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Review

Cannabinoids in intestinal inflammation and cancer

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ABSTRACT

Emerging evidence suggests that cannabinoids may exert beneficial effects in intestinal inflammation and cancer. Adaptive changes of the endocannabinoid system have been observed in intestinal biopsies from patients with inflammatory bowel disease and colon cancer. Studies on epithelial cells have shown that cannabinoids exert antiproliferative, antimetastatic and apoptotic effects as well as reducing cytokine release and promoting wound healing. *In vivo*, cannabinoids – via direct or indirect activation of CB₁ and/or CB₂ receptors – exert protective effects in well-established models of intestinal inflammation and colon cancer. Pharmacological elevation of endocannabinoid levels may be a promising strategy to counteract intestinal inflammation and colon cancer.

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Contents

1. Introduction.....	118
2. Intestinal inflammation.....	118
2.1. Studies on intestinal epithelial cells.....	118
2.2. Endocannabinoid and cannabinoid receptor changes in human intestinal biopsies.....	118
2.3. Effect of cannabinoid drugs in experimental models of IBD.....	119
2.4. Visceral sensation in the inflamed gut.....	120
2.5. Intestinal motility in the inflamed gut.....	120
2.6. Anandamide as an endovanilloid in the inflamed gut.....	120
2.7. Distribution of FAAH polymorphism in patients with Crohn's disease.....	120
3. Intestinal cancer.....	121
3.1. Studies on colorectal cancer cell lines.....	121
3.1.1. Antiproliferative/apoptotic effects CB ₁ or CB ₂ receptor activation.....	121
3.1.2. Antiproliferative/apoptotic effects via prostamides production.....	121
3.1.3. Antimetastatic actions of cannabinoids.....	122
3.1.4. Oestrogens and CB ₁ receptors.....	122
3.2. Endocannabinoid and cannabinoid receptor changes in human intestinal cancer biopsies.....	122
3.3. Effect of cannabinoid drugs in experimental models of colon cancer.....	122

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4.	Non-psychoactive plant cannabinoids in intestinal inflammation and cancer	122
4.1.	Intestinal inflammation	123
4.2.	Intestinal cancer	123
5.	Anandamide-related acylethanolamides and their role in intestinal inflammation and cancer	123
6.	Paradoxical beneficial effects of rimonabant in intestinal inflammation and cancer	123
7.	Conclusions	123
	Conflict of interest	123
	Acknowledgements	124
	References	124

1. Introduction

The marijuana plant *Cannabis sativa* is possibly one of the oldest plants cultivated by humans, but it has also been a source of controversy throughout history [1,2]. The plant has provided insights to medicine and has pointed the way in the last two decades toward a host of medical challenges from analgesia to weight loss through [1,2]. The main active ingredient in *Cannabis* is Δ^9 -tetrahydrocannabinol (Δ^9 -THC), which activates two Gi/o-coupled membrane receptors, named CB₁ and CB₂ receptors [3]. CB₁ receptors are located throughout the gastrointestinal tract, mainly in myenteric and submucosal neurons, but they are also expressed by non-neuronal cells such as epithelial cells (reviewed in Izzo and Camilleri [4]). CB₂ receptors are mainly located on inflammatory and epithelial cells, although recent evidence suggests the presence of CB₂ receptors in myenteric and submucosal neurons [5,6].

Endogenous ligands that activate cannabinoid receptors [i.e. the endocannabinoids, anandamide and 2-arachidonylglycerol (2-AG)] [7,8] have been identified in mammalian tissues, and their levels may increase in pathophysiological states affecting the intestine, such as inflammation and cancer [9]. Endocannabinoids are biosynthesized 'on demand' from membrane phospholipids and released from cells immediately after their production. Following receptor activation and induction of a biological response, endocannabinoids are inactivated through a reuptake process facilitated by a putative endocannabinoid membrane transporter (EMT) followed by enzymatic degradation catalysed by the fatty acid amide hydrolase (FAAH, in the case of anandamide) or by monoacylglycerol lipase (MGL, and possibly FAAH, in the case of 2-AG) [4,10,11]. These catalytic enzymes have also been identified in the digestive tract [12,13]. Apart from effects on cannabinoid receptors, the endocannabinoid anandamide may also activate the transient receptor potential (TRP) vanilloid type 1 (TRPV1), which is mainly expressed by primary afferent neurons and the orphan G-protein-coupled receptor GPR55 [3,14].

Although cannabinoids exert important physiological and pathophysiological actions in the digestive tract, including appetite regulation, emesis, protection of the gastric mucosa, intestinal ion transport, gastric emptying and intestinal motility [4,15–21], this review will focus on the role and the effects of cannabinoids in inflammation and cancer within the gut.

2. Intestinal inflammation

Some patients with inflammatory bowel disease (IBD) anecdotally report that they experience relief by smoking marijuana; in one series from Spain, about 10% of IBD patients consumed cannabis, typically before the diagnosis was made; one third of the patients informed their physician about use of Cannabis [22]. Enhancement of cannabinoid signalling, as revealed by the increased intestinal expression of CB₁/CB₂ receptors and/or endocannabinoid levels has been observed following inflammatory stimuli, both in animals and humans. Experiments on isolated epithelial cells and *in*

vivo studies using well-established models of IBD indicates that the endogenous cannabinoid system, via CB₁ or CB₂ receptor activation, mediates protective mechanisms counteracting intestinal inflammatory responses that are considered pathophysiological in IBD. Moreover, cannabinoids may reduce hypermotility and visceral hypersensitivity associated with intestinal inflammation [9,23], and thus impact on some of the clinical manifestations of IBD.

2.1. Studies on intestinal epithelial cells

Cannabinoids have been shown to exert pharmacological actions on epithelial cells; these effects may explain the benefits observed in experimental models of IBD.

Epithelial cells play a pivotal role in host defence against microorganisms in the intestinal lumen, and in inflammatory responses. In addition to their function as barriers preventing absorption of potentially deleterious substances, epithelial cells also express a variety of pro-inflammatory cytokines, which are up-regulated in IBD [24]. A number of cannabinoid receptor agonists, including the plant-derived Δ^9 -THC, have been shown to exert an inhibitory effect on the expression of TNF- α -induced interleukin-release from the human colonic epithelial cell line HT-29 [25]; this inhibition on inflammatory process is sensitive to CB₂ antagonist. Furthermore, delayed wound healing, a typical feature of IBD patients [26] may be modulated by cannabinoid drugs [27]. Thus, the endogenous cannabinoid ligands anandamide (non-selective cannabinoid agonist), noladin ether (CB₁ selective receptor agonist) as well as the synthetic selective CB₁ agonist arachidonylcyclopropylamide, ACPA (but not the synthetic CB₂ agonist JWH133) induced wound closure in HT29 and DLD1 epithelial cells [27].

Overall, studies on intestinal epithelial cells have shown that cannabinoids can exert protective effect by promoting wound healing via CB₁ receptors activation and by suppressing the release of pro-inflammatory cytokines via CB₂ receptors activation (Fig. 1).

2.2. Endocannabinoid and cannabinoid receptor changes in human intestinal biopsies

Increased expression of cannabinoid receptors and/or enhanced endocannabinoid levels have been generally observed in intestinal biopsies of patients with gut inflammatory diseases, including ulcerative colitis, Crohn's disease, diverticulitis and celiac disease (CD).

A more than 2-fold elevation of anandamide, but not 2-AG, levels was found in mucosal biopsies from patients with untreated ulcerative colitis relative to control biopsies. Anandamide levels significantly correlated with clinical activity of the disease, while no correlation was found between endocannabinoid levels and endoscopic and histologic activities [28]. In a different study, Wright et al. determined the location of both CB₁ and CB₂ receptors in normal and IBD human colonic tissue. Epithelial CB₁ immunoreactivity was evident in acute-phase IBD (not specified by author,

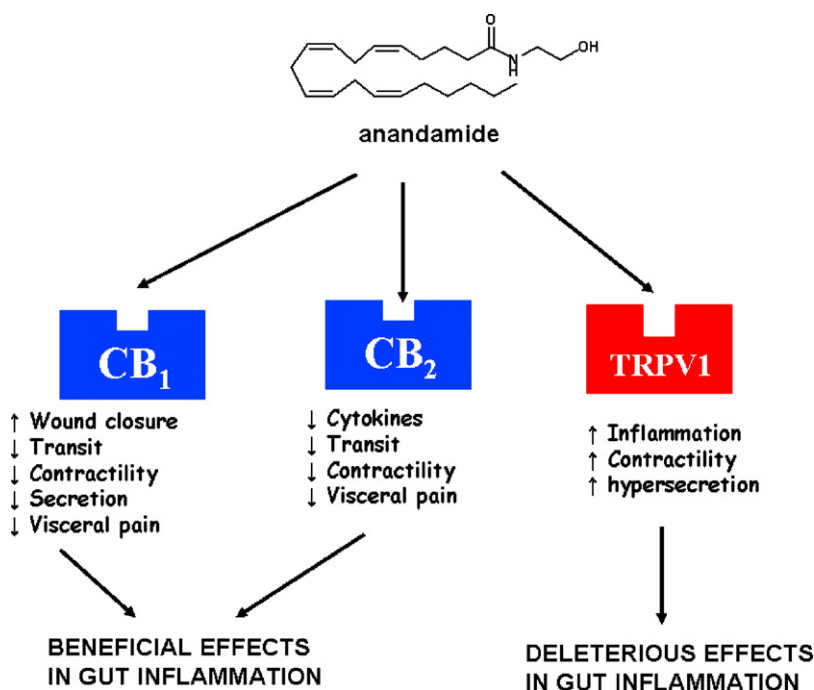


Fig. 1. Anandamide and intestinal inflammation. Anandamide may activate CB₁ and CB₂ receptors resulting in beneficial effects and, at higher concentrations, TRPV1, resulting in deleterious effects in the inflamed gut. Key information is reported in Refs. [28,33,34,55]. Note that this scheme reflects the action of anandamide and may not reflect the actions of other cannabinoid receptor agonists.

but most likely ulcerative colitis from the histological appearance of mucin depletion and neutrophil infiltration) and Crohn's disease, as seen in normal tissue, although with lower intensity. In contrast, epithelial CB₂ immunoreactivity, faintly detected in normal tissues, was always evident in colonic mucosa in acute-phase IBD, appeared more intense in the cytoplasm and was expressed in the membrane on the microvillus border. In Crohn's disease, intense CB₂ expression was evident in the epithelium of the crypts where ulceration had occurred [27].

Celiac disease is an intestinal disorder caused by intolerance to gluten (proteins in wheat) which causes inflammatory injury of the small intestinal mucosa [29]. The disease is associated with increased intestinal levels of anandamide and CB₁ receptors. The significantly elevated levels of anandamide in active celiac disease returned to normal after remission with a gluten-free diet. CB₁ receptors were detected mostly in elongated, fiber-like structures that may correspond possibly to neuronal fibres. Importantly fibers expressing CB₁ receptors were located in the subepithelial region, where gluten-reactive pro-inflammatory Th1 cells are present [30]. As in human studies, the experimental model of celiac disease induced by methotrexate, is associated with intestinal endocannabinoid levels that peak with atrophy and regress with remission [30].

Diverticulitis occurs when the mucosa of diverticula (small out-pouchings from the colonic lumen caused by mucosal herniations through the wall) becomes inflamed [31]. Guagnini et al. reported tissue levels of anandamide and TRPV1 were twice as high compared to control colon, whereas 2-AG levels were slightly lower in diverticulitis than in control segments. The expression levels of cannabinoid CB₁ receptors, measured by RT-PCR, were similar in colonic segments with diverticula and control segments. On the basis of functional *in vitro* experiments on intestinal contractility, the same authors suggest that variations in the endogenous cannabinoid system may lead to the altered neuronal control of motility observed in patients with diverticulitis [32].

2.3. Effect of cannabinoid drugs in experimental models of IBD

Direct activation of both CB₁ or CB₂ receptors is protective in experimental models of IBD. In the mustard oil model of colitis, the CB₁ agonist arachidonoyl-chloro-ethanolamide (ACEA) and the CB₂ selective agonist JWH-133 reduced colon shrinkage, colon inflammatory damage score, histological damage and diarrhoea [33]. Massa et al. showed that the non-selective cannabinoid receptor agonist HU-210 inhibited, while the CB₁ receptor antagonist, rimobant, exacerbated dinitrobenzene sulphonic acid (DNBS)-induced colonic inflammation [34]. Consistent with these pharmacological experiments, colitis was more severe in CB₁-deficient mice than in wild-types littermates [34].

Protection against inflammatory stimuli may be provided through direct activation of cannabinoid receptors, or indirectly, through the use of FAAH or EMT inhibitors which prevent anandamide inactivation. D'Argenio et al. found significant elevation of anandamide levels in the colon of DNBS-treated mice. The EMT inhibitor, VDM-11, further increased anandamide levels and concomitantly abolished inflammation, whereas the FAAH inhibitor, N-arachidonoyl-serotonin (AA-5-HT), did not affect endocannabinoid levels and was less efficacious at attenuating colitis [28]. More recently, this protective effect of the inhibitors of endocannabinoid inactivation was confirmed by experiments in CB₁- and CB₂-deficient mice. Thus blocking FAAH and EMT with URB597 and VDM11, respectively, protected against trinitrobenzene sulphonic acid (TNBS)-induced colitis in wild type but not in CB₁- or CB₂-deficient mice. Interestingly, the combination of both VDM11 and URB597 was found to be not superior to either given alone, suggesting a lack of additive effect [35].

To further address whether FAAH plays a pathophysiological role in colitis, Storr et al. evaluated FAAH mRNA expression in TNBS-treated mice. FAAH mRNA expression was reduced in colonic tissue 1 day after the TNBS (i.e. in the early stage of colonic inflammation), supporting the notion that FAAH is suppressed to protect against

colonic inflammation. The reduction of colonic FAAH mRNA in colitis is not model-specific, as it was also observed in the oxazolone and dextran sodium sulphate murine models of colitis. There was a tendency for recovery in mRNA levels as the diseases progress. Thus, 3 days after TNBS (when the damage is maximal), FAAH mRNA was increased in the colon [35] suggesting that FAAH levels alter during the course of intestinal inflammation, and contribute to the restorative or protective functions of this enzyme.

2.4. Visceral sensation in the inflamed gut

Abdominal pain is a common symptom of gastrointestinal diseases, such as IBD and irritable bowel syndrome (IBS). Animal models of visceral pain provided evidence that cannabinoids may modulate visceral sensation and pain, particularly in the inflamed gut [36,37]. Cannabinoids reduce the degree of visceral sensitivity (abdominal contractile response to colorectal distension) under basal conditions via activation of both CB₁ or CB₂ mechanisms [38,39]. However, after inducing hyperalgesia by rectal instillation of an inflammatory compound (i.e. TNBS), lower doses of cannabinoid agonists were needed to reduce sensitivity to colorectal distention [38,40]. The inhibition of visceral response by CB₂ receptor agonists appears to be due to inhibition of the pro-inflammatory/algic compound, bradykinin [41].

The role of the endocannabinoid system in endogenous antinociceptive pathways, has been investigated with the CB₁ receptor antagonist, rimonabant, which had no effect in control rats but enhanced colitis-induced hyperalgesia [38]. These results suggest involvement of the endocannabinoid system in inflammatory hyperalgesia, through CB₁ receptors.

Cannabinoids may act on TRPV1 – as well as other member of the TRP family – which are involved in visceral hypersensitivity [42,43]. The synthetic cannabinoid receptor agonists WIN55,212-2 and AM1241 exert peripheral analgesic effects in post-inflammatory pain models by activation of TRPA1 (TRP ankyrin type 1) on sensory neurons [44]. Interestingly, a selective functional interaction between cannabinoid CB₁ receptors and kappa-opioid receptors (KORs) in the inflamed gut *in vivo* may provide further evidence for anti-algic effects of CB₁ receptors [45]. This finding was mainly based on the observation that the inhibitory effect of both salvinorin A (plant-derived KOR agonist) and U-50488 (synthetic KOR agonist) on intestinal transit in the inflamed gut was counteracted both by the selective KOR receptor antagonist nor-binaltorphimine and by the CB₁ receptor antagonist [45]. Additionally, the inhibitory effect on motility of the selective CB₁ receptor agonist arachidonyl-2-chloroethylamide (ACEA) in the inflamed gut was not modified by the selective KOR antagonist nor-binaltorphimine, suggesting an ‘unidirectional’ cross-talk [45], that is the predominant effect on cannabinoid receptors is provided by CB₁ receptor antagonist, and that the interaction reported with KOR requires replication and further elucidation of the mechanisms.

2.5. Intestinal motility in the inflamed gut

The presence of dysmotility in inflammatory diseases of small or large intestine is manifested clinically as diarrhoea. Changes in the endogenous cannabinoid system during inflammation may alter and/or contribute the motility changes. Under physiological conditions, only CB₁ receptors are involved in the control of intestinal motility; in contrast, in inflammatory states, cannabinoids may reduce intestinal motility through activation of both CB₁ and CB₂ receptors.

In the model of intestinal ileitis induced in mice by the irritant croton oil, intestinal CB₁ receptors are hyper-expressed; consequently, cannabinoid agonists are more active in reducing transit compared to control mice [46,47]. CB₁ receptors, hyper-expressed

following DNBS administration, are involved in early protective mechanisms against disturbances in the neuromuscular unit initiated in the distal colon by an inflammatory insult [48].

Enhanced signalling at CB₁ receptors may contribute to the reduction of intestinal transit due to peritonitis-induced paralytic ileus. Reduced gastrointestinal motility associated with intraperitoneal acetic acid in mice was restored by the CB₁ receptor antagonist, rimonabant, and exaggerated by the cellular re-uptake inhibitor VDM11, which increases endogenous cannabinoid. Experimental paralytic ileus was characterised by increased intestinal levels of anandamide (but not 2-AG) and an increase in the number and density of CB₁ receptors on cholinergic and substance P-containing neurones [49]. Because CB₁ receptor activation reduced excitatory transmission, it was hypothesized that, following peritonitis-induced ileus, overactivity of CB₁ receptors on the enteric cholinergic/substance P neurones reduced the release of both excitatory neurotransmitters, with subsequent inhibition of motility.

Recent evidence also highlights the role of CB₂ receptors in the regulation abnormal motility [6,50,51]. In the LPS model of intestinal hypermotility in the rat, the control of intestinal motility is mediated almost completely by CB₂-mechanisms; hypermotility was normalized by a CB₂, but not by a CB₁ receptor agonist [50,52]. *In vitro*, the CB₂ receptor agonist JWH133 did not affect the electrically evoked twitch response of the ileum under basal conditions. In contrast, in the LPS-treated tissues, the CB₂ receptor agonist JWH133 was able to reduce the exaggerated contractile response in a concentration-dependent manner [5]. Similarly, the CB₂ receptor agonist JHW015 reduced (in a cannabinoid CB₂ antagonist-sensitive manner) motility in the inflamed gut (croton oil-induced ileitis), but not in control mice [53].

In summary, activation of both hyper-expressed CB₁ receptors and CB₂ receptors in the enteric nervous system of the gastrointestinal tract dampens inflammation-induced hypermotility.

2.6. Anandamide as an endovanilloid in the inflamed gut

Endovanilloids are defined as endogenous ligands of TRPV1. The first endovanilloid to be identified has been anandamide (AEA), which activates TRPV1 at concentration higher to those required for cannabinoid receptor activation [54]. In the digestive tract, TRPV1 are predominantly expressed by primary afferent neurons [43]. McVey et al. found that intraluminal anandamide stimulated fluid accumulation and myeloperoxidase activity (a marker of intestinal inflammation) in the rat ileum and that the endocannabinoid might mediate the intestinal inflammation caused by *Clostridium difficile* toxin A [55]. In a different study, it was found that anandamide stimulated acetylcholine release from guinea pig myenteric nerves [56]. Thus, TRPV1 activation by anandamide may cause effects on inflammation, hypersecretion, and hypercontractility which are opposite to those evoked by cannabinoid receptor activation. The possible dual role of anandamide in the inflamed gut is depicted in Fig. 1.

2.7. Distribution of FAAH polymorphism in patients with Crohn's disease

A single nucleotide polymorphism in the human FAAH gene (C385A) reduces FAAH expression, which would be expected to reduce the inactivation of the endocannabinoid anandamide, and therefore a greater synaptic level of the endocannabinoid which may impact on the efficacy of a cannabinoid receptor modulating drug. Associations of this polymorphism with drug abuse and obesity have been reported [57,58]. Also, a significant association of C825A variation in FAAH gene with symptom phenotype in diarrhea-predominant IBS (D-IBS) and mixed bowel function IBS phenotype and with faster colonic transit in D-IBS have been

reported [59]. Because FAAH expression may change in experimental colitis and reducing FAAH activity was protective, Storr et al. evaluated the frequency of FAAH gene polymorphism in patient with Crohn's disease [35]. The majority of the patients investigated had an early disease onset and, of the patients, 13.4% had a positive family history of IBD. The results of the genotype analysis in 202 Crohn's disease patients and 206 controls showed no significant difference in prevalence of the C385A polymorphism. Thus, it is unlikely that this FAAH polymorphism is involved in the pathogenesis of Crohn's disease [35]. The recent genome-wide association studies did not identify genes involved in cannabinoid metabolism as potential IBD susceptibility genes [60,61].

3. Intestinal cancer

Cannabinoids exert antiproliferative, antimetastatic and apoptotic actions in colorectal carcinoma epithelial cells as well as antitumoural effects in experimental models of colon cancer. The antitumour actions may be mediated by activation of CB₁, CB₂ or a non-cannabinoid receptor-mediated mechanism, such as prostamide production (Fig. 2). From detailed studies on colorectal cancer cells, the mechanism of the cannabinoid-mediated antitumour action involves the antiapoptotic factor survivin and the proapoptotic lipid ceramide. Estrogens may up-regulate CB₁ receptor expression; this may represent one of the mechanisms in estrogen-mediated colon cancer cell proliferation.

3.1. Studies on colorectal cancer cell lines

3.1.1. Antiproliferative/apoptotic effects CB₁ or CB₂ receptor activation

The antiproliferative/apoptotic effects of cannabinoid agents have been investigated in several cancer cell lines which express, to a different degree, both CB₁ and CB₂ receptors. These include SW480, HCT-15, HT29, CaCo-2, HCT116, LS174T and SW620 cells. Experiments suggest that CB₁ and possibly CB₂ receptor activation results in decreased in cell survival, an effect associated to increase of caspase-3 activity, which is suggestive of a pro-apoptotic mechanism.

The mechanism of the CB₁-mediated induction of tumour cell apoptosis has been investigated in detail by several groups and the following pathways appear to be involved:

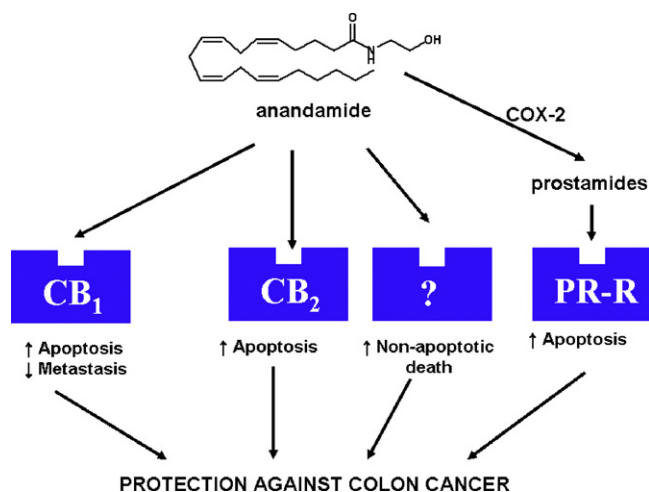


Fig. 2. Anandamide and colon cancer. Anandamide may exert antineoplastic actions via CB₁/CB₂ receptor activation or cause non-apoptotic death of cancer cell through a not-yet-identified mechanism. Moreover, anandamide may generate, via cyclooxygenase-2 (COX-2)—prostamides which exert apoptotic actions through activation of the putative prostamides receptor (PR-R). Key information is reported in Refs. [62,63,69,71,77,81].

- Inhibition of RAS-MAPK and PI3K-AKT pathways, in the CB₁-mediated apoptosis by Δ^9 -THC [62].
- Down-regulation of the antiapoptotic factor, surviving, by CB₁ was mediated by a cyclic AMP-dependent protein kinase A signalling pathway [63]. Bcl-2, an inhibitor of apoptosis protein (IAP) genes such as survivin, is known to control cell death. Survivin is unique among the IAP gene family in that, it is over-expressed in most every human tumour studied but is barely detectable in most normal adult tissues [64]. Over-expression of survivin is associated with a poor clinical outcome and reduced tumour cell apoptosis in patients with colorectal cancer [65,66]. Treatment of SW-480 cells with the CB₁ receptor agonist R1-methanandamide decreased survivin expression, but had not effect on Bcl-2 or PTEN expression [63].
- Activation of ceramide, a well known proapoptotic lipid which has been shown to act as a second messenger of cannabinoid action [67,68]. CB₁ receptor or CB₂ receptor activation stimulate ceramide *de novo* synthesis in different human tumours such as glioma, leukaemia and pancreatic cells. In DLD-1 and HT29 colorectal cancer cells, the effect of CB₁ and CB₂ receptor activation was associated to increased ceramide levels, while CB₁ and CB₂ receptor-induced apoptosis was prevented by the pharmacologic inhibition of ceramide *de novo* synthesis [69]. Additionally, the knockdown of TNF- α mRNA abrogated the ceramide increase and, therefore, the apoptotic effect induced by cannabinoid receptor activation. Thus, either CB₁ or CB₂ receptor activation induces apoptosis through ceramide *de novo* synthesis, with TNF- α acting as a link between cannabinoid receptor activation and ceramide production [69].

In conclusion, activation of both CB₁ and CB₂ receptor can induce apoptosis in colon cancer cells (Fig. 2). The mechanism of CB₁-mediated apoptosis involves inhibition of both RAS-MAPK/ERK and PI3K-AKT survival signalling cascade and down-regulation of the antiapoptotic factor survivin. The proapoptotic lipid ceramide could be involved in both CB₁- and CB₂-mediated antitumour effects.

3.1.2. Antiproliferative/apoptotic effects via prostamides production

The endocannabinoids anandamide and 2-AG are substrates for cyclooxygenase (COX-2), resulting in the generation of prostaglandin ethanolamides, named prostamides. These compounds may mediate an array of biological effects distinct from those of conventional prostanoids [70]. Patsos et al. found that anandamide inhibited the growth of colorectal carcinoma cell lines HT29 and HCA7/C29 (moderate and high COX-2 expressors, respectively) [71]; this effect was partially rescued by a COX-2 selective inhibitor, while prostamides were growth inhibitory. Expression of COX-2 is critical to the anandamide inhibition of colorectal cancer cell growth since it had little effect on the very low COX-2 expressing colorectal carcinoma cell line, SW480. Since cell death induced by anandamide was neither apoptosis nor necrosis and prostamides typically induce apoptosis, it suggests that anandamide may act through other COX-2 metabolites. Similarly, inhibition of FAAH potentiated non-apoptotic cell death, indicating that anandamide-induced cell death was mediated via metabolism of anandamide by COX-2, rather than its degradation into arachidonic acid and ethanolamine [71].

Collectively, these results suggest that anandamide induces cell death in COX-2 expressing colorectal tumour cells via production of COX-2 metabolites (i.e. prostamides). This raises the exciting possibility that colorectal tumour cells highly expressing COX-2 can be targeted for cell death by CB agonists while sparing normal cells which do not express COX-2.

3.1.3. Antimetastatic actions of cannabinoids

The migration of tumour cells is a prerequisite for tumour cell invasion and development of metastasis, which account for over 90% of cancer mortality [72]. Chemokines and neurotransmitters that bind to G-protein-coupled receptors (also known as serpentine receptors) are the most prominent of these factors that regulate tumour cell migration [72]. Cannabinoid receptor agonists anandamide and HU210 (both non-selective cannabinoid receptor agonists), docosatetraenoylethanolamide (CB₁ selective receptor agonist), but not JWH133 (CB₂ selective receptor agonist) inhibited the norepinephrine-induced migration of human colon carcinoma cell line SW480 [73]. Specific inhibition of tumour cell migration via CB₁ receptors might be a selective tool to prevent metastases formation without deleterious effects on the immune system of cancer patients.

3.1.4. Oestrogens and CB₁ receptors

The recent epidemiological and scientific data suggest a role of oestrogen in colon carcinogenesis [74]. Cellular signaling of estrogens is mediated through two oestrogen receptors (ERs), named ER α (NR3A1) and ER β (NR3A2), both belonging to the nuclear receptor family of transcription factors [75]. The effects of oestrogen on colonic cancer cell growth seem to be mediated predominantly through ER β in a combination of genomic and non-genomic mechanisms [74]. There is 17 β -estradiol-induced CB₁ gene expression in DLD-1 and HT-29 colorectal cancer cells, and in the lymph node metastatic colon cancer cell line, SW620. The early induction of CB₁ receptor mRNA was mediated by the oestrogen receptor because it was antagonised by the ER α /ER β oestrogen antagonist ICI182,780, which was ineffective in HT-29 cells, which are oestrogen receptor negative [76]. These observations suggest that up-regulation of CB₁ receptor expression by 17 β -estradiol could be a further mechanism whereby estrogens control colon cancer proliferation.

3.2. Endocannabinoid and cannabinoid receptor changes in human intestinal cancer biopsies

Adaptive changes of the endogenous cannabinoid system (i.e. increased in endocannabinoids level, down-regulation of CB₁ receptor expression via aberrant methylation of the promoter, and up-regulation of CB₂ receptor expression) have been observed in intestinal biopsies from colon cancer patients.

Levels of the endocannabinoids, anandamide and 2-AG, were 3- and 2-fold higher, respectively, in adenomas and colorectal cancer than in normal mucosa [77]. Quantitative real-time PCR revealed greatly reduced expression of CB₁ receptors in 18 of 19 cancer specimens as compared with adjacent normal mucosa [63]. Similarly, CB₁ protein was absent in 15 of 16 cancer specimens measured by Western blotting. In contrast, no recognizable pattern of mRNA CB₂ expression was found in tumour tissues. These results suggest that loss of CB₁ expression could be associated with colorectal cancer progression [63].

Inactivation of tumour suppressor genes in cancer results from epigenetic silencing as frequently as that due to genetic mutations [78]. Thus, epigenetic silencing (DNA methylation and histone modifications) of *Cnr 1* gene contributed to loss of its transcription; conversely, a demethylating agent restored *Cnr 1* mRNA expression and CB₁ protein expression, whereas an histone deacetylase inhibitor did not significantly affect *Cnr 1* mRNA expression. Collectively, these results suggest that aberrant methylation of the promoter results in transcriptional silencing of *Cnr 1* gene [63]. From a therapeutic point of view, it is postulated that initial treatment with a demethylating agent to boost CB₁ levels may be followed by administration of a CB₁ agonist to stimulate programmed cell death might be effective.

Down-regulation of protein CB₁ receptors expression in neoplastic epithelial cells from colon cancer biopsies [69] contrasts the CB₁ (but not CB₂) receptor expression by absorptive crypt epithelium in normal mucosa, and the increased expression of CB₂ in 22 of the 24 tumour specimens compared with paired normal mucosa. CB₁ and CB₂ positive staining was also found, respectively, in the subepithelial smooth muscle cells and subepithelial interstitial cells, most likely macrophages [69].

3.3. Effect of cannabinoid drugs in experimental models of colon cancer

The effect of cannabinoid drugs on colon cancer *in vivo* has been evaluated in mice using the azoxymethane model of colon cancer, in *Apc* mice and in tumour xenografts.

Aberrant crypt foci (ACF) are believed to be the earliest identifiable neoplastic lesions in the colon carcinogenic model. They exhibit a number of molecular mutations in regulatory genes consistent with the development of human colon cancer, most notably in the *ras* oncogene and APC tumour suppressor gene. In comparison to normal crypts, ACF have greater size, larger and often elongated openings, thicker lining of epithelial cells and compression of adjacent crypts [79,80]. Azoxymethane (AOM) is a carcinogenic compound which may induce ACF, polyps and tumours in the rodent colon. AOM-induced ACF are associated to increased levels of 2-AG and a non-significant increase of anandamide [81]. With 6-month treatment with the FAAH inhibitor, AA-5-HT, strongly reduces the formation of ACF in the mouse colon – via caspase-3 activation – and completely prevented the number of ACF with crypt multiplicity of greater than or equal to four. The latter is the marker best correlated with final tumour incidence. The effect of AA-5-HT was associated to increased colonic levels of both 2-AG and anandamide and was mimicked by the non-selective CB₁/CB₂ receptor agonist HU210, thus suggesting that AA-5-HT might exert protection through indirect activation of CB₁ and/or CB₂ receptors.

Apc mice are used to study colorectal cancer progression because they possess a germ-line mutation in the APC gene and, like humans, spontaneously develop multiple polyps in the intestine. CB₁-deficient *Apc* mice exhibited 2.5–3.8-fold increase in small intestinal and colonic polyp burden relative to littermate control mice. Similarly, *Apc* mice treated with the CB₁ receptor antagonist AM251 exhibited a 2–6-fold increase in small intestinal and colonic tumour burden relative to controls [63]. Conversely, the CB₁ receptor agonist R-1 methanandamide resulted in half to one sixth as many tumours in the small intestine and colon compared to control mice. Interestingly, genetic or pharmacological cannabinoid treatment mostly affected the number of large polyps (>1–2 mm), which are known to have a higher risk of progressing to carcinoma. Deletion of *Cnr 2* had no effect on intestinal polyp burden [63].

In tumour xenografts by sc injection of either DLD-1 or HT29 cells in immunodeficient mice, Western blot analysis showed that tumours obtained with DLD-cells showed a higher expression of CB₂ receptor than those obtained with HT29. Peritumoural treatment with the CB₂ receptor agonist CB13 significantly reduced the growth of tumours in DLD-1 colon cancer model [69].

In summary, cannabinoids might be protective in different stages of colon cancer progression either directly, through activation of CB₁ or CB₂ receptors, or indirectly, through elevation of endocannabinoid levels via FAAH inhibition.

4. Non-psychotropic plant cannabinoids in intestinal inflammation and cancer

The limitation of the therapeutic utility of *Cannabis* and of one of its major components, Δ^9 -tetrahydrocannabinol, is the

occurrence of psychoactive effects due to the activation of brain cannabinoid CB₁ receptors [82]. However, the plant *Cannabis* contains a number of non-psychoactive cannabinoids of pharmacological interest, including cannabigerol, cannabichromene, Δ^9 -tetrahydrocannabinol and cannabidiol [82–84]. Among these compounds, the most extensively studied is cannabidiol (CBD), which has antioxidant, anti-inflammatory, and immunomodulatory effects [82–85]. CBD has been shown to be safe in humans and, unlike Δ^9 -THC, has very low affinity for both cannabinoid CB₁ and CB₂ receptors [86] although it inhibits FAAH [87] and this may result in anti-inflammatory and anticancer effects within the gut [4,28,35,81].

4.1. Intestinal inflammation

CBD did not modify motility in control mice, but it normalized intestinal motility in the experimental model of ileitis induced in mice by the irritant croton oil [53]. Pharmacological studies aiming at investigating the mode of action have shown that the inhibitory effect of cannabidiol could involve, at least in part, FAAH inhibition, since CBD did not reduce motility in animals treated with the FAAH inhibitor AA-5-HT [53]. CBD inhibited FAAH expression in the inflamed – but not in the normal – mouse gut [88].

4.2. Intestinal cancer

Ligresti et al. evaluated a number of plant-derived cannabinoids including cannabigerol, cannabichromene, CBD, CBD-acid, Δ^9 -THC and Δ^9 -THC-acid in human colorectal carcinoma (Caco-2) cells. All compound tested, with the exception of CBD-acid (inactive up to 25 μ M), inhibited cell proliferation with an IC₅₀ in the 7.5–21.5 μ M range. CBD exhibited the highest potency with IC₅₀ value of 7.5 μ M, and maximal efficacy at 25 μ M concentration [89].

The oxidation of *Cannabis* constituents give rise to their corresponding quinones, which have been identified as cytotoxic agents. Out of these molecules, the quinone of cannabidiol (named HU-331), a synthetic compound, exerts antiangiogenic properties, induces apoptosis in endothelial cells and inhibits specifically topoisomerase II in nanomolar concentrations [90]. In a comparative *in vivo* study, it was found that HU-311 was more active but less toxic than doxorubicin in a HT-29 colon carcinoma model in nude mice [91].

5. Anandamide-related acylethanolamides and their role in intestinal inflammation and cancer

Acylethanolamides (AEs) are a group of lipids occurring both in plants and animals. Apart from anandamide, the anti-inflammatory compound palmitoylethanolamide (PEA) and the potent anorexigenic molecule oleoylethanolamide (OEA) are co-released together with anandamide and they represent the best AEs studied [92–98]. Both OEA and PEA have been identified in the digestive tract [99] and their molecular targets include TRPV1, activated by OEA, the nuclear receptor peroxisome proliferator-activated receptor- α (PPAR- α , activated by OEA and, to a less extent, by PEA) and the orphan G-coupled receptors GPR119 (activated by OEA) and GPR55 (activated by PEA and, with lower potency, by OEA) [14,100–103].

There is preliminary evidence that both OEA and PEA may have a role in the inflamed gut. PEA increases in intestinal biopsies of patient with celiac disease as well as in the experimental model of celiac-like disease induced in rats by methotrexate. In both rat muscle/serosa and mucosa layers, intestinal PEA levels peak with atrophy and regress with remission [30]. In a different study, PEA normalized the increase in motility associated to an experimental model of ileitis in mice [104]. However, whether or not the

effect of PEA was due to a direct effect on nerve/muscle activity or to an anti-inflammatory action within the gut was not determined. OEA, via PPAR- α activation, exhibits anti-inflammatory and analgesic effects in well-established models of somatic pain [105]. Recently, OEA has been shown to exert analgesic properties reducing the nociceptive responses produced by colonic administration of acetic acid, an experimental model of visceral pain associated with inflammation. OEA also inhibited the nociceptive response induced by the acetic acid in PPAR- α -null mice, suggesting an analgesic effect independent from PPAR- α activation [106].

Potential antitumoural effects of OEA and PEA have been not evaluated in the gut, either *in vitro* or *in vivo*. In non-intestinal cells (i.e. human breast cancer cells), PEA down-regulates FAAH expression, leading to enhancement AEA-induced, and CB₁ receptor-mediated, cytostatic effect [107].

6. Paradoxical beneficial effects of rimonabant in intestinal inflammation and cancer

Rimonabant is the first CB₁ receptor antagonist which had been approved for the treatment of obesity in humans. In several experimental assays, including the digestive tract, rimonabant exerts pharmacological actions in the gut which are opposite to those of CB₁ receptor agonists. For example, rimonabant increases gastric emptying [108], gastric acid secretion [109], small intestinal transit [47,110] and colonic propulsion [111], exacerbates DNBS-induced colitis [34], colitis-induced hyperalgesia [38] and cholera toxin-induced diarrhoea [112]. However, rimonabant has potentially beneficial anti-inflammatory and antiproliferative effects which are similar to those evoked by cannabinoid receptor agonists. These paradoxical effects do not seem to be mediated by cannabinoid receptors. Rimonabant reduced indomethacin-induced intestinal ulcers to a similar extent in wild-type, and in CB₁ receptor knock-out mice [113]. In addition, rimonabant inhibits the growth of human adenocarcinoma DLD1 cells [114], consistent with its anti-tumour action in thyroid tumours and breast cancer cells [115] as well as in peripheral blood mononuclear cells [116], in which rimonabant exerted immunomodulatory effects which were not CB₁-mediated; these effects appear mediated by down-regulation of iNOS and COX-2 [116]. It is important to highlight that the effect of rimonabant seems to be specific to that compound, and is not shared by other CB₁ receptor antagonists such as AM215 [62,63].

7. Conclusions

Cannabinoid mechanisms have significant potential in gastrointestinal disease models that involve inflammation and cancer (Figs. 1 and 2), including potentially, anti-metastasizing efficacy. As the pharmacology of cannabinoid mechanisms is increasingly understood, and more selective peripherally acting agents modulating CB₁ and CB₂ receptors, or for the inactivation of endogenous cannabinoids, are developed, there is great promise that this relatively new direction in clinical pharmacology will impact several diseases, including gastrointestinal inflammation and cancer. Given the disappointing experience with the withdrawal of the previously approved agent, rimonabant, in several countries, it will be essential for future drug development programs to screen psychotropic or depressant potential of this class of medications.

Conflict of interest

The authors state no conflict of interest.

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