



ELSEVIER

Contents lists available at ScienceDirect

# Biochemical Pharmacology

journal homepage: [www.elsevier.com/locate/biochempharm](http://www.elsevier.com/locate/biochempharm)

## Review

# Cannabinoid pharmacology and therapy in gut disorders

J.A. Uranga<sup>a,b,d</sup>, G. Vera<sup>a,b,c,d</sup>, R. Abalo<sup>a,b,c,d,\*</sup>

<sup>a</sup> Departamento de Ciencias Básicas de la Salud, Facultad de Ciencias de la Salud, Universidad Rey Juan Carlos, Alcorcón, Madrid, Spain

<sup>b</sup> Unidad Asociada I+D+i al Instituto de Investigación en Ciencias de la Alimentación,CIAL (CSIC), Spain

<sup>c</sup> Unidad Asociada I+D+i al Instituto de Química Médica, IQM (CSIC), Spain

<sup>d</sup> Grupo de Excelencia Investigadora URJC-Banco de Santander-Grupo Multidisciplinar de Investigación y Tratamiento del Dolor (i+DOL), Spain

## ARTICLE INFO

### Keywords:

Cannabinoid  
Chemotherapy-induced adverse effects  
Colorectal cancer  
Inflammatory bowel disease  
Irritable bowel syndrome

## 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).

## 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  $\Delta^9$ -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 vanilloid 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),

\* Corresponding author at: Departamento de Ciencias Básicas de la Salud, Facultad de Ciencias de la Salud, Universidad Rey Juan Carlos, Avda. de Atenas s/n, 28922 Alcorcón, Madrid, Spain.

E-mail address: [raquel.abalo@urjc.es](mailto:raquel.abalo@urjc.es) (R. Abalo).

<https://doi.org/10.1016/j.bcp.2018.07.048>

Received 31 May 2018; Accepted 31 July 2018

Available online 02 August 2018

0006-2952/ © 2018 Elsevier Inc. All rights reserved.

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 reuptaken 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 [17,18]. 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 ECS 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 nitroergic 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 [6,8,19]. Both receptors are expressed in the *lamina propria* by macrophages and plasma cells [8].

Regarding the other kind of receptors, TRPV1 binds AEA and, to a lesser extent, 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 [20]. Similarly to CB receptors, PPAR- $\alpha$  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- $\alpha$  receptors are also expressed on enteric glial cells, where they may be indirectly activated by PEA through TLR4-dependent pathways [21]. Other PPAR-family receptors, like PPAR- $\gamma$ , bind 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 [22]. On the contrary, GPR119 displays 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 [22].

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 [4,23]. As mentioned above, cannabinoids affect gut motility mainly by activating CB1 and CB2 receptors present on enteric neurons [6,23]. The activation of these receptors attenuates large and small bowel muscle tone, as shown *in vitro* using different preparations from different species [4,15,23]. Both receptors inhibit GI muscle contraction via the presynaptic reduction of excitatory neurotransmitter release (mainly acetylcholine and substance P) from the myenteric neurons [4,14,23]. As previously mentioned, the first experiments investigating the effects of cannabinoids 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  $\Delta^9$ -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 4,8,23].

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 [24]. 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 [25] may tonically modulate GI motility. In contrast, cannabinoid antagonists or inverse agonists such as rimonabant (SR141716A) increased intestinal motility *in vitro* [24,26]. However, 1,2,3-triazole derivatives, which have similar chemical structure to rimonabant, have demonstrated a multi-directional action in the mouse GI tract. Some compounds decreased ileal and colonic contractility, whereas others, depending on the concentration, increased or decreased ileal contractility [26].

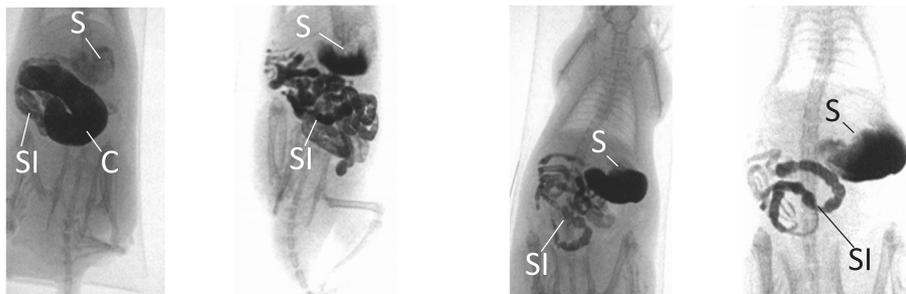
*In vivo*, synthetic and natural CB1 receptor agonists decrease intragastric pressure and inhibit gastric emptying, pyloric contraction, and intestinal transit and colonic propulsion [for review see 4]. In humans,  $\Delta^9$ -THC significantly reduced gastric emptying of solid food [27] and dronabinol decreased postprandial colonic tone and increased compliance [28], but did not affect colonic transit [29].

In addition to CB1 receptors, other “classical” and “nonclassical” cannabinoid receptors have been implicated in GI motility. CB2 receptors are suggested to play an important role in the regulation of gut motility under pathological conditions [30]. In this sense, a CB2 agonist, JWH-

133, attenuated accelerated gut transit in lipopolysaccharide-treated rats [31]. 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

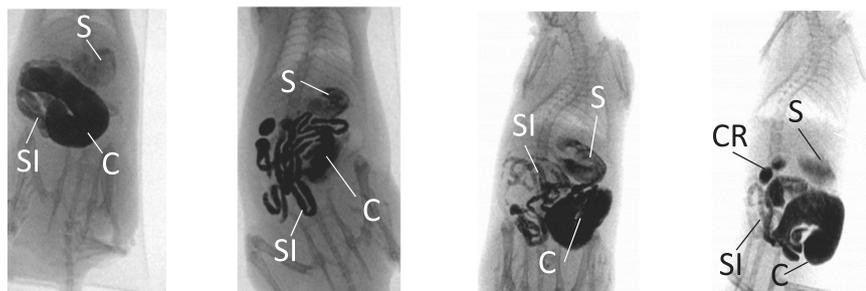
central effects [24]. Endocannabinoids can also stimulate neurons of the ENS via TRPV1, resulting in enteritis and enhanced motility [22]. In fact, exogenous and endogenous cannabinoids have a crucial role in states of gut inflammation [20], as discussed below.

**A**

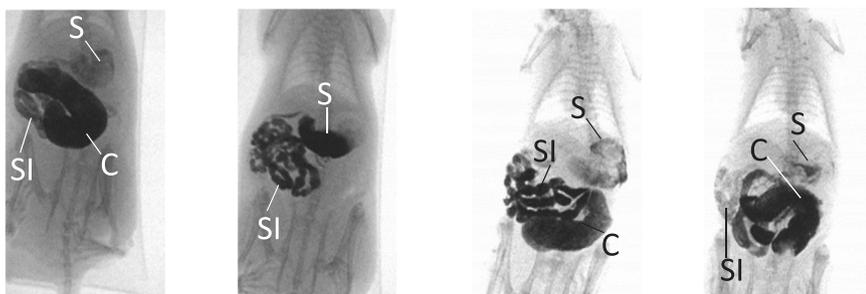


CANNABINOID TETRAD	VEHICLE	WIN 0.5	WIN 5	AM841
ANALGESIA	-	+	+	-
CATALEPSY	-	-	+	-
HYPOTHERMIA	-	-	+	-
HYPOLOCOMOTION	-	-	+	-
GI MOTOR FUNCTION	NORMAL	↓ GASTRIC EMPTYING ↓↓ SI TRANSIT	↓↓↓ GASTRIC EMPTYING ↓↓↓ SI TRANSIT	

**B**



VEHICLE WIN 5 AM251 AM251+WIN5



VEHICLE AM841 AM251 AM251+AM841

GASTRIC EMPTYING	NORMAL	↓↓↓	NORMAL	NORMAL
INTESTINAL TRANSIT	NORMAL	↓↓↓	NORMAL	NORMAL

(caption on next page)

**Fig. 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 fecal 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) slightly reduced gastric emptying (more barium was present in stomach compared to vehicle-treated animals), clearly 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 comparison, AM841 at 0.1 mg/kg depressed gastrointestinal motor function equipotently to WIN at 5 mg/kg, but did not induce any sign of the cannabinoid tetrad. In Panel B, the selective CB1 receptor antagonist AM251 (1 mg/kg, ip) was injected 20 min 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. Chemicals used in these experiments and their suppliers were as follows: WIN and Tocrisolve were obtained from Tocris Cookson (Bristol, UK); AM251 was obtained from Ascent Scientific (North Somerset, UK). AM841 was developed in Dr Makriyannis' laboratory (Northeastern University, Boston, USA); barium sulfate (Barigraf® AD) was purchased from Juste SAQF (Madrid, Spain).

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 [32]. Furthermore, intermittent (weekly) WIN administration enhanced the effect of WIN in the stomach [33]. 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 [34,35], might be particularly useful in the treatment of GI motility disorders. Fig. 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 decrease GI motility induced central effects in the case of WIN but not in the case of AM841 [34]. As shown 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 and alterations of gastric secretion

The main symptoms of gastroesophageal reflux disease (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 [4,36]. CB1 receptors have been located in brain areas related to the triggering of TLESRs in the ferret [37] and the expression of CB1 mRNA in patients with non-erosive esophageal reflux disease (NERD) was increased compared with erosive esophagitis [38]. Also, cannabinoid agonists reduced the occurrence of TLESRs in dogs and healthy volunteers [39,40]. Interestingly, the use of a CB1 receptor antagonist (rimonabant) in healthy

human subjects decreased TLESRs. On the other hand, rimonabant enhanced the rate of TLESRs and reflux events in dogs. 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 [41–43].

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) [44].

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. 45]. 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 [45].

#### 3.2. Nausea, emesis and gastric dysmotility

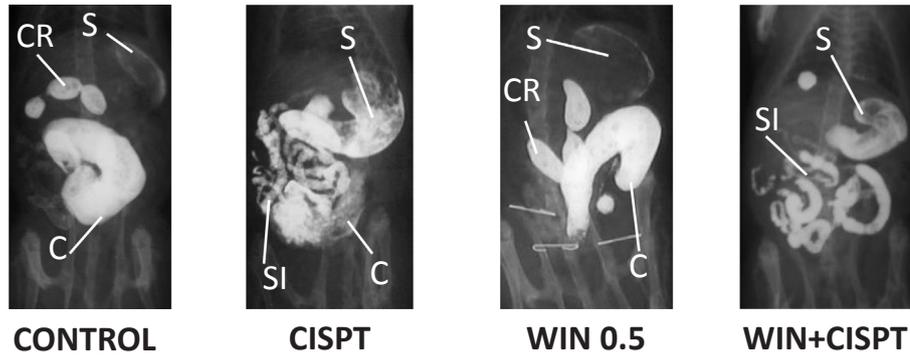
Nausea and vomiting are defense mechanisms against toxin ingestion, but they are also distressing side effects associated with some medications like chemotherapeutics. Since the introduction of antiemetics like 5-HT<sub>3</sub> antagonists, together with the corticoid dexamethasone and aprepitant (a 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 23]. 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 [46]. In species capable of vomiting (ferrets, least shrews, *Cryptotis parva*; house musk shrews, *Suncus murinus*...), cannabinoids have also been able to prevent the effect of different emetic stimuli: drugs (cisplatin, morphine, rimonabant, LiCl...), radiation and motion [for review, see 4,6,23,47]. In these animal models, AEA and 2-AG reduced emesis; but also inhibitors of FAAH (URB597) or MGL (JZL184) were able to reduce nausea and emesis without causing the typical cannabinoid agonist-induced central side effects [6,48–50]. In ferrets, the gold standard in the development of new antiemetic drugs, different CB1 and CB2 agonists (natural and synthetic) and TRPV1 agonists have been demonstrated to exert antiemetic effect [37,48,51].

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 different markers that can be assessed to overcome this technical problem, namely conditioned taste avoidance and conditioned gaping, changes in facial expression, pica and gastric distension. After pairing a novel flavored solution with the emetic stimuli (which induce malaise), rats not only avoid consumption of the flavored solution (conditioned taste avoidance), they also display

conditioned gaping reactions (the wide opening of the mouth) [52,53]. 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

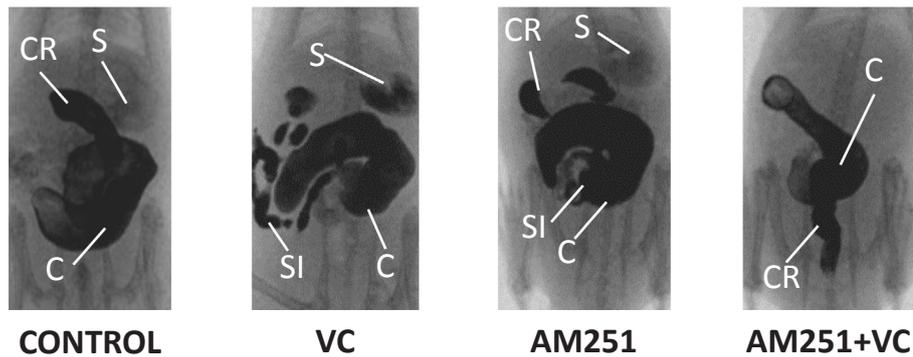
humans [54]. Pica, which is the consumption of non-nutritive substances (e.g., kaolin clay) in response to nausea-inducing agents, and gastric distension, temporally related with pica in rodents, can also be used [55,56].

**A**



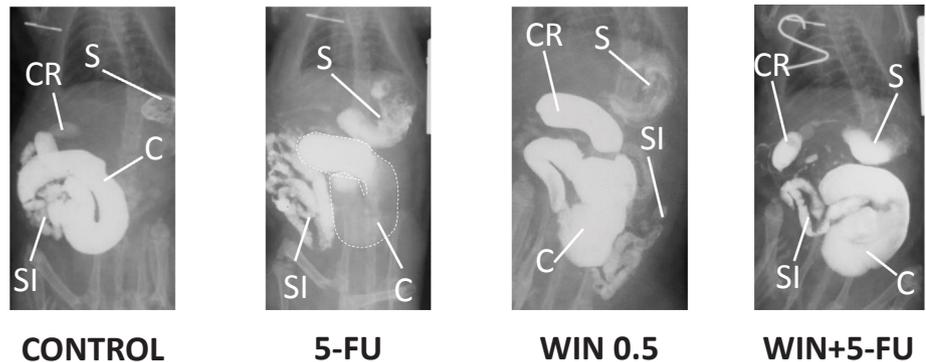
GASTRIC EMPTYING	NORMAL	↓↓↓	NORMAL	↓↓↓
INTESTINAL TRANSIT	NORMAL	NORMAL	NORMAL	↓↓

**B**



GASTRIC EMPTYING	NORMAL	↓↓	NORMAL	NORMAL
INTESTINAL TRANSIT	NORMAL	↓↓↓	NORMAL	NORMAL

**C**



GASTRIC EMPTYING	NORMAL	↓↓	NORMAL	↓↓
INTESTINAL TRANSIT	NORMAL	WATERY DIARRHEA	NORMAL	NORMAL

(caption on next page)

**Fig. 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 fecal 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 (a significant amount of barium was still present in the stomach at this time point, similarly to cisplatin-only treated animals, but in contrast with control and WIN-only treated rats). 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 and 24 h after VC). Barium sulfate was administered immediately after the last AM251/vehicle administration and X-rays were obtained afterwards. VC reduced gastric emptying, intestinal transit and production of fecal pellets, and the cannabinoid antagonist was able to block these effects (in rats treated with both drugs, at this time point of the study barium was not seen in the stomach and could be seen in the colorectum, in contrast with vincristine-only treated animals, and similarly to control and AM251-only treated rats). 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/vehicle 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 (a high amount of barium could still be seen in the stomach) but decreased diarrhea (caecum had a normal appearance and well-formed fecal pellets could be seen in the colorectum). Chemicals used in these experiments and their suppliers were as follows: Cisplatin, vincristine and 5-fluorouracil were purchased from Sigma-Aldrich (Spain). WIN and Tocrisolve were obtained from Tocris Cookson (Bristol, UK). AM251 was obtained from Ascent Scientific (North Somerset, UK). Barium sulfate (Barigraf® AD) was purchased from Juste SAQF (Madrid, Spain).

Cannabinoids reduced taste avoidance and gaping in rats, but not pica or delayed gastric emptying [review in 23]. Although cannabinoids are used in humans to prevent CINV, the synthetic cannabinoid WIN was not able to reduce pica, anorexia or delayed gastric emptying induced by cisplatin in rats [57,58]. Moreover, small intestinal transit was further delayed [57]. The effect of WIN on gastric motor dysfunction induced by cisplatin (the most emetogenic antitumoral drug) in the rat is illustrated in Fig. 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 paralytic ileus. Thus, constipation and paralytic ileus and, to a lesser extent, gastric dysmotility induced by this antineoplastic drug may be, at least partly, associated with an activation of the ECS [59].

Cannabinoids have been approved by the Food and Drug Administration (FDA) as treatment for CINV since 1985 [60]. 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 [61]. However, cannabinoids represent a valuable option for treating CINV, despite the adverse events related to treatment shown in some recent studies and metaanalysis [62–65].

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. [66]. CHS resolves with cannabis cessation, but recurs when patients resume the use of cannabinoids after hospital discharge [67]. It has been suggested that CHS could be due to a dysregulation of peripheral enteric nerves causing delayed gastric emptying and abdominal pain [6,67]. 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 [32] and increased when given weekly [33]. 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 [68]. 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 [69]. 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) [69]. IBS is difficult to diagnose, and current treatments are not always effective and usually treat the symptomatology but do not cure the disease [70]. These patients suffer from alterations in GI motility, abnormal visceral hypersensitivity, disruptions of brain-gut interactions, and abnormalities in processing of visceral afferent inputs [71]. 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 [23].

The ECS has mainly an inhibitory role in the GI tract: it reduces motility and secretion in physiological and pathophysiological states [23] 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) [18,72]. Other compounds like AM841 could be used in the treatment of IBS-D. As illustrated in Fig. 1, and shown by us and others, this compound reduces motility in a CB1 receptor-dependent manner, in both rats and mice [34,35,73]. Remarkably, the dose of AM841 used to inhibit GI motility did not produce the central side effects typical of other cannabinoid agonists (Fig. 1), and thus, this drug might be a milestone in the field of therapeutic application [34].

In the croton oil model that triggers ileitis, the CB1 receptor is overexpressed, and CB1 agonists reduce GI transit [5,74,75]. 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 [5,76]. In humans, the increase in colonic transit that occurs in IBS-D has been related to genetic variations in endocannabinoid metabolism [77]. 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 [78]. 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 [8,18,79,80].

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 [31]. 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 [31].

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 [81]. Finally, in patients with IBS-C there is an expression of the CB1 receptor higher than in patients with IBS-D or IBS-M [82].

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 [83]. 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 [84]. 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 [84].

In summary, 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. Cannabinoids and 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 [68,85].

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 3,68,85]. Also, the inhibition of AEA degradation led to an attenuated behavioral response to noxious stimuli in rodents [86]. 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 [87]. In a rat colitis model, a CB1, but not a CB2 receptor antagonist, produced an increase in visceral hyperalgesia [88]. Similarly, the non-selective cannabinoid agonist dronabinol, at relatively low doses, increased the colonic sensation due to distention in humans [29].

#### 5. Cannabinoids and inflammatory bowel disease

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 [89,90]. 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 [91]. 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 [92]. An enhanced ECS signaling has been shown to occur in intestinal inflammation, with an increased expression of receptors, altered endocannabinoid levels, and decreased expression of endocannabinoid degrading enzymes. Thus, increased expression of CB1 [93,94] and CB2

receptors [94,95], and of AEA [96] has been described. The activation of CB receptors by their ligands produces a protective effect in animal models [31,94,95,97]. On the contrary, mice lacking functional CB receptors are less resistant to colonic inflammation than wild type animals [93,95,98] and FAAH mRNA levels, that were reduced at the beginning of colitis after 2,4,6-trinitrobenzene sulfonic acid (TNBS) administration, increased when disease progressed [99]. According to this, several strategies to enhance endocannabinoid levels have been assayed, either by inhibition of endocannabinoid degradation [99,100] 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 [93,100–102]. At the same time, these authors observed raised levels of anandamide, PEA and OEA that most likely contributed to the beneficial effect [102]. In this way, it has been shown that inhibition of PEA degradation significantly improves the effects of experimental colitis [103]. In accordance, oral administration of THC and PEA resulted in anti-inflammatory effects in the gut [104]. Regarding membrane trafficking, the inhibition of AEA reuptake increased its concentration and abolished inflammation [96]. 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 [99]. Furthermore, the blockade of FAAH may even alter the levels of other CB receptor ligands, such as 2-AG, PGE2, and glycerol-derived lipids [100] (Table 1).

One concern about the translational use of *cannabis* is the psychoactive central effects of  $\Delta^9$ -THC. For this reason, other different non-psychoactive 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 in DNBS-induced experimental colitis [105]. CBD exerted similar effects when administered intraperitoneally or orally [106]. Likewise, it elicited anti-inflammatory effects on models of lipopolysaccharide-induced colitis and in biopsies from UC patients where it reduced TNF- $\alpha$  and iNOS expression in a way mediated by the PPAR- $\gamma$  receptor pathway [107]. In CD, the action of CBD was additive to that of THC in a dose-response manner, with a bell-shaped pattern [108]. Similarly, it has been shown that the synthetic analogue of CBD, O-1602, reportedly an agonist of the putative cannabinoid receptor GPR55, reduces the severity of dextran sulfate sodium (DSS) and TNBS-induced colitis by inhibiting neutrophil recruitment, without involving CB1, CB2 or GPR55 receptors [109]. However, the pro-inflammatory role of GPR55 was more recently demonstrated when treatment with its antagonist CID16020046 alleviated intestinal inflammation [110].

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 [111–113]. Unfortunately, placebo-controlled studies in IBD patients are scarce, although a beneficial response has been reported with up to 45% of clinical improvement after treatment with  $\Delta^9$ -THC [114]. 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* [115]. 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 [116]. However, other *Cannabis*-derived compounds, apart from THC, might also contribute to these effects. Thus, Nallathambi et al. [117] have shown that the anti-inflammatory activity of *Cannabis* could be attributed to the action of  $\Delta^9$ -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. [116] these

**Table 1**  
Summary of the effects of the ECS on IBD and expression of its components on human colorectal tumors.

ECS element	Experimental IBD/colitis method	Effects	References
CB1	DNBS/OM	Upregulation of receptor expression	[93,94]
CB2	TNBS/OM	Upregulation of receptor expression	[94,95]
CB-agonist binding	LPS/TNBS/DSS/OM	Protection against colitis	[31,94,95,97]
CB inhibition	DNBS/TNBS	Less resistance to colitis	[93,95,96]
AEA	TNBS/DNBS	Upregulation	
AEA reuptake inhibition	TNBS/DNBS	Increases AEA and abolishes inflammation	[96]
FAAH	TNBS	Upregulation with mucosa damage	[99]
FAAH inhibition	TNBS/DSS	Improves colitis and reduces inflammation	
		Increases levels of AEA, PEA and OEA	[100,102]
	TNBS	Protection against colitis (not in CB-KO mice)	[99]
	TNBS/DSS	Alteration of 2-AG levels	[100]
	DNBS	Protection against colitis	[93]
PEA activation	TNBS	Reduces colonic and systemic inflammation	[103]

ECS component	Findings	Patient number (n)	References	
Receptors	CB1	Downregulation	19	[134]
			24	[135]
		Downregulation with higher expression in poorer survival	534	[133]
		Upregulation	47	[131]
		Upregulation with tumor grade	487	[136]
		No change	15	[130]
	CB2	Intense expression	24	[135]
		Positive in poor prognosis	175	[137]
		No change	47	[131]
			15	[130]
Ligands	AEA	Upregulation	15	[130]
	2-AG	Upregulation	15	[130]
		No change	47	[131]
Synthesizing enzymes	NAPE-PLD	Upregulation	15	[130]
			47	[131]
Degrading enzymes	FAAH	Upregulation	47	[131]
		No change	15	[130]
			47	[131]
	MAGL	Upregulation	47	[131]

CS: 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; NAPE-PLD: N-acyl phosphatidylethanolamine phospholipase D; MAGL: monoacylglycerol lipase. DNBS: 2,4-dinitrobenzene sulfonic acid; OM: oil of mustard; TNBS: 2,4,6-trinitrobenzene sulfonic acid; LPS: lipopolysaccharide; DSS: dextran sulfate sodium.

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. [118].

Regarding human endocannabinoids, colonic biopsies derived from UC patients have also been analyzed by liquid chromatography-mass spectrometry showing high concentrations of AEA but not of 2-AG [96]. 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 [119]. Similarly, immunostaining for CB receptors in tissues from IBD patients revealed that CB2 receptor was significantly increased in colonic mucosal samples [19]. 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- $\alpha$ )-induced interleukin-8 (IL-8) release through activation of CB2 receptors [120].

Similarly, AEA was also protective while CB1 receptor agonism with ACEA was without effect [121]. 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 [19]. 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 h with cytokines to induce damage did not respond to CB2 or CB1 receptor activation [121]. 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 [122].

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 [118]. 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 [123].

## 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 the

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 CINV [see Refs. 1,63,64,124–126 for cancer-related reviews]. The more important epidemiological study so far has been recently published [127]. Authors recruited 2970 cancer patients for two years. After 6 months of follow up 1211 of them 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 [59,128]. Fig. 2 illustrates the effects of cannabinoid ligands on chemotherapy-induced dysmotility in rats: cisplatin-induced gastric dysmotility; vincristine-induced constipation; and 5-fluorouracil- (5-FU) induced diarrhea.

Moreover, new properties of endocannabinoids are arising that make them candidates to be considered as potential anticancer drugs [129]. 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 [130].

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 [131,132]. Levels of FAAH as well as MAGL were also increased [132]. Intriguingly, when MAGL was knocked down, tumor growth was inhibited by down-regulating cyclin D1 and Bcl-2 [133].

Contrary to ligands, CB1 receptor expression has been shown to be decreased in CRC patients compared to adjacent non-neoplastic mucosa [134–136]. This down-regulation of expression may be due to epigenetic silencing by CpG islands methylation around the transcription site of CB1 receptor [135]. 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 [134]. Similarly, an increase in CB1 expression has been reported to occur in Chinese patients [132]. 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 [137]. Finally, studies on CB2 receptor also showed conflicting results with either an intense immunoreactivity in CRC samples [136] or only in a 28.6% of cases correlating with poor prognostic markers of cancer progression [138]. No differences in CB2 expression have also been published [131,132].

Therefore, 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 is lacking (Table 1).

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. [139] 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 [136]. 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 TNF $\alpha$ -mediated ceramide synthesis [136]. In the same way, Greenhough et al. [140] 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 [141]. Similar results were obtained in the same model with the non-psychotropic CBD [142,143]. Likewise, GPR55 blockade with CBD elicited a decrease in adhesion to endothelial cells and migration of the CRC cell line HTC116 [144]. 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 [145]. More recently, Proto et al. [146] have shown that rimonabant inhibited, in cell lines and xenografts, the Wnt/ $\beta$ -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  $\beta$ -catenin gene regulation. Wnt/ $\beta$ -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 [147]. 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 NF $\kappa$ B and TNF $\alpha$  while pro-apoptotic factors were increased [148]. Other synthetic agonists like WIN induced apoptosis in colon cancer cell lines after reduction of PPAR- $\gamma$  levels, which blocked the pro-survival autophagic response of cancer cells [149]. Besides apoptosis, cannabinoids have been shown to theoretically prevent metastasis since treatment of CB1 receptor with its agonist docosate-traenoyl ethanolamide (DEA) inhibited the norepinephrine-induced migration of CRC cells [150]. 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-1 $\alpha$ ), and also of the vascular endothelial growth factor (VEGF), cyclooxygenase-2 (COX-2) and the Akt signalling pathway [151].

In summary, 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. Cannabinoids and their interaction with other endogenous systems

Although a deep comparison is beyond the scope of this review, it is important to note that the actions of cannabinoids on motility and other

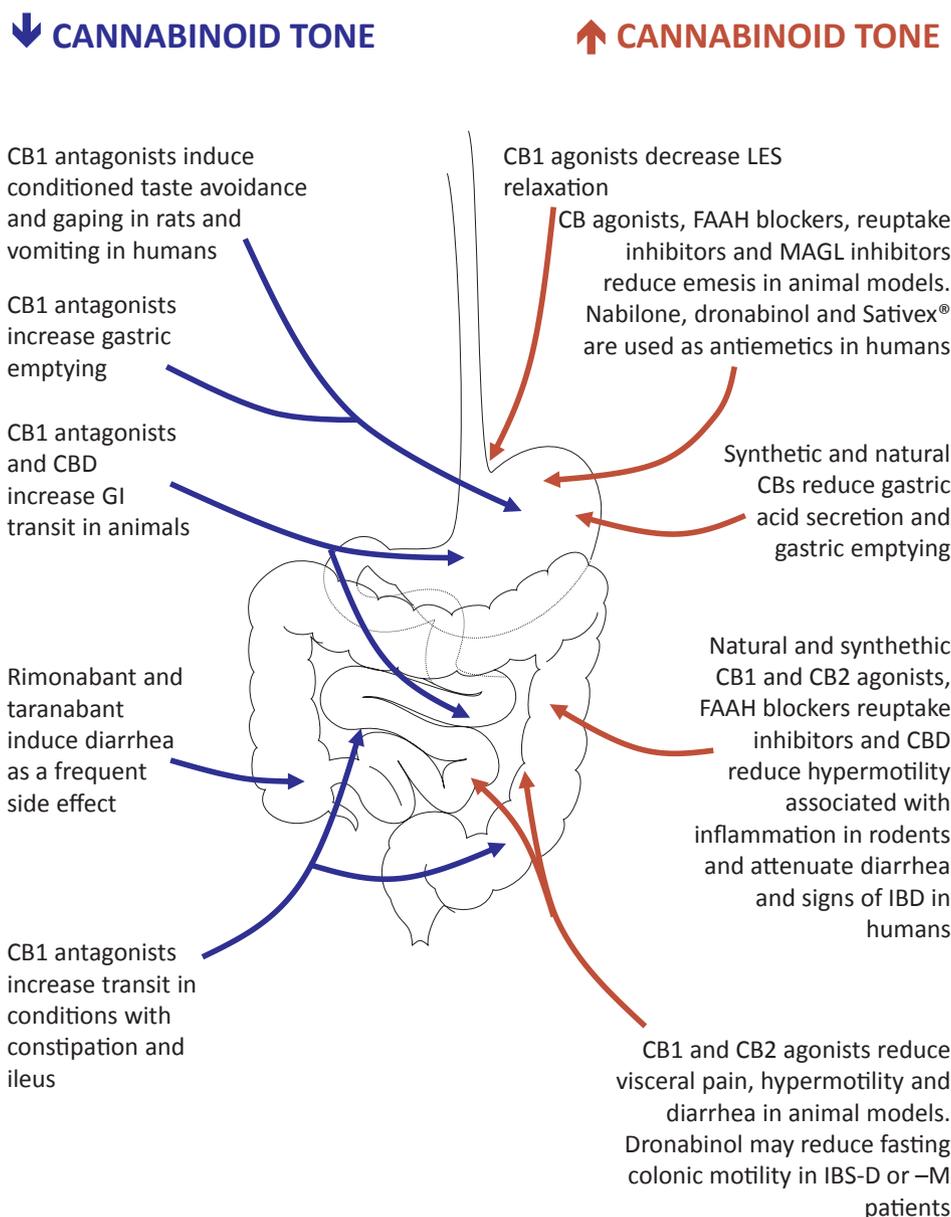
GI functions are similar to those induced by other drugs such as opioids or agonists acting upon alpha-2 adrenoceptors. Opioids are well-known to decrease gastric motility, pyloric tone, pancreatic and biliary secretion, and to reduce propulsion and increase fluid absorption in the small and large intestines. They also induce bloating, abdominal distension, constipation and abdominal cramps. Thus, opioid receptor agonists can be used to treat gut motor and secretory disorders, especially diarrhea. However, chronic administration, frequently for moderate-to-severe pain treatment, can cause the narcotic bowel syndrome (for review see [152–154]). The regulatory role of alpha 2-adrenoceptors on GI functions is also well-documented and, like cannabinoids, drugs acting on alpha 2-adrenoceptors might be useful for the treatment of different GI disorders, like IBS [155], functional dyspepsia [156] or IBD [157,158].

These similarities have important implications for treatment. For example, with the current opioid crisis, it has been suggested that cannabinoids, cannabinoid-like drugs or alpha-2B agonists may aid in the development of alternative new effective peripheral visceral

analgesics [159]. Additionally, the combination of mu opioid agonists with CB2 receptor agonists may synergistically attenuate chronic pain and reduce opioid-induced side effects, including rewarding behaviors and slowing of gastrointestinal motility, as shown in preclinical studies [160]. Finally, the use of strategies to increase the cannabinoid tone, like the inhibition of endogenous ligands degradation, may decrease CB1 cannabinoid receptor expression, and this may increase mu opioid receptor sensitivity [161].

### 8. 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 (Fig. 3, Table 1).



**Fig. 3.** Cannabinoids and the gut. Changes in the cannabinoid tone are associated with different disorders of the gastrointestinal tract. The figure shows the effects of reducing (left) or increasing (right) the cannabinoid tone with different endogenous ligands and exogenous drugs. 2-AG, 2-arachidonoylglycerol; CBD, cannabidiol; FAAH, fatty acid amide hydrolase; GI, gastrointestinal; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; IBS-D, predominant diarrhea IBS; IBS-M, mixed IBS; LES, lower esophageal sphincter; MAGL, monoacylglycerol lipase. See text for references.

The complexity of the endocannabinoid 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.

### References

- [1] D. Abrams, M. Guzman, Cannabis in cancer care, *Clin Pharmacol Ther.* 97 (2015) 575–586, <https://doi.org/10.1002/cpt.108>.
- [2] L. Borgelt, K. Franson, A. Nussbaum, G. Wang, The pharmacologic and clinical effects of medical cannabis, *Pharmacotherapy.* 33 (2013) 195–209, <https://doi.org/10.1002/phar.1187>.
- [3] R. Abalo, M.I. Martín-Fontelles, Cannabis, cannabinoids, and visceral pain, in: *Handbook of Cannabis and Related Pathologies, Biology, Pharmacology, Diagnosis, and Treatment*, 2017, pp. 439–449. <https://doi.org/10.1016/B978-0-12-800756-3.00051-X>.
- [4] G. Vera, J. Fichna, R. Cannabinoids and Effects on the Gastrointestinal Tract: A Focus on Motility. In: *Handbook of Cannabis and Related Pathologies. Biology, Pharmacology, Diagnosis, and Treatment*, 2017, pp. 947–957. <https://doi.org/10.1016/B978-0-12-800756-3.00114-9>.
- [5] M. Salaga, R. Abalo, J. Fichna, Cannabis and cannabinoids and the effects on gastrointestinal function: an overview, *Handbook of Cannabis and Related Pathologies, Biology, Pharmacology, Diagnosis, and Treatment*, 2017, pp. 471–480. <https://doi.org/10.1016/B978-0-12-800756-3.00056-9> (Chapter 49).
- [6] C. Hasenoeuhl, U. Taschler, M. Storr, R. Schicho, The gastrointestinal tract—a central organ of cannabinoid signaling in health and disease, *Neurogastroenterol. Motil.* 28 (2016) 1765–1780, <https://doi.org/10.1111/nmo.12931>.
- [7] M. Camilleri, Cannabinoids and gastrointestinal motility: pharmacology, clinical effects, and potential therapeutics in humans, *Neurogastroenterol. Motil.* (2018), <https://doi.org/10.1111/nmo.13370> e13370.
- [8] A. Izzo, K. Sharkey, Cannabinoids and the gut: new developments and emerging concepts, *Pharmacol. Therapeut.* 126 (2010) 21–38, <https://doi.org/10.1016/j.pharmthera.2009.12.005>.
- [9] L. Matsuda, S. Lolait, Young A Brownstein, T. Bonner, Structure of a cannabinoid receptor and functional expression of the cloned cDNA, *Nature* 346 (1990) 561–564, <https://doi.org/10.1038/346561a0>.
- [10] S. Munro, K. Thomas, M. Abu-Shaar, Molecular characterization of a peripheral receptor for cannabinoids, *Nature* 365 (1993) 61–65, <https://doi.org/10.1038/365061a0>.
- [11] S.E. O'Sullivan, D.A. Kendall, Cannabinoid activation of peroxisome proliferator-activated receptors: potential for modulation of inflammatory disease, *Immunobiology* 215 (2010) 611–616, <https://doi.org/10.1016/j.imbio.2009.09.007>.
- [12] H. Goyal, U. Singla, U. Gupta, E. May, Role of cannabis in digestive disorders, *Eur. J. Gastroenterol. Hepatol.* 29 (2017) 135–143, <https://doi.org/10.1097/MEG.0000000000000779>.
- [13] F. Borrelli, A. Izzo, Role of acylethanolamides in the gastrointestinal tract with special reference to food intake and energy balance, *Best Pract Res Clin. Endocrinol. Metab.* 23 (2009) 33–49, <https://doi.org/10.1016/j.beem.2008.10.003>.
- [14] V. Di Marzo, A. Fontana, Anandamide, an endogenous cannabinomimetic eicosanoid: 'killing two birds with one stone', *Prostaglandins Leukot. Essent. Fatty Acids* 53 (1995) 1–11 PMID: 7675818.
- [15] E. Gill, W. Paton, R. Pertwee, Preliminary experiments on the chemistry and pharmacology of cannabis, *Nature* 228 (1970) 134–136 PMID: 5466704.
- [16] S. Roth, Stereospecific presynaptic inhibitory effect of delta 9-tetrahydrocannabinol on cholinergic transmission in the myenteric plexus of the guinea pig, *Can. J. Physiol. Pharmacol.* 56 (1978) 968–975 PMID: 217512.
- [17] A.A. Izzo, N. Mascolo, F. Borrelli, F. Capasso, Excitatory transmission to the circular muscle of the guinea-pig ileum: evidence for the involvement of cannabinoid CB1 receptors, *British J. Pharmacol.* 124 (1998) 1363–1368.
- [18] L. Pinto, A.A. Izzo, M.G. Cascio, T. Bisogno, K. Hospodar-Scott, D.R. Brown, et al., Endocannabinoids as physiological regulators of colonic propulsion in mice, PMID: 12105851, *Gastroenterology* 123 (2002) 227–234.
- [19] K. Wright, N. Rooney, M. Feeney, J. Tate, D. Robertson, M. Welham, et al., Differential expression of cannabinoid receptors in the human colon: cannabinoids promote epithelial wound healing, *Gastroenterology* 129 (2005) 437–453, <https://doi.org/10.1016/j.gastro.2005.05.026>.
- [20] A. Izzo, M. Camilleri, Cannabinoids in intestinal inflammation and cancer, *Pharmacol. Res.* 60 (2009) 117–125, <https://doi.org/10.1016/j.phrs.2009.03.008>.
- [21] G. Esposito, E. Capocchia, F. Turco, L.L. Palumbo, J. Lu, A. Steardo, et al., Palmitoylethanolamide improves colon inflammation through an enteric glia/toll like receptor 4-dependent PPAR-alpha activation, *Gut* 63 (2013) 1300–1312, <https://doi.org/10.1136/gutjnl-2013-305005>.
- [22] G. Godlewski, L. Offertaler, J. Wagner, G. Kunos, Receptors for acylethanolamides—GPR55 and GPR119, *Prostaglandins Other Lipid Mediat.* 89 (2009) 105–111, <https://doi.org/10.1016/j.prostaglandins.2009.07.001>.
- [23] R. Abalo, G. Vera, A.E. López-Pérez, M. Martínez-Villaluenga, M.I. Martín-Fontelles, The gastrointestinal pharmacology of cannabinoids: focus on motility, *Pharmacology* 90 (2012) 1–10, <https://doi.org/10.1159/000339072>.
- [24] K. Li, J. Fichna, R. Schicho, D. Saur, M. Bashashati, K. Mackie, et al., A role for O-1602 and G protein-coupled receptor GPR55 in the control of colonic motility in mice, *Neuropharmacology* 71 (2013) 255–263, <https://doi.org/10.1016/j.neuropharm.2013.03.029>.
- [25] S.D. Smid, C.K. Bjorklund, K.M. Svensson, S. Heigis, A. Revesz, The endocannabinoids anandamide and 2-arachidonoylglycerol inhibit cholinergic contractility in the human colon, *Eur. J. Pharmacol.* 575 (2007) 168–176, <https://doi.org/10.1016/j.ejphar.2007.07.036>.
- [26] A. Szymaszkievicz, M. Zielinska, K. Li, M. Ramanathan, S. Alam, D.R. Hou, et al., Novel derivatives of 1,2,3-triazole, cannabinoid-1 receptor ligands modulate gastrointestinal motility in mice, *Naunyn Schmiedebergs Arch. Pharmacol.* 391 (2018) 435–444, <https://doi.org/10.1007/s00210-018-1465-9>.
- [27] R.W. McCallum, I. Soykan, K.R. Sridhar, D.A. Ricci, R.C. Lange, M.W. Plankey, Delta-9-tetrahydrocannabinol delays the gastric emptying of solid food in humans: a double-blind, randomized study, *Aliment Pharmacol. Ther.* 13 (1999) 77–80 PMID: 9892882.
- [28] T. Esfandyari, M. Camilleri, I. Busciglio, D. Burton, K. Baxter, A.R. Zinsmeister, Effects of a cannabinoid receptor agonist on colonic motor and sensory functions in humans: a randomized, placebo-controlled study, *Am. J. Physiol. Gastrointest. Liver Physiol.* 293 (2007) G137–G145, <https://doi.org/10.1152/ajpgi.00565.2006>.
- [29] T. Esfandyari, M. Camilleri, I. Ferber, D. Burton, K. Baxter, A.R. Zinsmeister, Effect of a cannabinoid agonist on gastrointestinal transit and postprandial satiation in healthy human subjects: a randomized, placebo-controlled study, *Neurogastroenterol. Motil.* 18 (2006) 831–838, <https://doi.org/10.1111/j.1365-2982.2006.00834.x>.
- [30] M. Storr, B. Yuce, C. Andrews, K.A. Sharkey, The role of the endocannabinoid system in the pathophysiology and treatment of irritable bowel syndrome, *Neurogastroenterol. Motil.* 20 (2008) 857–868, <https://doi.org/10.1111/j.1365-2982.2008.01175.x>.
- [31] R. Mathison, W. Ho, Q. Pittman, J.S. Davison, K.A. Sharkey, Effects of cannabinoid receptor-2 activation on accelerated gastrointestinal transit in lipopolysaccharide-treated rats, *Br. J. Pharmacol.* 142 (2004) 1247–1254, <https://doi.org/10.1038/sj.bjp.0705889>.
- [32] R. Abalo, P.A. Cabezas, V. López-Miranda, G. Vera, C. González, M. Castillo, et al., Selective lack of tolerance to delayed gastric emptying after daily administration of WIN 55,212-2 in the rat, 1002-e80, *Neurogastroenterol. Motil.* 21 (2009), <https://doi.org/10.1111/j.1365-2982.2009.01315.x>.
- [33] R. Abalo, P.A. Cabezas, G. Vera, V. López-Miranda, E. Herradón, M.I. Martín-Fontelles, Cannabinoid-induced delayed gastric emptying is selectively increased upon intermittent administration in the rat: role of CB1 receptors, *Neurogastroenterol. Motil.* 23 (2011) 457–467, <https://doi.org/10.1111/j.1365-2982.2011.01677.x>.
- [34] R. Abalo, C. Chen, G. Vera, J. Fichna, G.A. Thakur, A.E. López-Pérez, et al., In vitro and non-invasive in vivo effects of the cannabinoid-1 receptor agonist AM841 on gastrointestinal motor function in the rat, *Neurogastroenterol. Motil.* 27 (2015) 1721–1723, <https://doi.org/10.1111/nmo.12668>.
- [35] J. Fichna, M. Bawa, Thakur Gam, R. Tichkule, A. Makriyannis, D.M. McCafferty, et al., Cannabinoids alleviate experimentally induced intestinal inflammation by acting at central and peripheral receptors, *PLoS One.* 9 (2014) e109115, <https://doi.org/10.1371/journal.pone.0109115>.
- [36] P. Kuo, R.H. Holloway, Beyond acid suppression: new pharmacologic approaches for treatment of GERD, *Curr. Gastroenterol. Rep.* 12 (2010) 175–180, <https://doi.org/10.1007/s11894-010-0102-7>.
- [37] M. Van Sickle, L. Oland, W. Ho, C.J. Hillard, K. Mackie, J.S. Davison, et al., Cannabinoids inhibit emesis through CB1 receptors in the brainstem of the ferret, PMID: 11606489, *Gastroenterology* 121 (2001) 767–774.
- [38] C. Calabrese, E. Spisni, G. Liguori, G. Lazzarini, M.C. Valerii, A. Strillacci, et al., Potential role of the cannabinoid receptor CB1 in the pathogenesis of erosive and non-erosive gastro-oesophageal reflux disease, *Aliment Pharmacol. Ther.* 32 (2010) 603–611, <https://doi.org/10.1111/j.1365-2036.2010.04377.x>.
- [39] A. Lehmann, L.A. Blackshaw, L. Brändén, A. Carlsson, J. Jensen, E. Nygren, et al., Cannabinoid receptor agonism inhibits transient lower esophageal sphincter relaxations and reflux in dogs, PMID: 12360475, *Gastroenterology* 123 (2002) 1129–1134.
- [40] H. Beaumont, J. Jensen, A. Carlsson, M. Ruth, A. Lehmann, G. Boeckxstaens, et al., Effect of delta9-tetrahydrocannabinol, a cannabinoid receptor agonist, on the triggering of transient lower esophageal sphincter relaxations in dogs and humans, *Br. J. Pharmacol.* 156 (2009) 153–162, <https://doi.org/10.1111/j.1476->

- 5381.2008.00010.x.
- [41] J. Gottfried, R. Kataria, R. Schey, Review: the role of cannabinoids on esophageal function-what we know thus far, *Cannabis Cannabinoid Res.* 2 (2017) 252–258, <https://doi.org/10.1089/can.2017.0031>.
- [42] E. Scarpellini, K. Blondeau, V. Boeckstaens, R. Vos, A. Gasbarrini, R. Farré, et al., Effect of rimonabant on oesophageal motor function in man, *Aliment Pharmacol. Ther.* 33 (2011) 730–737, <https://doi.org/10.1111/j.1365-2036.2011.04576.x>.
- [43] M. Bifulco, C. Grimaldi, P. Gazzero, S. Pisanti, A. Santoro, Rimonabant: just an antiobesity drug? Current evidence on its pleiotropic effects, *Mol. Pharmacol.* 71 (2007) 1445–1456, <https://doi.org/10.1124/mol.106.033118>.
- [44] M.A. Mushref, S. Srinivasan, Effect of high fat-diet and obesity on gastrointestinal motility, *Ann. Transl. Med.* 1 (2013) 14, <https://doi.org/10.3978/j.issn.2305-5839.2012.11.01>.
- [45] K. Gyires, Z.S. Zádori, Role of cannabinoids in gastrointestinal mucosal defense and inflammation, PMID: 26935536, *Curr. Neuropharmacol.* 14 (2016) 935–951.
- [46] F.C. Machado Rocha, S.C. Stéfano, Haiek R De Cássia, L.M. Rosa-Oliveira, D.X. Da Silveira, Therapeutic use of Cannabis sativa on chemotherapy-induced nausea and vomiting among cancer patients: systematic review and meta-analysis, *Eur. J. Cancer Care (Engl)* 17 (2008) 431–443, <https://doi.org/10.1111/j.1365-2354.2008.00917.x>.
- [47] E.M. Rock, L.A. Parker, Cannabinoids as potential treatment for chemotherapy-induced nausea and vomiting, *Front. Pharmacol.* 7 (2016) 221, <https://doi.org/10.3389/fphar.2016.00221>.
- [48] M.D. Van Sickle, M. Duncan, P.J. Kingsley, A. Mouihate, P. Urbani, K. Mackie, N. Stella, A. Makriyannis, et al., Identification and functional characterization of brainstem cannabinoid CB2 receptors, *Science* 310 (2005) 329–332.
- [49] M.A. Sticht, J.Z. Long, E.M. Rock, C.L. Limebeer, R. Mechoulam, B.F. Cravatt, L.A. Parker, Inhibition of monoacylglycerol lipase attenuates vomiting in *Suncus murinus* and 2-arachidonoyl glycerol attenuates nausea in rats, *British J. Pharmacol.* 165 (2012) 2425–2435.
- [50] E.M. Rock, D. Bolognini, C.L. Limebeer, M.G. Cascio, S. Anavi-Goffer, P.J. Fletcher, R. Mechoulam, R.G. Pertwee, et al., Cannabidiol, a non-psychoactive component of cannabis, attenuates vomiting and nausea-like behaviour via indirect agonism of 5-HT<sub>1A</sub> somatodendritic autoreceptors in the dorsal raphe nucleus, *British J. Pharmacol.* 165 (2012) 2620–2634.
- [51] M.D. Van Sickle, L.D. Oland, K. Mackie, J.S. Davison, K.A. Sharkey, Delta9-tetrahydrocannabinol selectively acts on CB1 receptors in specific regions of dorsal vagal complex to inhibit emesis in ferrets, *Am. J. Physiol. Gastrointest Liver Physiol.* 285 (3) (2003) G566–G576.
- [52] L.A. Parker, Conditioned flavor avoidance and conditioned gaping: rat models of conditioned nausea, *Eur. J. Pharmacol.* 722 (2014) 122–133, <https://doi.org/10.1016/j.ejphar.2013.09.070>.
- [53] L. Parker, C. Limebeer, E. Rock, M.A. Sticht, J. Ward, G. Turvey, et al., A comparison of novel, selective fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MAGL) or dual FAAH/MAGL inhibitors to suppress acute and anticipatory nausea in rat models, *Psychopharmacology*. 233 (2016) 2265–2275, <https://doi.org/10.1007/s00213-016-4277-y>.
- [54] K. Yamamoto, S. Tatsutani, T. Ishida, Detection of nausea-like response in rats by monitoring facial expression, *Front. Pharmacol.* 7 (2017) 534, <https://doi.org/10.3389/fphar.2016.00534>.
- [55] P.A. Cabezas, G. Vera, M. Castillo, et al., Radiological study of gastrointestinal motor activity after acute cisplatin in the rat. Temporal Relationship with Pica, *Auton. Neurosci.* 141 (2008) 54–65, <https://doi.org/10.1016/j.autneu.2008.05.004>.
- [56] P.A. Cabezas, G. Vera, M.I. Martín-Fontelles, R. Fernández-Pujol, M.I. Martín, R. Abalo, Cisplatin-induced gastrointestinal dysmotility is aggravated after chronic administration in the rat. Comparison with pica, *Neurogastroenterol. Motil.* 22 (2010) 797–805, <https://doi.org/10.1111/j.1365-2982.2010.01483.x>.
- [57] R. Abalo, P.A. Cabezas, G. Vera, A.E. López-Pérez, M.I. Martín, Cannabinoids may worsen gastric dysmotility induced by chronic cisplatin in the rat, *Neurogastroenterol. Motil.* 25 (2013) 373–382, <https://doi.org/10.1111/nmo.12073>.
- [58] G. Vera, A. Chiarlone, P.A. Cabezas, WIN 55,212–2 prevents mechanical allodynia but not alterations in feeding behaviour induced by chronic cisplatin in the rat, *Life Sci.* 81 (2007) 468–479, <https://doi.org/10.1016/j.lfs.2007.06.012>.
- [59] G. Vera, A.E. López-Pérez, J.A. Uranga, R. Girón, M.I. Martín-Fontelles, R. Abalo, Involvement of cannabinoid signaling in vincristine-induced gastrointestinal dysmotility in the rat, *Front. Pharmacol.* 8 (2017) 37, <https://doi.org/10.3389/fphar.2017.00037>.
- [60] L.S. Schwartzberg, Chemotherapy-induced nausea and vomiting: clinician and patient perspectives, *J. Support Oncol.* 5 (2 Suppl. 1) (2007) 5–12 PMID: 17366928.
- [61] M. Duran, E. Pérez, S. Abanades, X. Vidal, C. Saura, M. Majem, et al., Preliminary efficacy and safety of an oromucosal standardized cannabis extract in chemotherapy-induced nausea and vomiting, *Br. J. Clin. Pharmacol.* 70 (2010) 656–663, <https://doi.org/10.1111/j.1365-2125.2010.03743.x>.
- [62] V. Schussel, L. Kenzo, A. Santos, J. Bueno, E. Yoshimura, Cruz C de Oliveira, et al., Cannabinoids for nausea and vomiting related to chemotherapy: Overview of systematic reviews, *Phytother. Res.* 32 (2018) 567–576, <https://doi.org/10.1002/ptr.5975>.
- [63] M. Badowski, A review of oral cannabinoids and medical marijuana for the treatment of chemotherapy induced nausea and vomiting: a focus on pharmacokinetic variability and pharmacodynamics, *Cancer Chemother. Pharmacol.* 80 (2017) 441–449, <https://doi.org/10.1007/s00280-017-3387-5>.
- [64] J.V. Pergolizzi, R. Taylor, Concise review of the management of iatrogenic emesis using cannabinoids: emphasis on nabilone for chemotherapy-induced nausea and vomiting, *Cancer Chemother. Pharmacol.* 79 (2017) 467–477, <https://doi.org/10.1007/s00280-017-3257-1>.
- [65] M. Morales, O. Corsi, J. Peña, Are cannabinoids effective for the management of chemotherapy induced nausea and vomiting? *Medwave* 17 (2017) e7119, <https://doi.org/10.5867/medwave.2017.09.7119>.
- [66] J.H. Allen, G.M. de Moore, R. Heddle, J.C. Twartz, Cannabinoid hyperemesis: cyclical hyperemesis in association with chronic cannabis abuse, *Gut* 53 (2004) 1566–1570, <https://doi.org/10.1136/gut.2003.036350>.
- [67] D.A. Simonetto, A.S. Oxentenko, M.L. Herman, J.H. Szostek, Cannabinoid hyperemesis: a case series of 98 patients, *Mayo Clin. Proc.* 87 (2012) 114–119, <https://doi.org/10.1016/j.mayocp.2011.10.005>.
- [68] Z.W. Reichenbach, R. Schey, Cannabinoids and GI disorders: endogenous and exogenous, *Curr. Treat Options Gastroenterol.* 14 (2016) 461–477, <https://doi.org/10.1007/s11938-016-0111-1>.
- [69] D.A. Drossman, Functional gastrointestinal disorders: history, pathophysiology, clinical features and Rome IV, *Gastroenterology* (2016), <https://doi.org/10.1053/j.gastro.2016.02.032> pii: S0016-5085(16)00223-7.
- [70] F. Mearin, C. Ciriza, M. Mínguez, E. Rey, J.J. Mascort, E. Peña, et al., Clinical Practice Guideline: Irritable bowel syndrome with constipation and functional constipation in the adult, *Rev. Esp. Enferm. Dig.* 108 (2016) 332–363, <https://doi.org/10.17235/reed.2016.4389/2016>.
- [71] J. Fichna, M.A. Storr, Brain-Gut interactions in IBS, *Front. Pharmacol.* 3 (2012) 127, <https://doi.org/10.3389/fphar.2012.00127>.
- [72] L. Pinto, R. Capasso, G. Di Carlo, A.A. Izzo, Endocannabinoids and the gut, *Prostaglandins Leukot. Essent Fatty Acids* 66 (2002) 333–341, <https://doi.org/10.1054/plef.2001.0345>.
- [73] C.M. Keenan, M.A. Storr, G.A. Thakur, J.T. Wood, J. Wager-Miller, A. Straiker, et al., AM841, a covalent cannabinoid ligand, powerfully slows gastrointestinal motility in normal and stressed mice in a peripherally restricted manner, *Br. J. Pharmacol.* 172 (2015) 2406–2411, <https://doi.org/10.1111/bph.13069>.
- [74] R. Capasso, F. Borrelli, M.G. Cascio, G. Avieello, K. Huben, J.K. Zjawiony, et al., Inhibitory effect of salvinorin A, from *Salvia divinorum*, on ileitis-induced hypermotility: cross-talk between kappa-opioid and cannabinoid CB<sub>1</sub> receptors, *Br. J. Pharmacol.* 155 (2008) 681–689, <https://doi.org/10.1038/bjp.2008.294>.
- [75] A. Izzo, F. Fezza, R. Capasso, T. Bisogno, L. Pinto, T. Iuvone, et al., Cannabinoid CB<sub>1</sub>-receptor mediated regulation of gastrointestinal motility in mice in a model of intestinal inflammation, *Br. J. Pharmacol.* 134 (2001) 563–570, <https://doi.org/10.1038/sj.bjp.0704293>.
- [76] J. Fichna, A. Sibaev, M. Sałaga, M. Sobczak, M. Storr, The cannabinoid-1 receptor inverse agonist taranabant reduces abdominal pain and increases intestinal transit in mice, *Neurogastroenterol. Motil.* 25 (2013) e550–e559, <https://doi.org/10.1111/nmo.12158>.
- [77] M. Camilleri, P. Carlson, S. McKinzie, A. Grudell, I. Busciglio, D. Burton, et al., Genetic variation in endocannabinoid metabolism, gastrointestinal motility, and sensation, *Am. J. Physiol. Gastrointest Liver Physiol.* 294 (2008) G13–G19, <https://doi.org/10.1152/ajpgi.00371.2007>.
- [78] J. Fichna, J.T. Wood, M. Papanastasiou, S.K. Vadel, P. Oprocha, M. Sałaga, et al., Endocannabinoid and cannabinoid-like fatty acid amide levels correlate with pain-related symptoms in patients with IBS-D and IBS-C: a pilot study, *PLoS One* 8 (2013) e85073, <https://doi.org/10.1371/journal.pone.0085073>.
- [79] J.R. Xu, J.Y. Luo, L. Shang, W.M. Kong, Effect of change in an inhibitory neurotransmitter of the myenteric plexus on the pathogenic mechanism of irritable bowel syndrome subgroups in rat models, *Chin. J. Dig. Dis.* 7 (2006) 89–96 PMID: 16643336.
- [80] A. Sibaev, B. Yüce, M. Kemmer, L. Van Nassauw, U. Broedl, H.D. Allescher, B. Göke, J.P. Timmermans, et al., Cannabinoid-1 (CB<sub>1</sub>) receptors regulate colonic propulsion by acting at motor neurons within the ascending motor pathways in mouse colon, *Am. J. Physiol. Gastrointest Liver Physiol.* 296 (2009) G119–G128.
- [81] M. Camilleri, G.J. Kolar, M.I. Vazquez-Roque, P. Carlson, D.D. Burton, A.R. Zinsmeister, Cannabinoid receptor 1 gene and irritable bowel syndrome: phenotype and quantitative traits, *Am. J. Physiol. Gastrointest Liver Physiol.* 304 (2013) G553–G560, <https://doi.org/10.1152/ajpgi.00376.2012>.
- [82] C. Cremon, V. Stanghellini, M.R. Barbaro, R.F. Cogliandro, L. Bellacosa, J. Santos, et al., Randomised clinical trial: the analgesic properties of dietary supplementation with palmitoylethanolamide and polydatin in irritable bowel syndrome, *Aliment Pharmacol. Ther.* 45 (2017) 909–922, <https://doi.org/10.1111/apt.13958>.
- [83] S.C. Zhang, W.L. Wang, P.J. Su, K.L. Jiang, Z.W. Yuan, Decreased enteric fatty acid amide hydrolase activity is associated with colonic inertia in slow transit constipation, *J. Gastroenterol. Hepatol.* 29 (2014) 276–283, <https://doi.org/10.1111/jgh.12346>.
- [84] M. Bashashati, Y. Nasser, C.M. Keenan, W. Ho, F. Piscitelli, M. Nalli, et al., Inhibiting endocannabinoid biosynthesis: a novel approach to the treatment of constipation, *Br. J. Pharmacol.* 172 (2015) 3099–3111, <https://doi.org/10.1111/bph.13114>.
- [85] Z. Malik, D. Baik, R. Schey, The role of cannabinoids in regulation of nausea and vomiting, and visceral pain, *Curr. Gastroenterol. Rep.* 17 (2015) 429, <https://doi.org/10.1007/s11894-015-0429-1>.
- [86] M. Bashashati, J. Fichna, F. Piscitelli, R. Capasso, A.A. Izzo, A. Sibaev, et al., Targeting fatty acid amide hydrolase and transient receptor potential vanilloid-1 simultaneously to modulate colonic motility and visceral sensation in the mouse: a pharmacological intervention with N-arachidonoyl-serotonin (AA-5-HT), *Neurogastroenterol. Motil.* 29 (2017), <https://doi.org/10.1111/nmo.13148>.
- [87] J. Fioramonti, L. Bueno, Role of cannabinoid receptors in the control of gastrointestinal motility and perception, *Expert Rev. Gastroenterol. Hepatol.* 2 (3) (2008) 385–397, <https://doi.org/10.1586/17474124.2.3.385>.

- [88] M. Sanson, L. Bueno, J. Fioramonti, Involvement of cannabinoid receptors in inflammatory hypersensitivity to colonic distension in rats, *Neurogastroenterol Motil.* 18 (2016) 949–956, <https://doi.org/10.1111/j.1365-2982.2006.00819.x>.
- [89] J. Cosnes, C. Gower-Rousseau, P. Seksik, A. Cortot, Epidemiology and natural history of inflammatory bowel diseases, *Gastroenterology* 140 (2011) 1785–1794, <https://doi.org/10.1053/j.gastro.2011.01.055>.
- [90] A. Schirbel, C. Fiocchi, Inflammatory bowel disease: established and evolving considerations on its etiopathogenesis and therapy, *J. Digest Dis.* 11 (2010) 266–276, <https://doi.org/10.1111/j.1751-2980.2010.00449.x>.
- [91] M. Scharl, G. Rogler, Inflammatory bowel disease pathogenesis: what is new? *Curr. Opin. Gastroenterol.* 28 (2012) 301–309, <https://doi.org/10.1097/MOG.0b013e328353e61e>.
- [92] J.R. Maxwell, W.A. Brown, C.L. Smith, F.R. Byrne, J.L. Viney, Methods of inducing inflammatory bowel disease in mice, *Curr. Protoc. Pharmacol.* (2009) (Chapter 5:Unit 5.58) <https://doi.org/10.1002/0471141755.ph0558s47>.
- [93] F. Massa, G. Marsicano, H. Hermann, A. Cannich, K. Monory, B.F. Cravatt, et al., The endogenous cannabinoid system protects against colonic inflammation, *J. Clin. Invest.* 113 (2004) 1202–1209, <https://doi.org/10.1172/JCI200419465>.
- [94] E.S. Kimball, C.R. Schneider, N. Wallace, P. Hornby, Agonists of cannabinoid receptor 1 and 2 inhibit experimental colitis induced by oil of mustard and by dextran sulfate sodium, *Am. J. Physiol. Gastrointestinal Liver Physiol.* 291 (2006) G364–G371, <https://doi.org/10.1152/ajpgi.00407.2005>.
- [95] M.A. Storr, C.M. Keenan, H. Zhang, K.D. Patel, A. Makriyannis, K.A. Sharkey, Activation of the cannabinoid 2 receptor (CB2) protects against experimental colitis, *Inflamm. Bowel Dis.* 15 (2009) 1678–1685, <https://doi.org/10.1002/ibd.20960>.
- [96] G. D'Argenio, M. Valenti, G. Scaglione, V. Cosenza, I. Sorrentini, V. Di Marzo, Up-regulation of anandamide levels as an endogenous mechanism and a pharmacological strategy to limit colon inflammation, *FASEB J.* 20 (2006) 568–570, <https://doi.org/10.1096/fj.05-4943fe>.
- [97] S. Lin, Y. Li, L. Shen, R. Zhang, L. Yang, M. Li, et al., 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.* 62 (2017) 372–386, <https://doi.org/10.1007/s10620-016-4404-y>.
- [98] M.A. Engel, C.A. Kellermann, G. Burnat, E.G. Hahn, T. Rau, P.C. Konturek, Mice lacking cannabinoid CB1-, CB2-receptors or both receptors show increased susceptibility to trinitrobenzene sulfonic acid (TNBS)-induced colitis, *J. Physiol. Pharmacol.* 61 (2010) 89–97 PMID: 20228420.
- [99] M.A. Storr, C.M. Keenan, D. Emmerding, H. Zhang, B. Yüce, A. Sibae, et al., Targeting endocannabinoid degradation protects against experimental colitis in mice: involvement of CB1 and CB2 receptors, *J. Mol. Med.* 86 (2008) 925–936, <https://doi.org/10.1007/s00109-008-0359-6>.
- [100] M. Salaga, A. Mokrowiecka, P.K. Zakrzewski, A. Cygankiewicz, E. Leishman, M. Sobczak, et al., 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* 8 (2014) 998–1009, <https://doi.org/10.1016/j.crohns.2014.01.025>.
- [101] H. Sharman, N.P. Singh, E.E. Zumbun, A. Murphy, D.D. Taub, M.K. Mishra, et al., Fatty acid amide hydrolase (FAAH) blockade ameliorates experimental colitis by altering microRNA expression and suppressing inflammation, *Brain Behav. Immun.* 59 (2017) 10–20, <https://doi.org/10.1016/j.bbi.2016.06.008>.
- [102] O. Sasso, M. Migliore, D. Habrant, A. Armirotti, C. Albani, M. Summa, et al., Multitarget fatty acid amide hydrolase/cyclooxygenase blockade suppresses intestinal inflammation and protects against nonsteroidal anti-inflammatory drug-dependent gastrointestinal damage, *The FASEB J.* 29 (2015) 2616–2627, <https://doi.org/10.1096/fj.15-270637>.
- [103] M. Alhouayek, P. Botteman, K.V. Subramanian, D.M. Lambert, A. Makriyannis, P.D. Cani, et al., N-Acylethanolamine-hydrolyzing acid amidase inhibition increases colon N-palmitoylethanolamine levels and counteracts murine colitis, *The FASEB J.* 29 (2015) 650–661, <https://doi.org/10.1096/fj.14-255208>.
- [104] F. Borrelli, B. Romano, S. Petrosino, E. Pagano, R. Capasso, D. Coppola, et al., Palmitoylethanolamide, a naturally occurring lipid, is an orally effective intestinal anti-inflammatory agent, *Br. J. Pharmacol.* 172 (2015) 142–158, <https://doi.org/10.1111/bph.12907>.
- [105] F. Borrelli, I. Fasolino, B. Romano, R. Capasso, F. Maiello, D. Coppola, et al., Beneficial effect of the non-psychoactive plant cannabinoid cannabigerol on experimental inflammatory bowel disease, *Biochem. Pharmacol.* 85 (2013) 1306–1316, <https://doi.org/10.1016/j.bcp.2013.01.017>.
- [106] E. Pagano, R. Capasso, F. Piscitelli, B. Romano, O.A. Parisi, S. Finizio, et al., An orally active cannabis extract with high content in cannabidiol attenuates chemically-induced intestinal inflammation and hypermotility in the mouse, *Front. Pharmacol.* 7 (2016) 341, <https://doi.org/10.3389/fphar.2016.00341>.
- [107] D. de Filippis, G. Esposito, C. Cirillo, M. Cipriano, B.Y. De Winter, C. Scuderi, et al., Cannabidiol reduces intestinal inflammation through the control of neuroimmune axis, *PLoS ONE* 6 (2011) 1–8, <https://doi.org/10.1371/journal.pone.0028159>.
- [108] J.M. Jamontt, A. Molleman, R.G. Pertwee, M.E. Parsons, The effects of delta 9-tetrahydrocannabinol and cannabidiol alone and in combination on damage, inflammation and in vitro motility disturbances in rat colitis, *Br. J. Pharmacol.* 160 (2010) 712–723, <https://doi.org/10.1111/j.1476-5381.2010.00791.x>.
- [109] R. Schicho, M. Bashashati, M. Bawa, D. McHugh, D. Saur, H.M. Hu, et al., The atypical cannabinoid O-1602 protects against experimental colitis and inhibits neutrophil recruitment, *Inflamm. Bowel Dis.* 17 (2011) 1651–1664, <https://doi.org/10.1002/ibd.21538>.
- [110] A. Stancić, K. Jandl, C. Hasenöhr, F. Reichmann, G. Marsche, R. Schuligoi, et al., The GPR55 antagonist CID16020046 protects against intestinal inflammation, *Neurogastroenterol. Motil.* 27 (2015) 1432–1445, <https://doi.org/10.1111/nmo.12639>.
- [111] M. Storr, S. Devlin, G.G. Kaplan, R. Panaccione, C.N. Andrews, 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.* 20 (2014) 472–480, <https://doi.org/10.1097/01.MIB.0000440982.79036.d6>.
- [112] J. Ravikoff Allegretti, A. Courtwright, M. Lucci, J. Levine, et al., Marijuana use patterns among patients with inflammatory bowel disease, *Inflamm. Bowel Dis.* 19 (2013) 2809–2814, <https://doi.org/10.1097/01.MIB.0000435851.94391.37>.
- [113] S. Lal, N. Prasad, M. Ryan, S. Tangri, M.S. Silverberg, A. Gordon, et al., Cannabis use amongst patients with inflammatory bowel disease, *Eur. J. Gastroenterol. Hepatol.* 23 (2011) 891–896, <https://doi.org/10.1097/MEG.0b013e328349bb4c>.
- [114] T. Naftali, L. Bar-Lev, I. Dotan, E.P. Lansky, F.S. Benjaminov, F.M. Meir Konikoff, Cannabis induces a clinical response in patients with Crohn's disease: a prospective placebo-controlled study, 1276–1280.e1, *Clin. Gastroenterol. Hepatol.* 11 (2013), <https://doi.org/10.1016/j.cgh.2013.04.034>.
- [115] A. Lahat, A. Lang, S. Ben-Horin, Impact of cannabis treatment on the quality of life, weight and clinical disease activity in inflammatory bowel disease patients: a pilot prospective study, *Digestion* 85 (2012) 1–8, <https://doi.org/10.1159/00032079>.
- [116] T. Naftali, R. Mechulam, A. Marii, G. Gabay, A. Stein, M. Bronshtain, et al., Low-dose cannabidiol is safe but not effective in the treatment for Crohn's disease, a randomized controlled trial, *Dig. Dis. Sci.* 62 (2017) 1615–1620, <https://doi.org/10.1007/s10620-017-4540-z>.
- [117] R. Nallathambi, M. Mazuz, A. Ion, G. Selvaraj, S. Weininger, M. Fridlender, et al., Anti-inflammatory activity in colon models is derived from D9-tetrahydrocannabinolic acid that interacts with additional compounds in Cannabis extracts, *Cannabis Cannabinoid Res.* 2 (2017) 167–182, <https://doi.org/10.1089/can.2017.0027>.
- [118] C. Hasenöhr, M. Storr, R. Schicho, Cannabinoids for treating inflammatory bowel diseases: where are we and where do we go? *Expert Rev. Gastroenterol. Hepatol.* 11 (2017) 329–337, <https://doi.org/10.1080/17474124.2017.1292851>.
- [119] L. Marquez, J. Suarez, M. Iglesias, F.J. Bernudez-Silva, F. Rodríguez de Fonseca, M. Andreu, Ulcerative colitis induces changes on the expression of the endocannabinoid system in the human colonic tissue, *PLoS ONE* 4 (2009) e6893, <https://doi.org/10.1371/journal.pone.0006893>.
- [120] K. Ihenetu, A. Molleman, M.E. Parsons, C.J. Whelan, Inhibition of interleukin-8 release in the human colonic epithelial cell line HT-29 by cannabinoids, *Eur. J. Pharmacol.* 458 (2003) 207–215 PMID: 12498928.
- [121] B.S. Harvey, L.L. Nicotra, M. Vu, S.D. Smid, Cannabinoid CB2 receptor activation attenuates cytokine-evoked mucosal damage in a human colonic explant model without changing epithelial permeability, *Cytokine* 63 (2013) 209–217, <https://doi.org/10.1016/j.cyto.2013.04.032>.
- [122] A. Alhamoruni, A.C. Lee, K.L. Wright, M. Larvin, M. Larvin, S.E. O'Sullivan, et al., Pharmacological effects of cannabinoids on the Caco-2 cell culture model of intestinal permeability, *J. Pharmacol. Exp. Ther.* 335 (2010) 92–102, <https://doi.org/10.1124/jpet.110.168237>.
- [123] P.D. Cani, H. Plovier, M.V. Hul, L. Geurts, N.M. Delzenne, C. Druart, et al., Endocannabinoids - at the crossroads between the gut microbiota and host metabolism, *Nat. Rev. Endocrinol.* 12 (2015) 133–143, <https://doi.org/10.1038/nrendo.2015.211>.
- [124] M.P. Davis, Cannabinoids for Symptom Management and Cancer Therapy: The Evidence, *J Natl Compr Canc Netw* 14 (2016) 915–922 PMID: 27407130.
- [125] M. Brafford May, A.E. Glode, Dronabinol for chemotherapy-induced nausea and vomiting unresponsive to antiemetics, *Cancer Manage. Res.* 8 (2016) 49–55, <https://doi.org/10.2147/CMAR.S81425>.
- [126] V. Maida, P.J. Daeninck, A user's guide to cannabinoid therapies in oncology, *Curr. Oncol.* 23 (2016) 398–406, <https://doi.org/10.3747/co.23.3487>.
- [127] L. Bar-Lev Schleidera, R. Mechoulam, V. Lederman, M. Hilou, O. Lencovsky, O. Betzalel, et al., Prospective analysis of safety and efficacy of medical cannabis in large unselected population of patients with cancer, *Eur. J. Int. Med.* 49 (2018) 37–43, <https://doi.org/10.1016/j.ejim.2018.01.023>.
- [128] R. Abalo, J.A. Uranga, I. Pérez-García, R. de Andrés, R. Girón, G. Vera, et al., May cannabinoids prevent the development of chemotherapy-induced diarrhea and intestinal mucositis? Experimental study in the rat, *Neurogastroenterol. Motil.* 29 (2017) e12952, <https://doi.org/10.1111/nmo.12952>.
- [129] L. De Petrocellis, D. Melck, T. Bisogno, V. Di Marzo, Endocannabinoids and fatty acid amides in cancer, inflammation and related disorders, *Chem Phys Lipids.* 108 (2000) 191–209 PMID: 11106791.
- [130] J.A. Uranga, J.C. Cámara, E. Herradón, G. Vera, N. Jagerovic, E. Quesada, et al., New strategies for treatment and prevention of colorectal cancer, in: A. Tyagi, S. Prasad (Eds.), *Gastrointestinal Cancers*, Nova publishers, 2017, pp. 103–170, ISBN: 978-1-53610-178-2.
- [131] A. Ligresti, T. Bisogno, I. Matias, L. De Petrocellis, M.G. Cascio, V. Cosenza, et al., Possible endocannabinoid control of colorectal cancer growth, *Gastroenterology* 125 (2003) 677–687 PMID: 12949714.
- [132] L. Chen, H. Chen, Y. Li, L. Li, Y. Qiu, J. Ren, et al., Endocannabinoid and ceramide levels are altered in patients with colorectal cancer, *Oncol Rep.* 34 (2015) 447–454, <https://doi.org/10.3892/or.2015.3973>.
- [133] L. Ye, B. Zhang, E.G. Seviour, K.X. Tao, X.H. Liu, Y. Ling, et al., Monoacylglycerol lipase (MAGL) knockdown inhibits tumor cells growth in colorectal cancer, *Cancer Lett.* 307 (2011) 6–17, <https://doi.org/10.1016/j.canlet.2011.03.007>.
- [134] C.K. Jung, W.K. Kang, J.M. Park, H.J. Ahn, S.W. Kim, S.T. Oh, Expression of the cannabinoid type I receptor and prognosis following surgery in colorectal cancer, *Oncol. Lett.* 505 (2013) 870–876, <https://doi.org/10.3892/ol.2012.1081>.
- [135] D. Wang, H. Wang, W. Ning, M.G. Backlund, S.K. Dey, R.N. DuBois, Loss of

- cannabinoid receptor 1 accelerates intestinal tumor growth, *Cancer Res.* 68 (2008) 6468–6476, <https://doi.org/10.1158/0008-5472.CAN-08-0896>.
- [136] F. Cianchi, L. Papucci, N. Schiavone, M. Lulli, L. Magnelli, M.C. Vinci, et al., Cannabinoid receptor activation induces apoptosis through tumor necrosis factor alpha-mediated ceramide de novo synthesis in colon cancer cells, *Clin. Cancer Res.* 14 (2008) 7691–7700, <https://doi.org/10.1158/1078-0432.CCR-08-0799>.
- [137] S.B. Gustafsson, R. Palmqvist, M.L. Henriksson, A. Dahlin, S. Edin, S. Jacobsson, et al., High tumour cannabinoid CB1 receptor immunoreactivity negatively impacts disease-specific survival in stage II microsatellite stable colorectal cancer, *PLoS ONE* 6 (2011) e23003, <https://doi.org/10.1371/journal.pone.0023003>.
- [138] E. Martínez-Martínez, I. Gómez, P. Martín, A. Sánchez, L. Román, E. Tejerina, et al., Cannabinoids receptor type 2, CB2, expression correlates with human colon cancer progression and predicts patient survival, *Oncoscience* 2 (2015) 131–141, <https://doi.org/10.18632/oncoscience.119>.
- [139] G. Velasco, C. Sánchez, M. Guzmán, Towards the use of cannabinoids as anti-tumour agents, *Nat. Rev. Cancer* 12 (2012) 436–444, <https://doi.org/10.1038/nrc3247>.
- [140] A. Greenhough, H.A. Patsos, A.C. Williams, C. Paraskeva, The cannabinoid delta (9)-tetrahydrocannabinol inhibits RAS-MAPK and PI3K-AKT survival signalling and induces BAD-mediated apoptosis in colorectal cancer cells, *Int. J. Cancer* 121 (2007) 2172–2180, <https://doi.org/10.1002/ijc.22917>.
- [141] A.A. Izzo, G. AvIELlo, S. Petrosino, P. Orlando, G. Marsicano, B. Lutz, et al., Increased endocannabinoid levels reduce the development of precancerous lesions in the mouse colon, *J. Mol. Med.* 86 (2008) 89–98, <https://doi.org/10.1007/s00109-007-0248-4>.
- [142] B. Romano, F. Borrelli, E. Pagano, M.G. Cascio, R.G. Pertwee, A.A. Izzo, Inhibition of colon carcinogenesis by a standardized Cannabis sativa extract with high content of cannabidiol, *Phytomedicine* 21 (2014) 631–639, <https://doi.org/10.1016/j.phymed.2013.11.006>.
- [143] G. AvIELlo, B. Romano, F. Borrelli, R. Capasso, L. Gallo, F. Piscitelli, et al., Chemopreventive effect of the non-psychotropic phytocannabinoid cannabidiol on experimental colon cancer, *J. Mol. Med.* 90 (2012) 925–934, <https://doi.org/10.1007/s00109-011-0856-x>.
- [144] J. Kargl, L. Andersen, C. Hasenöhrl, D. Feuersinger, A. Stančić, A. Fauland, et al., GPR55 promotes migration and adhesion of colon cancer cells indicating a role in metastasis, *Brit. J. Pharmacol.* 173 (2016) 142–154, <https://doi.org/10.1111/bph.13345>.
- [145] A. Santoro, S. Pisanti, C. Grimaldi, A.A. Izzo, F. Borrelli, M.C. Proto, et al., Rimonabant inhibits human colon cancer cell growth and reduces the formation of precancerous lesions in the mouse colon, *Int. J. Cancer* 125 (2009) 996–1003, <https://doi.org/10.1002/ijc.24483>.
- [146] M.C. Proto, D. Fiore, C. Piscopo, S. Franceschelli, V. Bizzarro, C. Laezza, et al., Inhibition of Wnt/ $\beta$ -Catenin pathway and Histone acetyltransferase activity by Rimonabant: a therapeutic target for colon cancer, *Sci. Rep.* 7 (2017) 11678, <https://doi.org/10.1038/s41598-017-11688-x>.
- [147] D. Fiore, P. Ramesh, M. Proto, C. Piscopo, S. Franceschelli, S. Anzelmo, et al., Rimonabant kills colon cancer stem cells without inducing toxicity in normal colon organoids, *Front. Pharmacol.* 8 (2018) 949, <https://doi.org/10.3389/fphar.2017.00949>.
- [148] J. Kargl, J. Haybaeck, A. Stančić, L. Andersen, G. Marsche, A. Heinemann, et al., O-1602, an atypical cannabinoid, inhibits tumor growth in colitis-associated colon cancer through multiple mechanisms, *J. Mol. Med.* 91 (2013) 449–458, <https://doi.org/10.1007/s00109-012-0957-1>.
- [149] O. Pellerito, A. Notaro, S. Sabella, A. de Blasio, R. Vento, G. Calvaruso, et al., WIN induces apoptotic cell death in human colon cancer cells through a block of autophagic flux dependent on PPAR gamma down-regulation, *Apoptosis* 19 (2014) 1029–1042, <https://doi.org/10.1007/s10495-014-0985-0>.
- [150] J. Joseph, B. Niggemann, K. Zaenker, F. Entschladen, Anandamide is an endogenous inhibitor for the migration of tumor cells and T lymphocytes, *Cancer Immunol. Immunother.* 53 (2004) 723–728, <https://doi.org/10.1007/s00262-004-0509-9>.
- [151] D. Thapa, Y. Kang, P. Park, et al., Anti-tumor activity of the novel hexahydrocannabinol analog LYR-8 in human colorectal tumor xenograft is mediated through the inhibition of Akt and hypoxia-inducible factor-1 $\alpha$  activation, *Biol. Pharmaceut. Bull.* 35 (2012) 924–932 PMID: 22687485.
- [152] J.J. Galligan, C. Sternini, Insights into the role of opioid receptors in the GI tract: experimental evidence and therapeutic relevance, *Handb. Exp. Pharmacol.* 239 (2017) 363–378, [https://doi.org/10.1007/164\\_2016\\_116](https://doi.org/10.1007/164_2016_116).
- [153] D.M. Grunkemeier, J.E. Cassara, C.B. Dalton, D.A. Drossman, The narcotic bowel syndrome: clinical features, pathophysiology, and management, *Clin. Gastroenterol. Hepatol.* 5 (10) (2007) 1126–1139 quiz 1121–2.
- [154] A. Kurz 1, D. Sessler, Opioid-induced bowel dysfunction: pathophysiology and potential new therapies, *Drugs* 63 (7) (2003) 649–671.
- [155] V. Andresen, M. Camilleri, Irritable bowel syndrome: recent and novel therapeutic approaches, *Drugs* 66 (8) (2006) 1073–1088.
- [156] J. Tack, R. Bisschops, G. Sarnelli, Pathophysiology and treatment of functional dyspepsia, *Gastroenterology* 127 (4) (2004) 1239–1255.
- [157] R. Furlan, S. Ardizzone, L. Palazzolo, A. Rimoldi, F. Perego, F. Barbic, M. Bevilacqua, L. Vago, G. Bianchi Porro, A. Malliani, Sympathetic overactivity in active ulcerative colitis: effects of clonidine, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 290 (1) (2006) R224–R232 Epub 2005 Aug 25.
- [158] F. Lechin, B. van der Dijks, C.L. Insausti, F. Gómez, S. Villa, A.E. Lechin, L. Arocha, O. Oramas, Treatment of ulcerative colitis with clonidine, *J. Clin. Pharmacol.* 25 (3) (1985) 219–226 PMID: 16789793.
- [159] M. Camilleri, Toward an effective peripheral visceral analgesic: responding to the national opioid crisis, *Am. J. Physiol. Gastrointest. Liver Physiol.* 314 (6) (2018) G637–G646, <https://doi.org/10.1152/ajpgi.00013.2018>.
- [160] S.A. Grenald, M.A. Young, Y. Wang, M.H. Ossipov, M.M. Ibrahim, T.M. Largent-Milnes, T.W. Vanderah, Synergistic attenuation of chronic pain using mu opioid and cannabinoid receptor 2 agonists, *Neuropharmacology* 116 (2017) 59–70, <https://doi.org/10.1016/j.neuropharm.2016.12.008>.
- [161] U. Taschler, T.O. Eichmann, F.P. Radner, G.F. Grabner, H. Wolinski, M. Storr, A. Lass, R. Schicho, R. Zimmermann, Monoglyceride lipase deficiency causes desensitization of intestinal cannabinoid receptor type 1 and increased colonic  $\mu$ -opioid receptor sensitivity, *Br. J. Pharmacol.* 172 (17) (2015) 4419–4429, <https://doi.org/10.1111/bph.13224>.