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Modulating the endocannabinoid system in human health and disease: successes and failures

Pál Pacher and **George Kunos**
Laboratory of Physiologic Studies, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland, USA

Abstract

The discovery of the endocannabinoid system (ECS; comprising of G-protein coupled cannabinoid 1 and 2 receptors, their endogenous lipid ligands or endocannabinoids, and synthetic and metabolizing enzymes, triggered an avalanche of experimental studies that have implicated the ECS in a growing number of physiological/pathological functions. They also suggested that modulating ECS activity holds therapeutic promise for a broad range of diseases, including neurodegenerative, cardiovascular and inflammatory disorders, obesity/metabolic syndrome, cachexia, chemotherapy-induced nausea and vomiting, tissue injury and pain, among others. However, clinical trials with globally acting CB_1 antagonists in obesity/metabolic syndrome, and other studies with peripherally restricted $CB_{1/2}$ agonists and inhibitors of the endocannabinoid metabolizing enzyme in pain introduced unexpected complexities, and suggested that better understanding of the pathophysiological role of the ECS is required in order to devise clinically successful treatment strategies, which will be critically reviewed in this brief synopsis.

Keywords

endocannabinoid system; disease; cannabinoids; human; clinical trials; therapeutic potential; pharmacology

Introduction

Although Cannabis sativa (marijuana plant) is one of the most ancient medicinal plants in the history of medicine[1], the clinical use of synthetic cannabinoids or medicinal plant extracts have been largely empirical and limited to a few specific indications related to pain, wasting disorders, and chemotherapy-induced nausea and vomiting, because of their socially undesirable psychoactive properties[2]. The discovery of endocannabinoids (ECs), which mimic some of the effects of synthetic cannabinoids in vivo, their G-protein coupled receptors (GPCR) as well as their synthetic and metabolizing enzymes, has prompted preclinical studies to explore the role of the ECS in health and disease[2–4]. These studies have been greatly facilitated by the introduction of mice deficient in cannabinoid receptors or the EC degrading enzymes, as well as selective cannabinoid receptor ligands and inhibitors of EC metabolism. The results of these studies have implicated the ECS in a variety of physiopathological processes, both in the peripheral and central nervous systems and in various peripheral organs[2]. They further suggested that modulating ECS activity may have therapeutic potential in almost all diseases affecting humans, including obesity/metabolic syndrome[5], diabetes and diabetic complications[6], neurodegenerative[7,8],

Correspondence: Pál Pacher M.D., Ph.D., Laboratory of Physiological Studies, National Institutes of Health/NIAAA, 5625 Fishers Lane, MSC-9413, Bethesda, Maryland 20892-9413, USA., Pacher@mail.nih.gov, Phone: (301)443-4830, Fax: (301)480-0257.

inflammatory[9], cardiovascular[10–12], liver[13,14], gastrointestinal[15], skin[16] diseases, pain[17,18], psychiatric disorders[19,20], cachexia[2], cancer[21,22], chemotherapy-induced nausea and vomiting[23], among many others[2]).

These investigations have also uncovered the remarkable complexity of the ECS, as exemplified by differences in the therapeutic profile of activating/inhibiting the same receptor in the CNS or in peripheral tissues, by the intriguing overlap between EC and eicosanoid signaling, or by the often opposite effects mediated by CB_1 and CB_2 receptors in disease models[2–4,6,24]. Similar complexities have emerged in clinical trials targeting the ECS. While globally acting (i.e. brain-penetrant) CB_1 antagonists/inverse agonists had therapeutic efficacy in obesity/metabolic syndrome, they elicited anxiety/depression in a small proportion of subjects, which has led to their withdrawal from the market worldwide and halted their further therapeutic development[5,25,26]. The first human trial with peripherally restricted mixed $CB_{1/2}$ agonist(s) for pain has failed because of cardiovascular and metabolic side effects and hepatotoxicity[27,28]. Amplifying ECS tone by inhibiting EC metabolism was ineffective in alleviating osteoarthritic pain in human subjects[29,30]. Thus, we need to better understand the pathophysiological function of the ECS in humans, and have to refine the indications and design of clinical trials in order to successfully translate recent progress in cannabinoid biology into clinically effective treatment strategies.

In this brief synopsis we will discuss preclinical evidence implicating the ECS in human disease, and review treatment strategies that target the ECS for therapeutic gain in humans. Because of space limitations, we will often refer readers to recent overviews on specific subjects instead of original papers.

The endocannabinoid system (ECS)

Δ9-tetrahydrocannabinol (THC), the putative psychoactive ingredient of marijuana, as well as its endogenous counterparts anandamide (arachidonoyl ethanolamide) and 2-arachidonoylglycerol (2-AG) exert their primary effects through cannabinoid 1 and 2 ($CB_{1/2}$) receptors; 2-AG favors CB_2, while AEA binds with higher affinity to CB_1[2], but at higher concentrations may also modulate $TRPV_1$ and other receptors. Signaling by cannabinoid receptors is complex, as it may involve both G protein-dependent pathways, such as inhibition of adenyl cyclase or modulation of ion channel function, and G protein-independent mechanisms, including activation of various MAPKs (p44/42MAPKs, p38, ERK and JNK) or ceramide signaling[2,31,32].

CB_1 receptors, the most abundant GPCR in the mammalian brain, mediate the socially undesirable psychoactive effects of Cannabis. Although their expression was initially considered to be restricted to the brain, more recent studies identified CB_1 receptors in virtually all peripheral tissues and cell types, albeit at much lower densities than in brain, and documented their important regulatory functions[2,3,5]. CB_2 receptors are largely restricted to immune and hematopoetic cells, although functionally relevant expression has been found in specific regions of the brain and in myocardium, gut, endothelial, vascular smooth muscle and Kupffer cells, exocrine and endocrine pancreas, bone, reproductive organs/cells, and in various tumors[4]. Both cannabinoid receptors may undergo rapid internalization and intracellular trafficking upon agonist exposure[33,34].

In the CNS, AEA and 2-AG are synthesized “on demand” and released to act as retrograde transmitters on CB_1 receptors[35–37]. They are not stored and are rapidly degraded after exerting a transient and localized effect[38]. The synthesis of ECs largely depends on the intracellular Ca^{2+}-concentration. AEA is mainly formed via a two step-pathway, involving a Ca^{2+}-dependent N-acyltranferase and N-acylphosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD), while diacylglycerol lipase and phospholipase Cβ are

mainly responsible for the biosynthesis of 2-AG[3,37]. The existence of additional, parallel biosynthetic pathways for AEA has also been proposed[39,40].

AEA and 2-AG are removed from the extracellular space by a process of cellular uptake and metabolism; however the putative transporter(s) involved have not yet been cloned, and are subjects of much recent controversy[41–43]. AEA is degraded primarily by fatty acid amide hydrolase (FAAH) and 2-AG is degraded by monoacylglycerol lipase (MAGL)[3,44], although additional enzymes have also been implicated in the degradation of both AEA and 2-AG[45,46]. Endocannabinoids may also be metabolized by cyclooxygenases, lipooxygenases and cytochrome P450, leading to the formation of bioactive metabolites which may activate CB receptor-independent mechanisms[24,47]. It is also important to note that FAAH and MAGL are also responsible for the degradation of numerous potentially bioactive lipids. Thus, the biological consequences of the inhibition of these enzymes are not necessarily due to enhanced EC levels. Some of the enzymes involved in EC synthesis/degradation may exist in several forms and their activity may vary in different tissues or even in different regions of the same tissue[3,37,48–52].

In addition to AEA and 2-AG, several other EC-like molecules have been discovered, but their activities have not been studied in sufficient detail[53,54]. Interestingly, recent studies have identified novel peptide allosteric negative modulators of CB_1 receptors[55], the biological significance of which is yet to be determined. Additionally, the anti-inflammatory lipid lipoxin A4 may be an endogenous allosteric enhancer of CB_1 receptors[56]. A comprehensive overview of the ECS is beyond the scope of this chapter; instead, the reader is referred to several detailed reviews on this subject[3,24,37,57].

The endocannabinoid system in health and disease

Despite the ubiquitous expression of the various components of the ECS, their genetic ablation or pharmacological blockade in normal, healthy animals has minimal functional consequences, which suggests that the ECS has minimal or no tonic activity under normal physiological conditions[2,4]. On the other hand, an increase or decrease in ECS tone is associated with various pathological states, as a result of altered expression of CB receptors, endocannabinoid metabolizing enzymes and/or synthetic pathways, in a tissue-specific and time-dependent manner. Examples of selected pathologies in which dysregulation of the ECS was reported (in most cases upregulation of $CB_{1/2}$ and/or increase in tissue levels of ECs) are shown in table 1, and have been summarized in more detail elsewhere[2–4,58,59]. In some cases, altered ECS activity is transient and forms part of the body's compensatory response to a particular insult, thus reducing symptoms and/or slowing progression of the disease (e.g. in neuropathic pain); in other cases, activation of the ECS may be pathogenic (e.g. in various forms of shock or diabetic complications) or may reflect a deficiency (e.g. in various tumors), the significance of which is yet to be determined[2].

From a therapeutic standpoint, identification of regional or tissue-specific changes in CB receptors is important, because of their possible selective targeting may mitigate unwanted side effects [59,60]. However, these changes can serve as a basis of successful drug development only as long as they are determined using appropriate tools (e.g. specific antibodies), the specificity of which needs to be carefully validated[4,61]. It is also very important to understand the underlying mechanisms of these alterations; for example, is the increase in the tissue level of an EC due to its increased biosynthesis or a decrease in its enzymatic degradation?

Cardiovascular consequences of targeting the ECS in health and disease

Since many promising drugs fail in clinical development because of cardiovascular side effects, it is important to briefly overview the cardiovascular consequences of modulating the ECS. ECs exert complex cardiovascular effects dominated by a decrease in blood pressure and myocardial contractility, mediated primarily by CB_1 receptors located in the myocardium, vasculature, and neurons in the central and autonomic nervous systems[2,62]. In cultured human coronary artery endothelial cells[63] and cardiomyocytes[64], CB_1 activation promotes stress signaling and cell death, and decreases contractility [10,12]. In contrast, activation of cardiovascular CB_2 receptors does not have adverse hemodynamic consequences[11]. CB_1, CB_2 or FAAH knockout mice have normal blood pressure, myocardial contractility and/or baroreflex sensitivity, indicating the minimal role of the ECS in normal cardiovascular regulation[2]. However, in several pathological conditions (e.g. shock, heart failure, cardiomyopathies, advanced liver cirrhosis) the ECS may become activated to promote hypotension/cardiodepression through cardiovascular CB_1 receptors[2,10]). CB_1 receptor signaling may also promote disease progression in preclinical models of heart failure[64–66] and atherosclerosis[67,68], and contributes to increased cardiovascular risk (e.g. plasma lipid alterations, abdominal obesity, hepatic steatosis, insulin and leptin resistance) in obesity/metabolic syndrome and diabetes, both in rodents and humans[5,69–71]. In contrast, CB_2 signaling in the heart and vasculature may activate cardioprotective mechanisms and limit inflammation[11].

Acute or chronic use of marijuana may decrease or increase heart rate and decrease blood pressure depending on the duration of the use, dose and route of administration[2,10]. Elevated resting heart rate is a known independent risk factor for cardiovascular disease in healthy men and women[72]. A recent controlled study at the National Institute on Drug Abuse evaluated the development of tolerance to the effects of oral synthetic THC in 13 healthy male daily cannabis smokers residing on a secure research unit over a period of 6 days[73]. Despite the development of tolerance to the subjective intoxicating effect of THC, no tolerance was observed to its hypotensive and tachycardic effects[73]. Another recent study of 72 young cannabis user men and 72 matched controls found increased heart rate variability in cannabis users[74]. Surinabant, a selective CB_1 antagonist, has recently been reported to inhibit THC-induced central nervous system and heart rate effects in humans, providing proof of principle that those effects were indeed mediated by CB_1 receptor activation[75]. At the 20^{th} ICRS meeting in Sweden, AstraZeneca presented data from the first clinical studies with two novel, peripherally restricted, orally active mixed $CB_{1/2}$ agonists (AZD1940 & AZD1704). The study was terminated due to adverse cardiovascular effects, weight gain and mild hepatotoxicity[27,28].

An increasing number of case reports associates marijuana smoking with precipitation of acute coronary syndrome (ACS)[76]. Alarmingly, this occurs mostly in young healthy subjects without any prior cardiovascular disease[77,78]. A retrospective study assessed the risk of ACS after exposure to marijuana smoke. It was found that the risk of myocardial infarction was highest during the first hour of exposure[79]. The effect of marijuana use on mortality following acute myocardial infarction was assessed in a prospective study involving 1913 adults hospitalized with myocardial infarction at 45 US hospitals between 1989 and 1994, with a median follow-up of 3.8 years. The results indicated that marijuana use may pose increased risk of infarction in susceptible individuals with coronary heart disease[80]. A more recent study evaluated the consequences of marijuana use and long-term mortality among survivors of acute myocardial infarction, and found that habitual marijuana use among patients presenting with acute MI was associated with an apparent increase in mortality rate (29% higher) over the following 18 years, though this did not reach statistical significance because of the limited sample size[81]. In the absence of large

scale, long term controlled studies with repeated measures of marijuana use, a firm conclusion on the long term impact of cannabis use on cardiovascular mortality cannot be drawn. Nevertheless, the above findings are of concern. Because THC is a relatively weak CB_1 agonist compared to many synthetic ligands, also activates cardioprotective CB_2 receptors and is a potent antioxidant, one may predict that the uncontrolled spread and use of mixtures of potent synthetic CB_1 agonists (spice, K2, etc.) used as recreational drugs, would lead to significantly greater cardiovascular morbidity. Indeed, in a recent case series in healthy children, myocardial infarction was precipitated by synthetic cannabinoid use[82], and another paper reported tachycardia, loss of consciousness and diffuse pain in two adolescents[83].

What is the situation regarding the ECS and cardiovascular pathology? As mentioned before, EC/CB_1 receptor signaling has been implicated as a pathogenic factor in rodent models of cardiovascular diseases, including atherosclerosis, shock and various forms of cardiomyopathy. However, ECs were also reported to exert protective effects, based mostly on ex vivo and indirect studies, via CB_2 and CB-receptor independent mechanisms. Clearly, selective CB_2 agonists exert beneficial effects in rodent models of myocardial infarction by limiting inflammatory cell infiltration (in cardiomyocytes the expression of CB_2 is very low, if any)[11]. To analyze the role of the ECS more directly, a recent study employed FAAH knockout mice with a 2.5–3-fold increase in myocardial AEA content. When such mice were used to induce various experimental models of cardiomyopathy, they displayed increased mortality, tissue injury and neutrophil infiltration in the heart, which could be partially rescued by CB_1 antagonists[66]. Consistently with this report, a recent study showed that FAAH deficiency enhanced intraplaque neutrophil recruitment in atherosclerotic mice and increased a proinflammatory immune response[84]. These findings indicate that the primary cardiovascular effects of elevated EC tone are deletorious and are mediated by CB_1 receptors.

In obese human subjects, increased plasma levels of AEA and 2-AG were strongly associated with coronary circulatory dysfunction, suggesting that plasma EC levels may be used as biomarkers of cardiovascular risk in obesity[85]. In another study, increased plasma AEA and 2-AG levels positively correlated with impaired coronary endothelial function in obese subjects[86]. In samples of epicardial fat from ischemic human hearts, upregulation of CB_1 was accompanied by downregulation of CB_2 and FAAH, compared to non-ischemic hearts[87]. CB_1 receptor density was significantly higher in atherosclerotic coronary artery sections from patients with unstable angina compared to those with stable angina[67]. G1359A polymorphism in the CB_1 receptor gene was also associated with coronary artery disease in the Chinese Han population, although the effect of this polymorphism on receptor function is unknown[88]. Both ECs were reported to inhibit human cardiac Kv4.3 channels at fairly low concentrations in ovary cells expressing Kv4.3 or in human cardiomyocytes in a receptor independent manner[89], a harbinger of pro-arrhythmic risk.

Thus it is clear that activation of CB_1 receptors by synthetic ligands or ECs is associated with adverse cardiovascular consequences, which must be very carefully weighed during preclinical/clinical development of new drugs targeting the ECS.

Activation of $CB_{1/2}$ receptors: THC, synthetic agonists and cannabinoid extracts

THC (Dronabinol; Marinol) and its synthetic analogue Nabilone (Cesamet) have been approved by the FDA for treatment of chemotherapy-induced nausea and vomiting and for stimulating appetite in wasting disorders (e.g. AIDS, tumor cachexia, etc). Sativex, an oromucosal spray containing THC and the non-psychoactive plant cannabinoid, cannabidiol,

has recently been approved in Canada, the UK and several other European countries for the symptomatic relief of neuropathic pain and spasticity associated with multiple sclerosis, and as adjunctive analgesic treatment for adults with advanced cancer. However, the therapeutic utility of THC and its synthetic analogs are limited by their unwanted psychotropic effects mediated by central CB_1 receptors. Here, we will summarize only the clinically most relevant indications.

Earlier preclinical studies suggested that ECs or plant-derived cannabinoids exert neuroprotective effects in the CNS by: 1) modulating excitability and calcium homeostasis via effects on various ion channels (Ca^{2+}, Na^{+}, K^{+}), intracellular Ca^{2+} stores and gap junctions and *N*-methyl d-aspartate (NMDA) receptors; 2) attenuating excitatory glutamatergic transmissions and modulating synaptic plasticity via presynaptic CB_1 receptors; 3) inducing CB_1 receptor-mediated hypothermia; 4) exerting antioxidant effects; 5) modulating immune responses and the release of pro-inflammatory mediators by CB_1, CB_2, and non CB_1/CB_2 receptors on microglia, astrocytes, macrophages, neutrophils, lymphocytes and neurons[2]. Numerous recent studies have suggested that many of the previously described protective effects of synthetic CB_1 ligands were in fact attributable to centrally-mediated hypothermia and/or receptor-independent antioxidant/anti-inflammatory effects of the compounds, and ECs through the activation of CB_1 receptors may also promote tissue injury and neurodegeneration (for example in stroke and other forms of I/R injury)[6,90–92].

Historical documents reveal that one of the earliest uses of cannabis was to treat pain [93]. Studies in modern times initially focused on CB_1 receptors and demonstrated beneficial effects of cannabinoids in rodent models of acute and chronic pain. The results suggested that the observed antinociceptive effects have complex mechanisms involving actions in the CNS, spinal cord, and peripheral sensory nerves[2,94]. Recent evidence also implicates CB_2 receptors in the antihyperalgesic activity of cannabinoids[95,96], however the exact mechanisms and cellular targets are elusive because of the lack of reliable antibodies for CB_2[4].

In humans, the analgesic activity of THC and other cannabinoids is less clear-cut, as cannabinoids are relatively weak analgesics compared to opiates, even when they do show efficacy[2]. The clinical data on THC, CBD and their combinations have been comprehensively reviewed elsewhere[97,98]. The primary focus of these studies has been the safety/efficacy and symptom relief (e.g. bladder incontinence, limb spasticity, pain and sleep quality) in multiple sclerosis (MS) or other pain-related conditions. Three studies demonstrated that cannabis extract in MS patients improved urinary incontinence[98]. A number of controlled and blinded trials evaluating the efficacy of oral or sublingual cannabis/Sativex on spasticity in MS found that at doses that lack overt psychoactivity, these drugs show no or minimal efficacy, as assessed by the objective outcomes using the Ashworth Scale. However, the treatment consistently improved subjective, patient-assessed endpoints (spasms, pain, spasticity, sleep quality). Follow-up studies using a patient assessed Numeric Rating Scale for spasticity showed significant benefits of Sativex compared to placebo[98]. One could argue that some of the benefits observed could be due mood improvement (patients feel subjective improvement), but since only some of the symptoms were improved (spasticity, pain and sleep quality), this may not be the case. In patients treated with THC for one year, improvements using the Ashworth Scale were reported[98]. Zhornitsky and Potvin meta-analyzed the data of 33 studies with cannabidiol alone or in various combinations with THC, the rationale for combining THC and CBD being to attenuate the psychoactive effects of THC by CBD, based on empirical evidence obtained in some studies. Among these studies, 16 had been conducted in healthy subjects and 17 in clinical populations, including 4 in MS, 3 in neuropathic and cancer pain, 4 in schizophrenia

and bipolar mania, 2 in social anxiety disorder, and one each in cancer-related anorexia, Huntington's disease, insomnia, and epilepsy [97]. The authors concluded that depending on the study and on the THC/CBD ratio, CBD may prolong/intensify or inhibit THC-induced effects. In some of these studies THC or CBD+THC was more effective in reducing pain, but in others CBD alone also exerted (or completely lacked) analgesic properties. Notably, several of these studies used multiple pain assessment scores, and the treatments were effective when evaluated by some, but not by other scales[97]. In one of the studies in which oral administration of CBD+THC in MS was not effective in improving symptoms, immunological analysis surprisingly revealed a certain pro-inflammatory effect of the drug[97]. The authors also concluded that preliminary clinical evidence suggests that high-dose oral CBD may have therapeutic benefits in social anxiety disorder, insomnia and epilepsy, but may also cause mental sedation[97].

Taken together, the above mentioned studies in MS show consistent improvements in subjective rather than quantitative symptomatic outcome measures (including pain), which supports the beneficial effects of cannabinoid based medicines in neuropathic pain associated with MS. The relatively poor efficacy observed in some clinical studies may be attributable to pharmacokinetic problems such as first pass effects via liver and slow absorption via the oral route of administration, which may also limit the success of self-titration[98]. In most of these studies, formulations containing THC frequently caused generally mild to moderate side effects. However, with individual dose-titration, which can be better achieved by using the oromucosal Sativex spray, side effects can be further attenuated. Initial dose-titration may also help in select responders and excluding non-responders early. Future clinical studies should explore how cannabinoid-based medicines affect MS progression. In light of the preclinical data, the combination of THC with CBD appears to be the most promising, given the neuroprotective effects of CBD observed in numerous preclinical studies[99].

There is considerable interest in developing THC-based medicines for other forms of pain, such as pain associated with cancer or diabetic neuropathy. However, under these conditions we should also carefully weigh the potential effect of the treatment on cancer and/or diabetes progression. Regarding cancer, although numerous studies suggest that THC may slow down the growth/progression of certain types of cancers in preclinical models, others suggest that THC may in fact promote cancer growth, and cannabinoid receptor deletion or inhibition is beneficial[2,4,22]. In addition, results of a clinical study evaluating the association between ECS activity and survival and pain in pancreatic cancer indicate that although patients with high CB_1 receptor expression in enlarged nerves in pancreatic ductal adenocarcinoma had a lower combined pain score (intensity, frequency, duration), they had significantly shorter survival[100]. For CBD, the evidence more clearly suggests potential benefits in multiple preclinical tumor models[99]. In the case of diabetes and diabetic complications, there is strong evidence (both preclinical and clinical) that CB_1 activation promotes primary diabetes and also contributes to all diabetic complications (including neuropathy), and CB_1 antagonists can prevent or reverse these changes as well as insulin resistance[6,69,101].

Interestingly, analysis of cross-sectional data from the National Health and Nutrition Examination Survey (NHANES III, 1988–1994) indicated that marijuana use was independently associated with a lower prevalence of diabetes mellitus [102], and glucose tolerance and insulin sensitivity were found unchaged in chronic marijuana smokers [103]. In view of the demonstrated ability of acute marijuana smoking to induce insulin resistance[104], these findings may reflect desensitization of peripheral CB_1 receptors in chronic users. Further clinical studies are needed to analyze the differential mechanisms involved in the acute and chronic effects of marijuana use on glycemic control.

Nevertheless, in light of the overwhelming preclinical and clinical evidence suggesting that CB_1 receptor activation contributes to diabetes development and its complications (cardiovascular, neuropathy, retinopathy, and nephropathy)[6], and a recent study by Centers for Disease Control and Prevention (CDC) associating cases of acute kidney injury with synthetic cannabinoid use [105], the use of THC would be risky from a clinical point of view in patients with established diabetes. Diabetic patients also have impaired immune functions and wound healing, which could be adversely affected by immunosuppressive/immunomodulatory drugs such as THC. In contrast, CBD demonstrated beneficial effects due to its anti-inflammatory and antioxidant properties both in preclinical models of primary diabetes and in models of all major diabetic complications, which is encouraging for its potential testing in diabetic patients[6].

As mentioned above, THC and its synthetic analogue Nabilone are used to treat chemotherapy-induced nausea and vomiting and to stimulate appetite in cachexia associated with AIDS or terminal tumors[2]. In case of AIDS, recent controlled studies in non-human primates showed unexpectedly that chronic THC administration prior to and during simian immunodeficiency virus infection ameliorates disease progression, attenuates viral load and tissue inflammation, significantly reducing morbidity and mortality of virus-infected macaques[106], which is very encouraging.

There is considerable preclinical and clinical evidence that the combination of THC with opioids or non-steroidal anti-inflammatory drugs may enhance their efficacy in pain and also limit their side effects,[2,95,96]. Recently, it has become clear that cannabinoid analgesia is predominantly mediated via peripheral CB_1 receptors in nociceptors[107], providing the rationale for selectively targeting peripheral CB_1 receptors by peripherally restricted (brain impermeable) agonists, thereby eliminating the undesirable CNS consequences of CB_1 stimulation[71]. Astra Zeneca developed 2 novel peripherally restricted, orally bioavailable $CB_{1/2}$ agonists (AZD1940 & AZD1704). Despite their mixed agonist activity at CB_1 and CB_2 receptors, analgesic efficacy in rodent models was mainly driven by CB_1 receptors, validated through the use of CB_1 selective antagonist and knockout mice[27]. The clinical efficacy of AZD1940 as a pain reliever was tested in two single-dose, phase II studies (human capsaicin and 3rd molar extraction models) and in a multiple ascending doses (MAD) study performed in subjects with chronic low-back pain. The 2 single-dose, phase II studies showed no efficacy at the primary endpoints (pain intensity and heat pain threshold for capsaicin study)[28]. In the multiple ascending dose study where AZD1940 was administered for 12 days, repeated dosing led to slow compound accumulation, significant weight gain and elevation of hepatic transaminases. AZD1704 also induced profound hypotensive effects[28]. Thus, the analgesic efficacy of peripherally restricted CB_1 agonists remains to be established in humans. Whereas their cardiovascular and metabolic side effects confirm the role of CB_1 receptors in these functions in humans, they further limit their usefulness as therapeutic agents. Whereas the above studies of Astra Zeneca with novel, peripherally restricted, orally bioavailable $CB_{1/2}$ agonists did not indicate CB_2 involvement in preclinical models of analgesia, other studies suggest that CB_2 activation may attenuate certain types of pain[95,96]. CB_2-selective peripherally restricted agonists (instead of mixed $CB_{1/2}$ agonists) may offer better optimization of dosing in humans, as metabolic and cardiovascular side effects are less likely to occur.

Inhibition of the CB_1 receptors: global and peripherally restricted CB_1 antagonists

Recent preclinical studies provided compelling evidence that ECs modulate food intake, energy balance, glucose and lipid metabolism through CB_1 receptors expressed in the brain and various peripheral tissues, such as fat, liver, and skeletal muscle[5,70,108,109].

Treatment with brain-penetrant CB_1 receptor antagonists/inverse agonists resulted in improvements of multiple cardiovascular risk factors both in preclinical studies and in clinical trials in obese/overweight subjects[110–116]. Parallel preclinical studies clearly demonstrated that reduced food intake was not the primary mechanism responsible for the weight reducing effect of CB_1 antagonists, and suggested that peripheral energy metabolism might be directly under EC control[5]. These studies demonstrated that ECs promote lipogenesis in adipose tissue and liver, but inhibit fatty acid oxidation and mitochondrial biogenesis, while CB_1 antagonists exert opposite effects[5]. Meanwhile, clinical trials revealed that a small but statistically significant fraction of subjects treated with the CB_1 inverse agonist rimonabant exhibited anxiety, depression and/or suicidal ideations, eventually leading to withdrawal of rimonabant from the market in over 50 countries and discontinuation of the therapeutic development of this class of compounds[117].

By that time, several lines of evidence strongly suggested that selective inhibition of peripheral CB_1 receptors may preserve much of the metabolic benefit of global CB_1 blockade while minimizing side effects due to blockade of CB_1 receptors in the CNS[5]. A proof of principle study by Tam et al.[118] demonstrated that chronic treatment of DIO mice with AM6545, the first high affinity, selective, peripherally restricted neutral CB_1 antagonist, improved glucose tolerance, insulin sensitivity, plasma lipid profile, and also reversed fatty liver, but was less effective than its parent compound rimonabant in reducing body weight, as it did not affect caloric intake. This study also provided evidence for the importance of CB_1 receptors in hepatocytes in the development of diet-induced insulin resistance. A subsequent study provided additional mechanistic insight by demonstrating that CB_1-mediated hepatic insulin resistance involves ER stress-dependent impairment of insulin signaling as well as reduced insulin clearance[119]. In a follow-up study a highly potent, selective, and brain impermeable CB_1 receptor inverse agonist, JD5037, was even more effective in improving metabolic parameters in mouse models of obesity, and it not only improved cardiometabolic risk but had antiobesity and hypophagic effects by reversing leptin resistance[101]. This compound is currently undergoing toxicology screening as a prelude to its clinical testing.

As discussed above, we have learned important lessons from the first clinical trials aiming to attenuate pain with the peripherally restricted mixed $CB_{1/2}$ agonists, which were terminated because of the excessive weight gain, hepatotoxicity, and cardiovascular adverse effects. Interestingly, this side effect profile strongly supports the rationale for the development and therapeutic use of peripherally restricted CB_1 antagonists in humans[27,28].

Activation of CB_2 receptors by selective agonists

Overwhelming evidence for the therapeutic potential of EC/CB_2 receptor signaling in some of the major pathologies affecting humans have been reviewed recently[4]. An important consideration for the therapeutic development of selective CB_2 receptor agonists is the absence of psychoactive effects, coupled with anti-inflammatory and tissue protective activity of these ligands in numerous preclinical disease models[4].

CB_2 receptors are predominantly expressed in peripheral blood immune cells where the level of their expression is strongly modulated by pro-inflammatory and other stimuli, largely depending on the experimental conditions[120]. Initial studies focusing on the immunomodulatory effects of THC and other cannabinoid ligands *in vivo* in rodents and *in vitro* in human immune cell cultures demonstrated immunosuppressive effects in T and B lymphocytes, NK cells and macrophages, and most likely involved both CB_1 and CB_2 receptors as well as CB receptor-independent mechanisms[9,120,121]. ECs were also found to modulate T and B cell proliferation and apoptosis, immune cell activation and

inflammatory cytokine production, chemotaxis and inflammatory cell migration, and macrophage-mediated killing of sensitized cells[9,120,122]. These generally inhibitory effects were ligand- and cell type-dependent and were also influenced by the experimental conditions used[9,120,123,124]. A complicating factor is the agonist-induced rapid internalization and trafficking of CB_2 receptors in vitro, which can confound the interpretation of results[33,34]. The effects of ECs or synthetic analogs on microglia activation/migration also appear to be largely experimental condition-dependent[123].

An important recent development has been the identification of low levels of CB_2 receptor expression in tissues previously thought to be devoid of these receptors. These include specific regions of the brain[125–127], spinal cord and dorsal root ganglia[17,95,128], neurons in the myenteric and submucosal plexus of the enteric nervous system[129–131], in myocardium or cardiomyocytes[64,65,132], human vascular smooth muscle and endothelium[25,133–135], activated hepatic stellate cells[136,137], Kupffer cells[138], in reproductive organs/cells[139,140], colonic epithelial cells[141], bone[142–144], mouse and human exocrine and endocrine pancreas[145–148], and in various human tumors[149]. Further studies are needed to fully explore the function of CB_2 receptors at these sites.

More importantly, disease-induced changes – usually increases - in CB_2 receptor expression have been reported (Table 1), and synthetic CB_2 receptor agonists exerted protective effects in a variety of preclinical disease models and pathological conditions[4], ranging from cardiovascular disorders[11], various forms of ischemic-reperfusion injury[90], gastrointestinal and liver inflammation[13,150,151], autoimmune and neurodegenerative disorders[7,152–154], kidney[4] and bone disorders[143,144], cancer[149,155–157], and pain[17,95].

As for the therapeutic potential of CB_2 agonists, it is important to point out that while under conditions of a sterile inflammatory response CB_2 agonists may limit injury, in pathogen-induced inflammation the immunosuppressive effects of the CB_2 receptor activation may enhance or even inflict tissue damage, and may also lead to accelerated cancer growth in certain types of tumors, as reviewed recently[4]. In order to successfully target CB_2 in selected human diseases it is imperative to identify the exact cellular location and disease-induced, time-dependent changes in the expression of CB_2 receptors. This will necessitate the development of improved research tools, such as more reliable and specific antibodies. This is particularly important, because in many injury models CB_2 agonists appear to be most effective when given before the initiation of the insult, and may lose their efficacy or even promote inflammation when given at later time points[4]. Thus, a better understanding of the underlying pathology and its effects on CB_2 expression is required for the development of meaningful therapeutic approaches. Before going to clinical development for a particular indication, it is also important to confirm previous preclinical findings with novel and more selective CB_2 agonists, since currently available ligands may not be entirely specific. Better knowledge of the pharmacokinetics and metabolism of ligands is also essential, particularly given the bell-shaped dose-response often seen with recently available CB_2 agonists in various disease models[4]. The reason for the latter may be that, when used at higher doses, currently used CB_2 agonists may also activate CB_1 receptors, particularly when the relative expression of CB_1 over CB_2 is high. Our understanding of the complexities of CB_2 receptor signaling is still limited, and one must also consider important interspecies differences in CB_2 receptor signaling and in the pharmacology of CB_2 ligands[158].

Problems with the use of peripherally restricted $CB_{1/2}$ agonists for pain relief due to cardiovascular and metabolic side effects have been discussed above. A plausible alternative could be the testing of peripherally restricted selective CB_2 agonists for analgesia in

humans, as such compounds would be expected to be devoid of cardiometabolic liabilities. However, the preclinical data with AZD1940 & AZD1704 indicate that the analgesic efficacy of this class of compounds was mainly driven by the CB_1 receptor[27] which, if confirmed in humans, would limit the promise of this approach. Nevertheless, the therapeutic development of selective CB_2 receptor ligands (agonists or inverse agonists/antagonists depending on the pathology and its stage) is still a promising strategy for a number of disease conditions, provided the issues discussed above are successfully resolved[4].

Inhibition of EC metabolism, cellular uptake or biosyntheses

The hypothesis behind the therapeutic inhibition of EC degradation was that increasing EC tissue levels would be less likely to cause psychoactive effects than would the use of synthetic CB_1 ligands (endocannabinoids are biosynthesized and degraded in a site and time-dependent manner), while the beneficial effects of $CB_{1/2}$ activation, such as analgesia, would be maintained[159]. In support of this, FAAH knockout mice or mice treated with a FAAH inhibitor have elevated AEA levels in the brain and other tissues, are supersensitive to exogenous AEA and exhibit CB_1 receptor-mediated hypoalgesia[160,161] and reduced anxiety, but do not display catalepsy, a marker for psychoactivity in humans[162]. The antinociceptive effect of FAAH inhibitors, likely mediated through increases in AEA and PEA levels which activate $CB_{1/2}$, PPARα, and/or TRPV1 [163], was investigated in acute and chronic rodent models of pain[164]. Most of the initial results were based on using URB597, which irreversibly inhibit FAAH both in the CNS and a periphery[164]. Recent studies with a peripherally restricted FAAH inhibitor, URB937, showed efficacy in neuropathic and inflammatory pain[165], confirming that the analgesic effects of AEA are initiated at the peripheral sites[107]. However, similar to direct acting peripheral $CB_{1/2}$ agonists, URB597 has both hypotensive[166] and diabetogenic effects[167] mediated by CB_1 receptors, and FAAH knockout mice are also prone to diet-induced obesity and diabetes[168]. The diabetogenic effect of URB597 has been attributed to blocking FAAH in the liver, and the novel FAAH inhibitor AM3506, which does not block FAAH in the liver due to its rapid uptake and metabolism by hepatocytes, was found to be devoid of glycemic side effects in rodents[167]. FAAH antagonism may also promote fat accumulation and insulin resistance through centrally mediated hypothyroidism[169].

The analgesic effects of FAAH inhibition in preclinical models prompted the development PF-04457845, an irreversible FAAH inhibitor with excellent analgesic efficacy in animal models[29,170], which was selected for clinical development. In a randomised, placebo-controlled, phase II clinical trial PF-04457845 was recently evaluated in patients with osteoarthritic pain of the knee[30]. The results clearly demonstrated that PF-04457845 inhibited FAAH activity in white blood cells and raised the concentrations of various fatty acid amides 3.5–10 fold, which persisted for up to 2 weeks after discontinuation of the drug, and did not affect cognitive functions in test subjects. However, the study failed to show any analgesic efficacy of PF-04457845, while the NSAID naproxen, used as positive control, was effective[30]. These results were also highlighted and discussed in a recent editorial[171].

A promising alternative indication for the therapeutic use of FAAH antagonists is post-traumatic stress syndrome (PTSD). The FAAH inhibitor AM3506 was recently found effective in increasing fear extinction in a CB_1 receptor-dependent manner in a mouse model of PTSD, and human carriers of a low-expressing FAAH variant displayed quicker habituation of amygdala reactivity to threat, as detected by brain imaging[172].

The main rationale for the development of MAGL inhibitors, which metabolize 2-AG, is similar to the rationale for FAAH inhibitors. Numerous recent studies demonstrated that MAGL inhibition or genetic deletion exerts antiemetic[173], antineoplastic[174], and anxiolytic and antinociceptive effects in rodents[175], protects against brain injury[176,177], acute liver injury/inflammation[138] and colitis either via enhancing $CB_{1/2}$ signaling or by attenuating eicosanoid synthesis in specific tissues, such as the brain and the liver[178], or by the combination of both. In case of cancer, MAGL inhibition modulates fatty acid release for the synthesis of protumorigenic signaling lipids[174]), as reviewed recently[179,180].

While the above preclinical findings are indeed exciting, they also highlight important limitations. 1) Raising the tissue levels of ECs may promote the formation of cyclooxygenase-, lipoxygenase- and cytochrome P450-derived pro-inflammatory metabolites[47,181]. 2) Some of the prostaglandins which were attenuated by MAGL inhibitors have well documented tissue protective functions. 3) While the dual effect of MAGL inhibition on attenuating eicosanoid and enhancing EC signaling can be beneficial in certain tissues (e.g. brain and liver) where MAGL links the EC and eicosanoid systems through hydrolysis of 2-AG, in other tissues it can promote inflammation and injury (e.g. in the myocardium) through the non-CB mechanisms described above (the cardiotoxicity of COX-2 inhibitors is well documented in humans). 4) Chronic MAGL inhibition leads to functional antagonism of the ECS[175]. 5) As previously discussed, very strong preclinical and clinical evidence suggests that in cardiovascular disease and diabetes/diabetic complications endocannabinoids through CB_1 and most likely through the first two mechanisms described above promote cardiovascular injury. 6) There is growing evidence that ECs exert proinflammatory effects in various disease models through both CB_1-dependent and -independent mechanisms[6]. This is supported by a recent study demonstrating that inhibition of EC synthesis is anti-inflammatory in macrophages[182]; 7) Various isoforms of metabolizing enzymes (e.g. FAAH) may have distinct functions[52], and the functional properties of rodent and human FAAH may also be different[183]. 8) Most of the benefits observed with inhibitors of FAAH or MAGL were reported in acute models; the safety of chronic inhibition of these enzymes has not yet been determined, particularly in pathological situations. 9) The use of irreversible inhibitors of FAAH and MAGL could be a disadvantage for accurate dose titration and would make it difficult to treat toxicity[164].

Conclusions and future directions

Recent clinical studies provided evidence that cannabinoid based medicines with controlled doses of plant derived cannabinoids can provide symptomatic relief in a subset of patients suffering from pain and spasticity associated with MS and certain other types of pain, and there is hope based on preclinical studies that these medications would also positively modulate disease progression. Synthetic cannabinoids are also useful in subset of patients with wasting disorders and chemotherapy-induced nausea and vomiting. There are numerous promising new targets (plant-derived cannabinoids, peripherally restricted CB_1 antagonists, selective CB_2 agonists, inhibitors of endocannabinoid metabolism/transport) “in waiting” which have been reviewed here. However, it is clear that for the successful translation of preclinical findings to clinical practice, better understanding of the pathological role of the ECS in various diseases, of potential side effects of targeting this system, and of endocannabinoid pharmacology is required, coupled with the development of improved research tools to dissect these processes (see also Figure 1 and Table 2).

Future studies should focus on rigorous evaluation of the CB receptor dependent/independent, and hypothermia-independent effects of THC in preclinical models (e.g. in

tissue injury, cancer, inflammation, etc.) using global and tissue/cell specific knockout mice and to identify potential novel targets/mechanisms of action of THC and other plant derived cannabinoid, coupled with identification of non-psychoactive constituents in cannabis extracts with potential therapeutic effects. Novel highly selective, orally available non-toxic cannabinoid ligands should be developed and evaluated in preclinical disease models. Large animal studies (e.g. canine, pig, primate) should confirm the efficacy of cannabinoid ligands obtained in rodent disease models before initiating human trials. Development of specific novel antibodies for $CB_{1/2}$ receptors and endocannabinoid metabolic enzymes (FAAH, MAGL, DAGLα/β) validated by using positive and negative controls is essential to accurately assess the time-dependent changes in $CB_{1/2}$ receptors and metabolic enzyme expressions in diseased animal and human tissues, in order to understand the human relevance of these changes. Our limited knowledge should be expanded in understanding the $CB_{1/2}$ receptor trafficking, signaling and their interspecies differences. Development of reliable radio-ligands suitable for human imaging studies and research could contribute to our better understanding the role of ECS in human health and disease.

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List of abbreviations

2-AG	2-arachidonoylglycerol
AEA	anandamide or arachidonoyl ethanolamide
$CB_{1/2}$	cannabinoid receptor 1 or 2
CBD	cannabidiol
EC(s)	endocannabinoid(s)
ECS	the endocannabinoid system
FAAH	fatty acid amide hydrolase
GPCR	G-protein coupled receptor
MAGL	monoacylglycerol lipase
MAPKs	mitogen-activated protein kinases
NAPE-PLD	N-acyltranferase and N-acylphosphatidylethanolamine-hydrolyzing phospholipase D
PPARα	peroxisome proliferator-activated receptorα
THC	Δ9-tetrahydrocannabinol
$TRPV_1$	transient receptor potential cation channel subfamily V member 1

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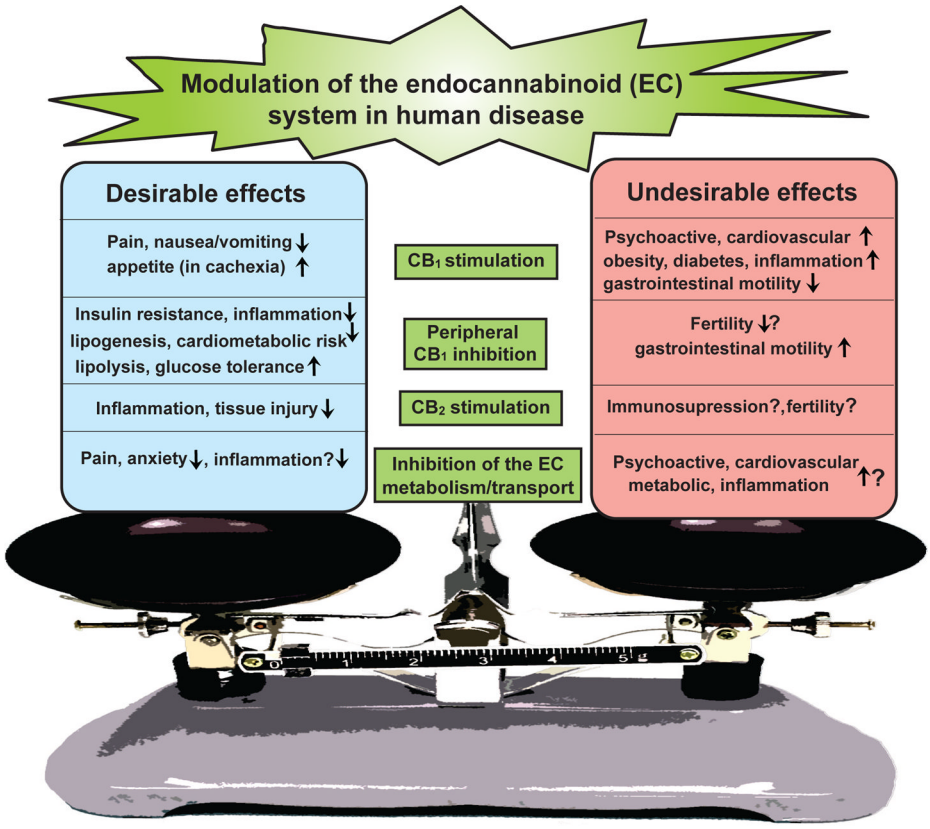

Figure 1.
Cannabinoid therapeutics: finding the right balance

Table 1

Examples of the dysregulation of the ECS in disease

Disease, sample (R: rodent; P: pig; C: canine; H: human)	Expression/changes in $CB_{1/2}$	Changes in endocannabinoid levels	Proposed role of CB receptors in disease	Reference
Myocardial infarction ischemia/reperfusion injury) (R, P, H)	Myocardium. In human epicardial adipose tissues of ischemic hearts upregulation of CB_1 and PKA, accompanied by CB_2 and FAAH downregulation, increased iNOS/eNOS ration and reduced cell survival signaling	Increase in circulating immune cells or in serum of obese patients with adverse cardiovascular events. Elevated endocannabinoid plasma levels are strongly associated with coronary dysfunction in obese human subjects.	CB_2: decrease in leukocyte infiltration and enhancement of pro-survival pathways; CB_1: contribution to cardiovascular dysfunction, cell death/dysfunction in human endothelial cells and cardiomyocytes; central hypothermia (the latter is only in rodents and can be protective)	[11,12,76,85– 87,90,184–187]
Heart failure, cardiomyopathies (R, H)	Myocardium, cardiomyocytes, endothelial cells	Myocardium, cardiomyocytes, circulating immune cells and platelets	CB_2: attenuation of inflammation/ injury; CB_1: promotion of cardiac dysfunction and cell death in cardiomyocytes and endothelial cells	[64,65,186,188–192]
Atherosclerosis, restenosis (R, H)	Infiltrating and other immune cells, vascular smooth muscle and endothelium	Serum, atherosclerotic plaques	CB_2: context dependent attenuation or promotion of vascular inflammation (monocyte chemotaxis, infiltration and activation) and factors of plaque stability; attenuation of vascular smooth muscle proliferation; CB_1: increase of vascular inflammation and/ or plaque vulnerability	[67,84,133,134,193–198]
Stroke, spinal cord injury (R, H)	Brain, microglia, infiltrating immune cells, endothelium	Serum, brain	CB_2: attenuation of inflammation (endothelial activation, leukocyte infiltration), and tissue injury; attenuation of motor and autonomic deficits in a mouse model of spinal cord injury;	[90,199–206]

Disease, sample (R: rodent; P: pig; C: canine; H: human)	Expression/changes in $CB_{1/2}$	Changes in endocannabinoid levels	Proposed role of CB receptors in disease	Reference
			CB_1: promotes hypothermia-dependent protection, but if hypothermia is compensated ineffective or enhances injury	
Cirrhotic cardiomyopathy (R, H)	N.D.	Myocardium, circulating immune cells and platelets	CB_2: attenuation of hypotension by decreasing liver inflammation; CB_1: contribution to cardiovascular dysfunction	[189–192]
Septic shock by live bacteria (R, H)	N.D.	Serum	CB_2: decrease or increase in inflammation and tissue injury most likely by affecting bacterial load; CB_1: contribution to cardiovascular collapse	[10,207–210]
Hepatic ischemia-reperfusion injury (R, P, H)	Inflammatory immune cells, activated endothelium	Liver, serum, hepatocytes, Kupffer and endothelial cells	CB_2: attenuation of inflammation (endothelial activation, leukocyte chemotaxis, infiltration and activation), oxidative stress, and tissue injury; CB_1: promotion of liver injury	[135,138,211–213]
Obesity, nonalcoholic fatty liver disease, diabetic complications (R, H)	Hepatocytes, inflammatory cells, adipocytes, certain neurons, sites of diabetic complications (kidneys, retina and myocardium)	Liver, adipose tissue, brain, skeletal muscle, diabetic kidneys, hearts, retinas, serum	CB_2: Enhancement of high fat diet-induced steatosis and inflammation or attenuation of obesity associated one with age; CB_1: increase in fat storage, decrease in metabolism, promotion of insulin and leptin resistance and inflammation in adipose tissue and in the liver	[5,6,70,101,108,214–221]
Liver fibrosis, cirrhosis, alcohol-induced liver injury (R, H)	Activated Stellate cells, inflammatory cells, hepatocytes, Kupffer cells	Liver, serum, inflammatory cells	CB_2: Attenuation of fibrosis and injury/ inflammation; CB_1: increase in fibrosis/injury	[14,136,137,191,222,223]
Pancreatitis (R, H)	Pancreas	Inflamed pancreas	CB_2: Attenuation of inflammation; CB_1: context dependent effect	[145,146,148,224]

Disease, sample (R: rodent; P: pig; C: canine; H: human)	Expression/changes in $CB_{1/2}$	Changes in endocannabinoid levels	Proposed role of CB receptors in disease	Reference
Inflammatory bowel disease, colitis, diverticulitis (R, H)	Epithelial cells, infiltrating inflammatory cells, enteric nerves	Inflamed gut	Attenuation of inflammation and visceral sensitivity	[130,151,225 –229]
Nephropathy (R, H)	Kidney, human proximal tubular cells, podocytes	Kidney	CB_2: attenuation of inflammation (chemokine signaling and chemotaxis, inflammatory cell infiltration and endothelial activation) and oxidative stress; CB_1: promotion of inflammation/ injury	[105,219,220,230–233]
Neurodegenerative/ neuroinflammatory disorders (multiple sclerosis, Alzheimer's, Parkinson's and Huntington's disease, spinal cord injury) (R, H)	Microglia, inflammatory cells, brain lesions, neurons?	Brain, spinal fluid	CB_2: attenuation of inflammation (microglia activation, secondary immune cell infiltration), facilitation of neurogenesis; CB_1: attenuation of excitotoxicity, hypothermia; context dependent effect on injury/ inflammation	[2,7,91,92,152,205,234–250]
Pain (R)	Inflammatory cells, certain neurons	Site of induced chronic inflammatory pain	CB_2: attenuation of inflammatory pain via unknown mechanism(s); CB_1: attenuation of various forms of pain by inhibiting neurotransmission	[17,95,96,251–266]
Psychiatric disorders (anxiety and depression, schizophrenia) (R, H)	Glial, inflammatory cells, neurons?	Blood, cerebrospinal fluid, brain (increased in schizophrenia, but decreased in brain in depression)	CB_2: largely unexplored, In rodent models of depression/anxiety it may modulate CNS inflammation and either attenuate or promote anxiety like behavior; CB_1: context dependent effect on anxiety, improved sleep	[19,267–277]
Rheumatoid arthritis (H)	N.D.	Synovial fluid, synovia	CB_2: attenuation of the autoimmune inflammatory response; CB_1: attenuation of pain	[278]
Cancer (R, H)	In various tumors or cancer cells	Various tumors	$CB_{1/2}$: context dependent attenuation or promotion of tumor growth (apoptosis,	[279–282] [2,22,149,155,157]

Disease, sample (R: rodent; P: pig; C: canine; H: human)	Expression/changes in $CB_{1/2}$	Changes in endocannabinoid levels	Proposed role of CB receptors in disease	Reference
			angiogenesis, proliferation, etc.)	

Table 2

Potential approaches/directions for future success

Therapeutic approach *(target)*	**Possible directions/ approaches for success**	**Possibly therapeutic indications in humans (realistic)**	**Potential/expected adverse effects**
THC based medicines, cannabinoid based extracts *(CB_1, CB_2 and unrelated antioxidant anti-inflammatory mechanisms)*	• Optimization of route of administration, dosing and indication • Better selection criteria for trials, identification of potential positive responders by initial titration • Placebo controlled trials to establish short and long term efficacy in given indications • Long term controlled studies to determine possible disease modifying effects (e.g. in multiple sclerosis) and adverse consequences (e.g. immune and/or cardiovascular effects, etc.) • Combination approaches in pain to achieve better efficacy and fewer side effects (e.g. with opioids, non-steroid anti-inflammatory drugs, etc.) • Optimization of the extract composition for improved benefit/ risk profile	• Symptomatic relief in certain forms of pain and spasticity (as in neurodegenerative disorders such as multiple sclerosis) • Stimulation of appetite in patients with wasting disorders • Attenuation of chemotherapy-induced nausea and vomiting • Topical administration in certain skin disorders? • Non-psychoactive constituents of marijuana, such as cannabidiol or their analogs, may have therapeutic utility in certain forms of acute tissue injury, inflammatory disorders, diabetes and diabetic complications	• In case of THC- containing formulations, effects related to CB_1 stimulation at higher doses (e.g. psychoactive, cardiovascular, metabolic side effects) and potential modulation of immune responses
Peripherally restricted CB_1 agonists *(peripheral CB_1)*	• Evaluation of the feasibility of the topical/local use of peripherally restricted CB_1 agonists in certain forms of pain and skin conditions (e.g. pruritus)	• Topical/local use in certain forms of pain and skin conditions/ diseases? (the systematic administration/ use is not likely because of the established adverse cardiovascular and metabolic consequences of this approach)	• Cardiovascular • Metabolic • Kidney • Gastrointestinal (decreased motility) • Pro-inflammatory?

Therapeutic approach *(target)*	**Possible directions/ approaches for success**	**Possibly therapeutic indications in humans (realistic)**	**Potential/expected adverse effects**
Peripherally restricted or global CB_2 agonists *(peripheral CB_2)*	• Reevaluation of human indications based on previous failures of trials with mixed peripherally restricted $CB_{1/2}$ agonists • Search for new indications • More preclinical and clinical research to understand the significance of tissue and time specific changes in CB_2 receptor expression in pathological conditions • Development of novel, specific and orally available ligands for proof of the principle studies; evaluation of toxicology and pharmacokinetics	• Various forms of acute tissue injuries associated with inflammation (stroke, myocardial infarction, traumatic injury, organ transplantation, etc,) • Various forms of inflammatory diseases if the antiinflammatory effects are confirmed in humans	• Most likely related to effects on immune and hematopoietic system • Effects on fertility?
Peripherally restricted CB_1 antagonists, inverse agonists *(peripheral CB_1)*	• Development and testing of new ligands, toxicology and safety studies in rodents, large animals, and humans • Proof of the principle studies in large animals and humans	• Diabetes and diabetic complications, • Cardiometabolic syndrome • Kidney disease?	• Gastrointestinal (increased motility) • Effects on fertility?
Inhibition of EC metabolism, cellular uptake or biosynthesis *($CB_{1/2}$, $TRPV_1$ and nuclear receptors, prostaglandin and leukotriene signaling)*	• Preclinical research to identify the putative endocannabinoid transporter(s), and to better understand the tissue, time, and disease specific metabolism of endocannabinoids to various other bioactive mediators (e.g. prostaglandins, leukotriens, etc.) • Reevaluation of human indications based on previous	• Pain? • Certain disorders associated with anxiety? • Certain forms of acute tissue injury?	• Similar, but acutely less pronounced than with CB_1 agonists. However, long term use may be associated with adverse effects similar to COX2 inhibitors (e.g. cardiovascular). • Pro-inflammatory effects in certain cases?

Therapeutic approach *(target)*	**Possible directions/ approaches for success**	**Possibly therapeutic indications in humans (realistic)**	**Potential/expected adverse effects**
	failures of trials with FAAH inhibitors in pain • Search for new indications, better and more selective ligands		