contents in caecum (a hatched line has been drawn to make it easier to distinguish the border of the caecum with increased water contents in the second X-ray of this raw), and the cannabinoid agonist did not improve altered gastric emptying but decreased diarrhea.

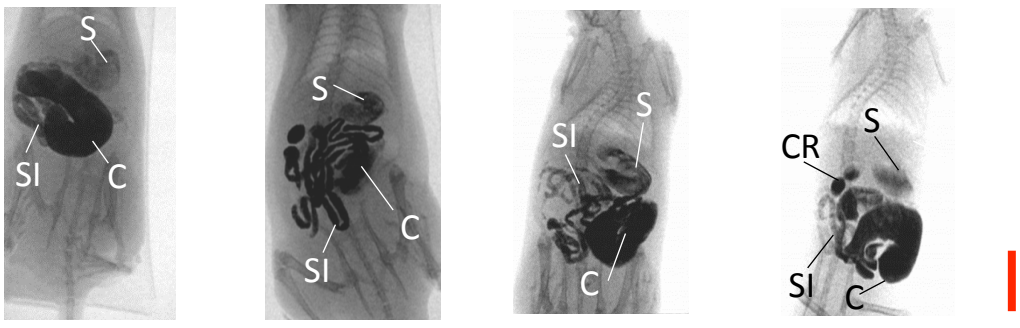
Figure

A

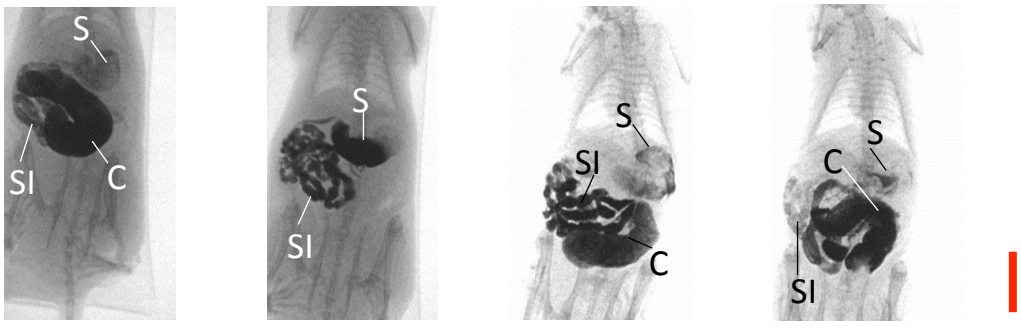


CANNABINOID TETRAD	VEHICLE	WIN 0.5	WIN 5	AM841
ANALGESIA	-	+	+	-
CATALEPSY	-	-	+	-
HYPOTHERMIA	-	-	+	-
HYPOLOCOMOTION	-	-	+	-

B

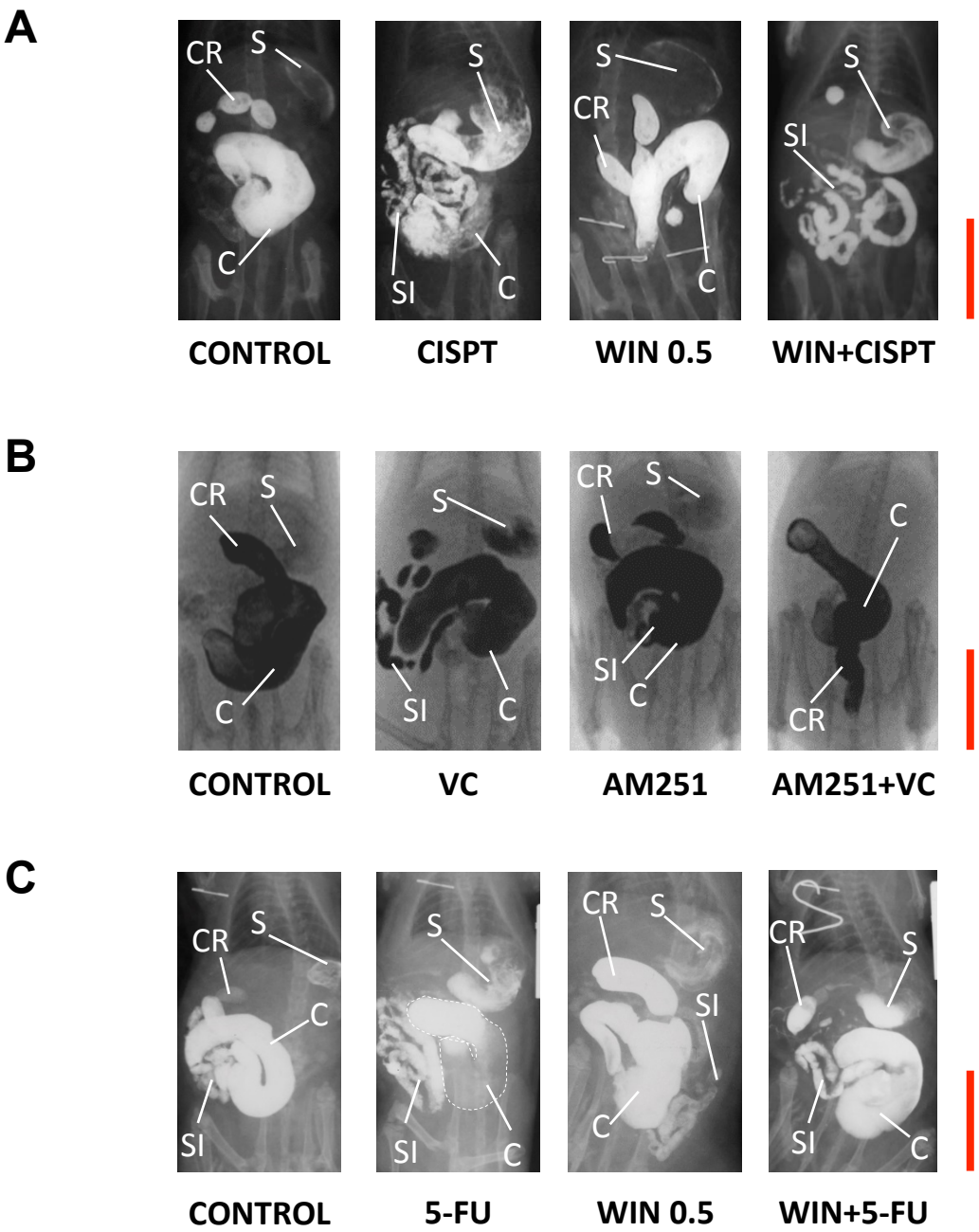


VEHICLE WIN 5 AM251 AM251+WIN5



VEHICLE AM841 AM251 AM251+AM841

Figure


A
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AM251+VC
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5-FU
WIN 0.5
WIN+5-FU

Table

Table 1: Summary of the effects of the endocannabinoid system on inflammatory bowel disease

ECS element	Experimental IBD/colitis method	Effects	References
CB1	DNBS/OM	Upregulation of receptor expression	[87, 88]
CB2	TNBS/OM	Upregulation of receptor expression	[88, 89]
CB-agonist binding	LPS/TNBS/DSS/OM	Protection against colitis	[29, 88, 89, 91]
CB inhibition	DNBS/TNBS	Less resistance to colitis	[87, 89, 92]
AEA	TNBS/DNBS	Upregulation	
AEA reuptake inhibition	TNBS/DNBS	Increases AEA and abolishes inflammation	[90]
FAAH	TNBS	Upregulation with mucosa damage	[93]
FAAH inhibition	TNBS/DSS	Improves colitis and reduces inflammation	
		Increases levels of AEA, PEA and OEA	[94, 96]
	TNBS	Protection against colitis	
		(not in CB-KO mice)	[93]
	TNBS/DSS	Alteration of 2-AG levels	[94]
	DNBS	Protection against colitis	[87]
PEA activation	TNBS	Reduces colonic and systemic inflammation	[97]

ECS: endocannabinoid system; CB1: cannabinoid receptor 1; CB2: cannabinoid receptor 2; AEA: anandamide; 2-AG: 2-arachidonoylglycerol; FAAH: fatty acid amide hydrolase; PEA: palmitoylethanolamide; OEA: oleoylethanolamide; DNBS: 2,4-dinitrobenzene sulfonic acid; OM: oil of mustard; TNBS: 2,4,6-trinitrobenzene sulfonic acid; LPS: lipopolysaccharide; DSS: dextran sulfate sodium.

Table

Table 2: Expression of components of the endocannabinoid system on human colorectal tumors

ECS component		Findings	Patient number (n)	References
Receptors	CB1	Downregulation	19	[128]
			24	[129]
		Downregulation with higher expression in poorer survival	534	[127]
		Upregulation	47	[125]
		Upregulation with tumor grade	487	[130]
		No change	15	[124]
	CB2	Intense expression	24	[129]
		Positive in poor prognosis	175	[131]
		No change	47	[125]
			15	[124]
Ligands	AEA	Upregulation	15	[124]
	2-AG	Upregulation	15	[124]
		No change	47	[125]
Synthesizing enzymes	NAPE-PLD	Upregulation	15	[124]
			47	[125]
Degrading enzymes	FAAH	Upregulation	47	[125]
		No change	15	[124]
	MAGL	Upregulation	47	[125]

ECS: endocannabinoid system; CB1: cannabinoid receptor 1; CB2: cannabinoid receptor 2; AEA: anandamide; 2-AG: 2-arachidonoylglycerol; NAPE-PLD: N-acyl phosphatidylethanolamine phospholipase D; FAAH: fatty acid amide hydrolase; MAGL: monoacylglycerol lipase.