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Endocannabinoids in the treatment of gasytrointestinal inflammation and symptoms

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The evolving policies regarding the use of therapeutic Cannabis have steadily increased the public interest in its use as a complementary and alternative medicine in several disorders, including inflammatory bowel disease. Endocannabinoids represent both an appealing therapeutic strategy and a captivating scientific dilemma. Results from clinical trials have to be carefully interpreted owing to possible reporting-biases related to cannabinoids psychotropic effects. Moreover, discriminating between symptomatic improvement and the real gain on the underlying inflammatory process is often challenging. This review summarizes the advances and latest discovery in this ever-changing field of investigation, highlighting the main limitations in the current use of these drugs in clinical practice and the possible future perspectives to overcome these flaws.

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Introduction

Ever since the first description of its beneficial effects in the Chinese pharmacology book 'Pen-Ts'ao Ching' (2838–2698 BCE), the use of therapeutic Cannabis in gastrointestinal diseases has aroused a particular interest, by means of modulating at once abdominal pain and bowel function [1]. The main marijuana component is the 'classical' phytocannabinoid Δ^9-THC that functions by targeting specific cannabinoid receptors (CB1 and CB2) [2], which are physiologically activated by a heterogeneous group of endogenous ligands, the endocannabinoids (ECs). Alongside with their synthetizing and degrading enzymes, this complex network has been collectively labelled with the term of Endocannabinoid System (ECS) [3]. In inflammatory bowel disease (IBD), the ubiquitous expression of the ECS offers the unique prospect of providing symptomatic relief, while also targeting several mechanisms underlying its pathophysiology [4,5•]. The ECS is, indeed, a highly integrated system expressed at every level of the so-called 'brain–gut–microbiota axis' and its homeostatic role ranges from the control of mucosal integrity and permeability, microbiota–host interactions to the modulation of the neuroinflammatory response (Table 1) [4,5•,6–10].

Currently, three main classes of cannabinoid-related drugs are considered for their potential therapeutic role in IBD (Figure 1):

- 'Classical' cannabinoid receptor agonists.
- Inhibitors of enzymes involved in ECs catabolism.
- 'Non-classical' cannabinoid receptor agonists.

We will discuss the effectiveness of the above-listed therapeutic agents, pinpointing the main flaws of current therapeutic trials evaluating their efficacy in treating IBD. We will then, focus on the possible solutions to overcome the current failings limiting their use in clinical practice.

Classical cannabinoid receptor agonists

Despite the increasing amount of pre-clinical data assessing the effectiveness of CB receptor agonists in IBD [5•], evidences in humans are still sparse and generally limited to retrospective studies. Two small-sized prospective studies form Israel, where medical use of cannabinoids is allowed, have suggested their beneficial effects in Crohn's disease (CD) [11,12••]. In a small pilot study on 13 CD patients, inhalation of Cannabis 'on demand' (i.e. when patients were in pain) was reported to significantly improve patients' quality of life (QoL) [11]. The same group also demonstrated the efficacy of cigarettes containing THC in inducing a significant reduction in Crohn's disease activity index (CDAI) over placebo [12••]. Of note, despite the positive results in terms of clinical response and improvement in QoL, there was no significant difference in C-reactive protein levels among the placebo and treatment arms. This observation suggests that these results might be heavily biased by the beneficial effects of Cannabis on several items impacting on the CDAI score (including appetite loss, abdominal pain and diarrhea), rather than on intestinal inflammation *per se*. Another phase II randomized-controlled trial has

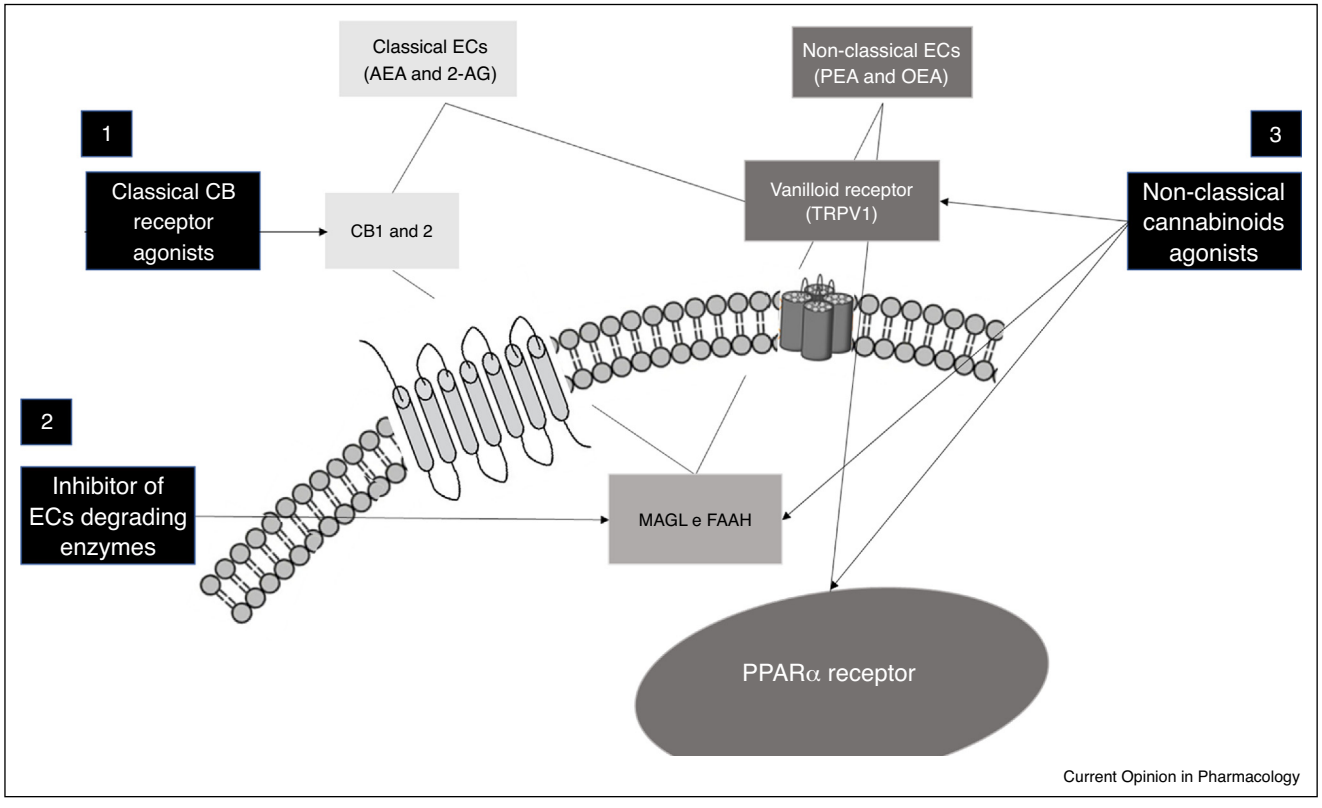
Table 1

Proposed mechanisms underlying endocannabinoids anti-inflammatory effects. *Abbreviations*: CB, cannabinoid; TRPV1, transient receptor potential cation channel subfamily V member 1; EGCs, entero-glial cells; LPS, lipopolysaccharide

	Proposed mechanisms of action	Studied model	Reference
Gut microbiota–host interaction	• VSL#3 displays anti-inflammatory properties through an upregulation of both CBs and TRPV1 receptors and down-regulation of ECs degrading enzymes	Zebrafish	[9]
Mucosal immunity	• Modulation of inflammatory cells infiltration, pro-inflammatory cytokines release and degranulation of mast cells	Murine models of colitis and cultured biopsies	[4,5•,6–8,34••]
Enteric glial cells and neurons	• Reduced activation of members of the toll-like receptors (TLRs) superfamily on EGCs	Murine models of colitis and cultured biopsies	[4,5•,6–8,34••]
Mucosal permeability	• CB1 receptors activation increases plasma levels of LPS	Normal and obese mice	[4,5•,6–8,10]

evaluated the efficacy of the drug GWP42003, an oral capsule containing both cannabidiol (CBD) and Δ^9-THC, in ulcerative colitis (UC). The results of this study (ClinicalTrials.gov Identifier: NCT01562314) showed a non-superiority of GWP42003 over placebo in inducing clinical remission in UC. Surprisingly, out of 29 patients randomized into the treatment arm, 12 patients dropped out due to minor THC-related adverse events, such as dizziness. Furthermore, Cannabis use has been retrospectively associated with an increased surgical risk in CD

Figure 1



Overview of the ECS and cannabinoid-related drugs with their molecular targets. Classical endocannabinoids, like anandamide (AEA) and 2-acylglycerol (2-AG), directly activate the G-coupled receptors CB1 and CB2 and the vanilloid receptor, TRPV1. After binding their receptors, they are internalized and metabolized by fatty acid amide hydrolase and monoacylglycerol lipase (FAAH and MAGL, respectively). Non-classical ECs, like PEA and OEA, are not able to directly activate CB receptors and exert their activity by modulating several 'non-CB' receptors (including the peroxisome proliferator-activated receptor, PPARα and TRPV1), but they share the same catabolic enzymes of stereotypical ECs. Possible therapeutic targets able of modulating the ECS signalling system in IBD are: (1) 'Classical' cannabinoid receptor agonists, that display their activity by binding, with different affinity, the two G-protein coupled receptors CB1 and CB2. (2) Inhibitors of MAGL and FAAH, that act as indirect agonist, increasing the level of both classical and non-classical cannabinoids by inhibiting their degrading enzymes. (3) 'Non-classical' cannabinoid receptor agonists that modulate several 'non-CB' receptors and can potentiate the activity of stereotypical ECs.

patients [13], questioning whether its consumption is actually safe in IBD patients. In this perspective, CBD, the major non-psychotropic constituent of Cannabis represents an attractive option in IBD, given the lack of central effects [14]. In a recent placebo-controlled trial on 19 CD patients, oral administration CBD demonstrated a placebo-like tolerability during an 8-week trial. However, the authors failed to demonstrate a significant improvement in the CDAI scores over placebo in CBD-treated patients [15••]. Once again, these negative results should be carefully considered, as they could be secondary to the small sample size or to the employed doses of CBD. Overall, the inconsistent results produced in clinical trials, so far, outline the importance of designing further larger randomized trials, prior to draw any certain conclusion.

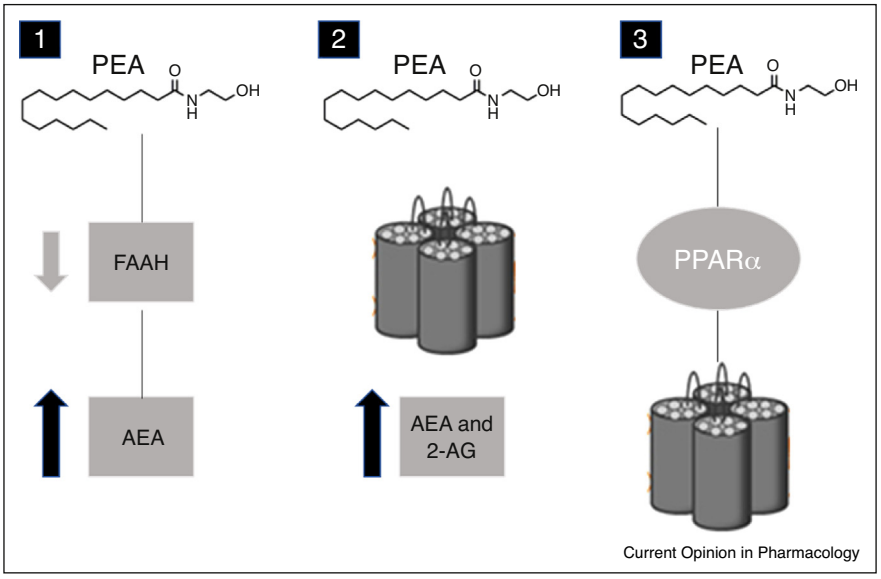
Inhibitors of ECs catabolism

Another appealing therapeutic strategy is to increase the relative levels of ECs by inhibiting their degrading enzymes. The two best recognized ECs, Anandamide and 2-acylglycerol are both short-lived compounds. They are indeed rapidly converted into arachidonic acid (AA) by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively [16]. Inhibitors of these two degrading enzymes could, therefore, have a dual beneficial effect on pain and inflammation. On one hand, they could act as indirect agonists, by increasing the levels of both classical and non-classical ECs. On the other hand, by decreasing AA bioavailability, they could also reduce prostaglandin synthesis. Selective inhibitors of MAGL and FAAH could therefore have the potential to be used as analgesic [17], neuroprotective [18], immunomodulatory and anti-inflammatory drugs [19]. Several selective FAAH and MAGL inhibitors have been developed and tested in animal models, demonstrating potent anti-nociceptive and anti-inflammatory effects, through the relative change in ECs levels. However, evidences from genetically engineered animals, knocked out for either FAAH or MAGL, have suggested that the chronically elevated ECs levels may, ultimately, cause desensitization of CB receptors; resembling what occurs in chronic Cannabis abuse [20,21]. MAGL-deficiency has been indeed associated with decreased CB1 receptor density, reduced CB1 receptor ligand binding [22,23] and resistance to cannabimimetic effects of CB receptor agonists [24]. The complexity and plasticity of the ECS turnover machinery takes account of the difficulty in designing selective FAAH and MAGL inhibitors that could be used in clinical practice [25]. Moreover, a recent clinical trial, studying a FAAH inhibitor (BIA 10-2474) as an analgesic in humans, was aborted after 6 subjects developed significant neurologic side effects [26]. Even though subsequent studies on other FAAH inhibitors have suggested that these events are questionably a class effect, this led to a setback in the development of FAAH inhibitors as potential therapeutics in humans [25,27].

Non-classical cannabinoid receptor agonists

Recently, several lipid-derived mediators, [including *N*-oleoylethanolamine (OEA) and *N*-palmitoylethanolamine (PEA) have been shown to act synergistically with stereotypical ECs. In fact, even if unable to directly activate CB receptors, they can indirectly potentiate ECS signalling, by either competing with prototypic ECs for enzymatic degradation or increasing their receptor binding affinity, configuring the so-called 'entourage effect' [28] (Figure 2). Aside from these synergistic effects, these compounds may also activate several non-CB receptors, namely PPARα and PPARγ, the G-coupled receptor GPR119 and the vanilloid receptor, TRPV1 [29–31]. The firstly described anti-inflammatory effects of PEA, known as the ALIA (autacoid local inflammation antagonism) mechanism, were mainly related to its ability to modulate mast cell activation and degranulation [32,33]. PEA has also been proven to significantly reduce macroscopic signs of colitis, with a significant decreased expression and release of pro-inflammatory cytokines as well as neutrophil infiltration, in both animal models of colitis and in cultured biopsies deriving from UC patients [34••]. Interestingly, the anti-inflammatory effect of PEA was dependent by its ability to inhibit the expression of S100B and Toll-like Receptor 4 on enteric glial cells, by selectively binding PPARα receptors. Moreover, PEA treatment significantly dampened colitis-associated angiogenesis by decreasing VEGF release, in both mice with DSS-induced colitis and in UC patients [35]. Altogether these evidences underline the importance of PEA in modulating intestinal inflammation and suggest that, by reducing the inflammatory-driven mucosal damage, PEA could also prevent the shift towards colonic carcinogenesis [36]. Since several PEA formulations are already approved for treating neuropathic pain, showing a good efficacy and safety profile, clinical trials aimed at evaluating its therapeutic role in IBD are clearly required. To date, there is only a single randomised, double-blind, placebo-controlled study assessing the effect of PEA/polydatin (200 mg/20 mg) in patients with irritable bowel syndrome (IBS) [37]. IBS pathophysiology is thought to be related to a state of low-grade mucosal inflammation, with an increased number of mucosal mast cells. Although the authors failed to prove a significant effect on microscopic inflammation and on mast cell count, the treatment with PEA significantly improved abdominal pain severity over placebo [37]. One of the main factors, limiting the use of PEA in humans, is its poor oral absorption and bioavailability, together with its short-lived action. A recent paper by Petrosino *et al.* [38] showed that PEA bioavailability could be successfully increased by administering micronized and ultramicronized formulations of PEA (m-PEA and um-PEA) in both human volunteers and beagle dogs. Nonetheless, there was a disappointing discrepancy between the plasma levels of PEA reached in animals (up to 6-fold)

Figure 2



PEA molecular targets and proposed synergistic mechanisms with typical ECs. PEA can indirectly potentiate the activity of AEA and 2-AG, by either competing for their degrading enzymes (1) or by increasing the receptor affinity through an allosteric modulation of TRPV1 channels (entourage effect) (2). Finally, PEA may also activate TRPV1 channels through PPARα receptors.

and healthy volunteers (nearly 2-fold increase) 1 and 2 h after the oral administration of PEA. The smaller peak in PEA levels in humans could be related to the lower total amount pro-kg (5 mg kg vs 30 mg kg in dogs), to the different formulations employed (m-PEA and um-PEA in humans and dogs, respectively), or even to the fact that the dogs used for the study were allergic, thus suggesting that, under pathological conditions, PEA bioavailability might be significantly affected [38,39,40••]. Complete pharmacokinetic/pharmacodynamic studies comparing the efficacy of these oral formulations of PEA are presently lacking and are eagerly awaited, in order to translate its promising anti-inflammatory effects into clinical practice.

Conclusions

Convincing evidence from *in vitro* and *in vivo* animal models shows that the ECS signalling system offers the unique possibility of targeting a number of pathophysiological mechanisms in IBD. In humans, Δ-9 THC, the major psychotropic component of Marijuana, has shown some potential therapeutic benefits. Cannabis is the most used recreational drug worldwide [41] and that the public interest in its use as a complementary and alternative medicine has continuously grown, owing to the evolving liberalization policies for medical use in some countries. In fact, despite the inconsistent data produced, in a large Canadian survey, nearly 40% of IBD patients, who were regularly consuming Cannabis, believed that it was superior to corticosteroids for IBD management, and nearly 87% of them would recommend Cannabis to other IBD patients [13,41–43]. At present, googling the search terms 'cannabinoid' AND 'IBD' yields to over 22 000 hits, reinforcing in some patients the pre-existing beliefs that Cannabis use is effective and has virtually no side-effects. Yet, a retrospective study has also indicated that Cannabis use is associated with an increased surgical risk in CD patients, questioning whether the perceived beneficial effects outweighs the risk of Cannabis use [44,45]. These alarming figures urge the scientific community to successfully design novel therapeutics lacking of central side-effects. CBD and FAAH/MAGL inhibitors have shown encouraging results in pre-clinical models of intestinal inflammation; however, clinical trials demonstrated the lack of significant clinical response for the former drug and the induction of permanent neurological side effects, for at least one of the FAAH inhibitors. Contrariwise, non-classical ECs, like PEA, show high safety and excellent tolerability profile [46]. Despite the lack of controlled trials assessing ALIAmides efficacy in IBD, these compounds represent very promising candidate-drugs and the main factor limiting their medical use is the often-unpredictable concentrations, following oral administration. One possibility to efficiently increase PEA tissue exposure could be the use of formulation enhancing its contact surface, like m-PEA and um-PEA or alternatively, the co-administration with anti-oxidants, like polydatin. A very intriguing alternative strategy could be the oral administration of genetically engineered probiotics, able to successfully colonise the intestinal surface and to locally

produce PEA, enhancing the likelihood of its tissue exposure. Remarkably, PEA and OEA-producing bacteria have been recently developed (*E. coli Nissle* and *Lactobacillus Paracasei F19* European Patent number: 3040070A1) [47•,48]. Preliminary data shows an excellent pharmacokinetic profile in animal models and very promising results, in terms of anti-inflammatory and anti-diarrheal properties. Although still at a pre-clinical stage, it is the authors' opinion that the synergistic targeting of the microbiota–host interactions and the ECS signalling pathway in IBD is a very fascinating therapeutic strategy. It would indeed allow to overcome some of the main flaws shown by the previous trials evaluating cannabinoids-related drugs, given PEA virtually absent adverse events and the possibility of enhancing its delivery at the mucosal surface.

Altogether these evidences attest a real advance in the development of cannabinoids-related drugs and now, millennia after the first description of Cannabis beneficial effects, the prospect of moving cannabinoids-based therapy into the clinical era is finally within touching distance.

Conflict of interest

Nothing declared.

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