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MINIREVIEW

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

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The discovery of the endocannabinoid system, comprising the G-protein coupled cannabinoid 1 and 2 receptors ($CB_{1/2}$), their endogenous lipid ligands or endocannabinoids, and synthetic and metabolizing enzymes, has triggered an avalanche of experimental studies implicating the endocannabinoid system in a growing number of physiological/pathological functions. These studies have also suggested that modulating the activity of the endocannabinoid system holds therapeutic promise for a broad range of diseases, including neurodegenerative, cardiovascular and inflammatory disorders; obesity/metabolic syndrome; cachexia; chemotherapy-induced nausea and vomiting; and tissue injury and pain, amongst 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, have introduced unexpected complexities, suggesting that a better understanding of the pathophysiological role of the endocannabinoid system is required to devise clinically successful treatment strategies.

Introduction

Although *Cannabis sativa* (the 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 has been largely empirical and limited to a few specific indications related to pain, wasting disorders, and chemotherapy-induced nausea and vomiting, as a result 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, as well as their synthetic and metabolizing enzymes, has prompted preclinical studies aiming to explore the role of the endocannabinoid system (ECS) in health and disease [2–4]. These studies have been greatly facilitated by the introduction of mice deficient in cannabinoid receptors or 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]. Such studies have 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], inflammatory [9], cardiovascular [10–12], liver [13,14], gastrointestinal [15] and skin [16] diseases; pain [17,18]; psychiatric disorders [19,20]; cachexia [2]; cancer [21,22]; and chemotherapy-induced nausea and vomiting [23], amongst many others [2].

Abbreviations
2-AG, 2-arachidonoylglycerol; AEA, anandamide or arachidonoyl ethanolamide; $CB_{1/2}$, cannabinoid receptor 1 or 2; CBD, cannabidiol; CNS, central nervous system; EC, endocannabinoid; ECS, endocannabinoid system; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; MS, multiple sclerosis; THC, Δ9-tetrahydrocannabinol; $TRPV_1$, transient receptor potential cation channel subfamily V member 1.

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 central nervous system (CNS) or in peripheral tissues, by the intriguing overlap between EC and eicosanoid signalling, or by the often opposite effects mediated by cannabinoid 1 and 2 receptors ($CB_{1/2}$) receptors in disease models [2–4,6,24]. Similar complexities have emerged in clinical trials targeting the ECS. Although globally acting (i.e. brain-penetrant) CB_1 antagonists/inverse agonists were shown to have 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 failed as a result of cardiovascular and metabolic side effects and hepatotoxicity [27,28]. Amplifying the 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, as well as refine the indications and design of clinical trials, so that it is possible to successfully translate recent progress in cannabinoid biology into clinically effective treatment strategies.

The present minireview discusses preclinical evidence implicating the ECS in human disease, and reviews the treatment strategies that target the ECS for therapeutic gain in humans. Because of limitations of space, reference is also made to recent overviews on specific subjects, rather than to original papers.

The ECS

Δ9-Tetrahydrocannabinol (THC), the putative psychoactive ingredient of marijuana, and its endogenous counterparts, anandamide (arachidonoyl ethanolamide) (AEA) and 2-arachidonoylglycerol (2-AG), exert their primary effects through $CB_{1/2}$ receptors; 2-AG favours CB_2, whereas AEA binds with higher affinity to CB_1 [2], although, at higher concentrations, it may also modulate transient receptor potential cation channel subfamily V member 1 ($TRPV_1$) and other receptors. Signalling by cannabinoid receptors is complex because it may involve both G protein-dependent pathways, such as inhibition of adenyl cyclase or the modulation of ion channel function, and G protein-independent mechanisms, including the activation of various mitogen-activated protein kinases (p44/42 mitogen-activated protein kinases, p38, extracellular signal-regulated kinase and c-Jun N-terminal kinase) or ceramide signalling [2,31,32].

CB_1 receptors, the most abundant G-protein coupled receptor 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 have identified CB_1 receptors in almost all peripheral tissues and cell types, albeit at much lower densities than in the brain, and documented their important regulatory functions [2,3,5]. CB_2 receptors are largely restricted to immune and haematopoetic cells, although functionally relevant expression has been found in specific regions of the brain and in the myocardium, gut, endothelial, vascular smooth muscle and Kupffer cells, exocrine and endocrine pancreas, bone, and reproductive organs/cells, as well as in various tumours [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, whereas 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 the subject of much 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 that 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 a result of 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, although 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 the present minireview; instead, several detailed reviews are available on this subject [3,24,37,57].

The ECS 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 the 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, up-regulation of $CB_{1/2}$ and/or an 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 tumours) of unknown significance [2].

From a therapeutic standpoint, the identification of regional or tissue-specific changes in CB receptors is important because their possible selective targeting may mitigate unwanted side effects [59,60]. However, these changes can serve as a basis for 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 the result of its increased biosynthesis or a decrease in its enzymatic degradation?

Cardiovascular consequences of targeting the ECS in health and disease

Because many promising drugs fail in clinical development as a result of cardiovascular side effects, it is important to briefly overview the cardiovascular consequences of modulating the ECS. ECs exert complex cardiovascular effects that are dominated by a decrease in blood pressure and myocardial contractility, mediated primarily by CB_1 receptors located in the myocardium, vasculature and neurones 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 signalling and cell death, and decreases contractility [10,12]. By contrast, activation of cardiovascular CB_2 receptors does not have adverse haemodynamic 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 signalling 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]. By contrast, CB_2 signalling in the heart and vasculature may activate cardioprotective mechanisms and limit inflammation [11].

Acute or chronic use of marijuana may decrease or increase the heart rate and decrease blood pressure depending on the duration of the use, dose and route of administration [2,10]. An 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 who were 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 male cannabis users and 72 matched controls reported an increased heart rate variability in cannabis users [74]. Surinabant, a selective CB_1 antagonist, has

Table 1. Examples of the dysregulation of the ECS in disease. C, canine; H, human; P, pig; R, rodent. ND, not determined.

Disease, sample	Expression/changes in $CB_{1/2}$	Changes in endocannabinoid levels	Proposed role of CB receptors in disease	Reference
Myocardial infarction (ischaemia-reperfusion injury) (R, P, H)	Myocardium, in human epicardial adipose tissues of ischaemic hearts, up-regulation of CB_1 and protein kinase A, accompanied by CB_2 and FAAH down-regulation, increased inducible NOS/endothelial NOS ration and reduced cell survival signalling	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; CB_1: promotes hypothermia-dependent protection but, if hypothermia is compensated, ineffective or enhances injury	90,199–206
Cirrhotic cardiomyopathy (R, H)	ND	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)	ND	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 ischaemia-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, non-alcoholic fatty liver disease, diabetic complications (R, H)	Hepatocytes, inflammatory cells, adipocytes, certain neurones, 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

Table 1. (Continued).

Disease, sample	Expression/changes in $CB_{1/2}$	Changes in endocannabinoid levels	Proposed role of CB receptors in disease	Reference
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
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 signalling 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, neurones?	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 neurones	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, neurones?	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 behaviour; CB_1: context-dependent effect on anxiety, improved sleep	19,267–277
Rheumatoid arthritis (H)	ND	Synovial fluid, synovia	CB_2: attenuation of the autoimmune inflammatory response; CB_1: attenuation of pain	278
Cancer (R, H)	In various tumours or cancer cells	Various tumours	$CB_{1/2}$: context-dependent attenuation or promotion of tumour growth (apoptosis, angiogenesis, proliferation, etc.)	279–282, 2,22,149, 155,157

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 20th International Cannabinoid Research Society meeting in Sweden, AstraZeneca presented data from the first clinical studies investigating two novel, peripherally-restricted, orally active mixed $CB_{1/2}$ agonists (AZD1940 and AZD1704). The study was terminated as a result of adverse cardiovascular effects, weight gain and mild hepatotoxicity [27,28].

An increasing number of case reports associates marijuana smoking with the precipitation of acute coronary syndrome [76]. Alarmingly, this occurs mostly in young healthy subjects without any previous cardiovascular disease [77,78]. A retrospective study assessed the risk of acute coronary syndrome 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 after acute myocardial infarction was assessed in a prospective study involving 1913 adults who were 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 an 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 myocardial infarction was associated with an apparent increase in mortality rate (29% higher) over the subsequent 18 years, although 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, and also activates cardioprotective CB_2 receptors and is a potent antioxidant, it may be predicted that the uncontrolled spread and use of mixtures of potent synthetic CB_1 agonists (spice, K2, etc.) employed 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 study reported tachycardia, loss of consciousness and diffuse pain in two adolescents [83].

What is the situation regarding the ECS and cardiovascular pathology? As noted previously, EC/CB_1 receptor signalling 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- to three-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]. Consistent with this report, a recent study showed that FAAH deficiency enhanced intraplaque neutrophil recruitment in atherosclerotic mice and increased a pro-inflammatory 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 ischaemic human hearts, the up-regulation of CB_1 was accompanied by down-regulation of CB_2 and FAAH compared to non-ischaemic 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]. A 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 the activation of CB_1 receptors by synthetic ligands or ECs is associated with adverse cardiovascular consequences, which must be given very careful consideration during the preclinical/clinical development of new drugs targeting the ECS.

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

THC (dronabinol; Marinol; Solvay Pharmaceuticals, Brussels, Belgium) and its synthetic analogue nabilone (Cesamet; Valeant Pharmaceuticals, Irvine, CA, USA) have been approved by the Food and Drug Administration for treatment of chemotherapy-induced nausea and vomiting and for stimulating appetite in wasting disorders (e.g. AIDS, tumour cachexia, etc). Sativex (GW Pharmaceuticals, Salisbury, Wiltshire, UK), an oromucosal spray containing THC and the nonpsychoactive plant cannabinoid, cannabidiol (CBD), 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 an adjunctive analgesic treatment for adults with advanced cancer. However, the therapeutic utility of THC and its synthetic analogues are limited because of their unwanted psychotropic effects mediated by central CB_1 receptors. The present minireview summarizes only the clinically most relevant indications.

Earlier preclinical studies suggested that ECs or plant-derived cannabinoids exert neuroprotective effects in the CNS by: (a) 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 receptors; (b) attenuating excitatory glutamatergic transmissions and modulating synaptic plasticity via presynaptic CB_1 receptors; (c) inducing CB_1 receptor-mediated hypothermia; (d) exerting antioxidant effects; and (e) modulating immune responses and the release of proinflammatory mediators by CB_1, CB_2 and non-CB_1/CB_2 receptors on microglia, astrocytes, macrophages, neutrophils, lymphocytes and neurones [2]. Numerous recent studies have suggested that many of the previously described protective effects of synthetic CB_1 ligands were attributable to centrally-mediated hypothermia and/or receptor-independent antioxidant/anti-inflammatory effects of the compounds, and that ECs through the activation of CB_1 receptors may also promote tissue injury and neurodegeneration (e.g. 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 a lack of reliable antibodies for CB_2 [4].

In humans, the analgesic activity of THC and other cannabinoids is less clear-cut because 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 have 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 lacking 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]. It could be argued that some of the benefits observed were a result of mood improvement (patients feel subjective improvement) but, because only some of the symptoms were improved (spasticity, pain and sleep quality), this may not be the case. In patients treated with THC for 1 year, improvements using the Ashworth scale were reported [98]. Zhornitsky and Potvin [97] performed a meta-analyses of the data from 33 studies with CBD alone or in various combinations with THC, with 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 four in MS, three in neuropathic and cancer pain, four in schizophrenia and bipolar mania, two in social anxiety disorder, and one each in cancer-related anorexia, Huntington's disease, insomnia and epilepsy [97]. It was 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 at reducing pain, although, in other studies, 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 the oral administration of CBD+THC in MS was not effective in improving symptoms, immunological analysis unexpectedly revealed a certain pro-inflammatory effect of the drug [97]. The preliminary clinical evidence was concluded to suggest that high-dose oral CBD may have therapeutic benefits in social anxiety disorder, insomnia and epilepsy, although it may also cause mental sedation [97].

Taken together, the above 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 the 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 the early selection of responders and exclusion of nonresponders. 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, the 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 tumour models [99]. In the case of diabetes and diabetic complications, there is strong evidence (both preclinical and clinical) indicating that CB_1 activation promotes primary diabetes and also contributes to all diabetic complications (including neuropathy), and that 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 to be 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 glycaemic 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 the Centers for Disease Control and Prevention 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. By contrast, CBD demonstrated beneficial effects as a result of 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 noted above, THC and its synthetic analogue Nabilone are used to treat chemotherapy-induced nausea and vomiting, as well as to stimulate appetite in cachexia associated with AIDS or terminal tumours [2]. In the case of AIDS, recent controlled studies in nonhuman primates showed unexpectedly that chronic THC administration before and during simian immunodeficiency virus infection ameliorates disease progression, and also attenuates viral load and tissue inflammation, significantly reducing the morbidity and mortality of virus-infected macaques [106], which is very encouraging.

There is considerable preclinical and clinical evidence showing that the combination of THC with

opioids or nonsteroidal anti-inflammatory drugs may enhance their efficacy in pain and also limit their side effects [2,95,96]. 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 (London, UK) has developed two novel peripherally-restricted, orally bioavailable $CB_{1/2}$ agonists (AZD1940 and AZD1704). Despite their mixed agonist activity at CB_1 and CB_2 receptors, the analgesic efficacy in rodent models was mainly driven by CB_1 receptors, as 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 third molar extraction models) and in a multiple ascending doses study performed in subjects with chronic low-back pain. The two 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. Although 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. 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, whereas 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 the better optimization of dosing in humans because 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 have 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, whereas CB_1 antagonists exert the opposite effects [5]. Meanwhile, clinical trials have 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 the 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, there were several lines of evidence strongly suggesting that selective inhibition of peripheral CB_1 receptors may preserve much of the metabolic benefit of global CB_1 blockade at the same time as minimizing side effects as a result of the 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 and the plasma lipid profile, and also reversed fatty liver, although it was less effective than its parent compound rimonabant in reducing body weight because it did not affect caloric intake. The same 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 signalling, 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 also 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 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 signalling in some of the major pathologies affecting humans has 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 the 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, natural killer cells and macrophages, which 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 any interpretation of the results [33,34]. The effects of ECs or synthetic analogues on microglia activation/migration also appear to be largely experimental condition-dependent [123].

One important recent development has been the identification of low levels of CB_2 receptor expression in tissues previously considered to be devoid of these receptors. These include specific regions of the brain [125–127], spinal cord and dorsal root ganglia [17,95,128], neurones in the myenteric and submucosal plexus of the enteric nervous system [129–131], myocardium or cardiomyocytes [64,65,132], human vascular smooth muscle and endothelium [25,133–135], activated hepatic stellate cells [136,137], Kupffer cells [138], reproductive organs/cells [139,140], colonic epithelial cells [141], bone [142–144], mouse and human exocrine and endocrine pancreas [145–148], and various human tumours [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 ischaemic-reperfusion injury [90], gastrointestinal and liver inflammation [13,150,151], autoimmune and neurodegenerative disorders [7,152–154], kidney disorders [4], 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 note that, although, 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 tumours [4]. 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 [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, because 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 signalling is still limited, and important interspecies differences in CB_2 receptor signalling and in the pharmacology of CB_2 ligands must also be considered [158].

Problems with the use of peripherally-restricted $CB_{1/2}$ agonists for pain relief as a result of 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 because such compounds would be expected to be devoid of cardiometabolic liabilities. However, the preclinical data with AZD1940 and 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 that 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), whereas 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, although they do not display catalepsy, an indicator of psychoactivity in humans [162]. The antinociceptive effect of FAAH inhibitors, likely mediated through increases in AEA and PEA levels that activate $CB_{1/2}$, peroxisome proliferator-activated receptor α 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 inhibits FAAH both in the CNS and 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 as a result of its rapid uptake and metabolism by hepatocytes, was found to be devoid of glycaemic 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 of PF-04457845, an irreversible FAAH inhibitor with excellent analgesic efficacy in animal models [29,170], which was selected for clinical development. In a randomized, 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 function in test subjects. However, the study failed to show any analgesic efficacy of PF-04457845, whereas the nonsteroidal anti-inflammatory drug naproxen, used as a 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. The FAAH inhibitor AM3506 was recently found to be effective in increasing fear extinction in a CB_1 receptor-dependent manner in a mouse model of post-traumatic stress syndrome, 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 have demonstrated that MAGL inhibition or genetic deletion exerts anti-emetic [173], antineoplastic [174], and anxiolytic and antinociceptive effects in rodents [175], and also protects against brain injury [176,177], acute liver injury/inflammation [138] and colitis either via enhancing $CB_{1/2}$ signalling or by attenuating eicosanoid synthesis in specific tissues, such as the brain and liver [178], or by a combination of both. In the case of cancer, MAGL inhibition modulates fatty acid release for the synthesis of protumourigenic signalling lipids [174], as reviewed recently [179,180].

Although the above preclinical findings are indeed exciting, they also highlight important limitations. (a) Raising the tissue levels of ECs may promote the formation of cyclooxygenase-, lipoxygenase- and

cytochrome P450-derived pro-inflammatory metabolites [47,181]. (b) Some of the prostaglandins that were attenuated by MAGL inhibitors have well documented tissue protective functions. (c) Although the dual effect of MAGL inhibition on attenuating eicosanoid and enhancing EC signalling can be beneficial in certain tissues (e.g. the brain and liver) where MAGL links the EC and eicosanoid systems through the 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). (d) Chronic MAGL inhibition leads to functional antagonism of the ECS [175]. (e) 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. (f) There is growing evidence that ECs exert pro-inflammatory effects in various disease models through both CB_1-dependent and -independent mechanisms [6]. This is supported by a recent study demonstrating that the inhibition of EC synthesis is anti-inflammatory in macrophages [182]. (g) 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]. (h) 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. (i) 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 show 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', as discussed in the present

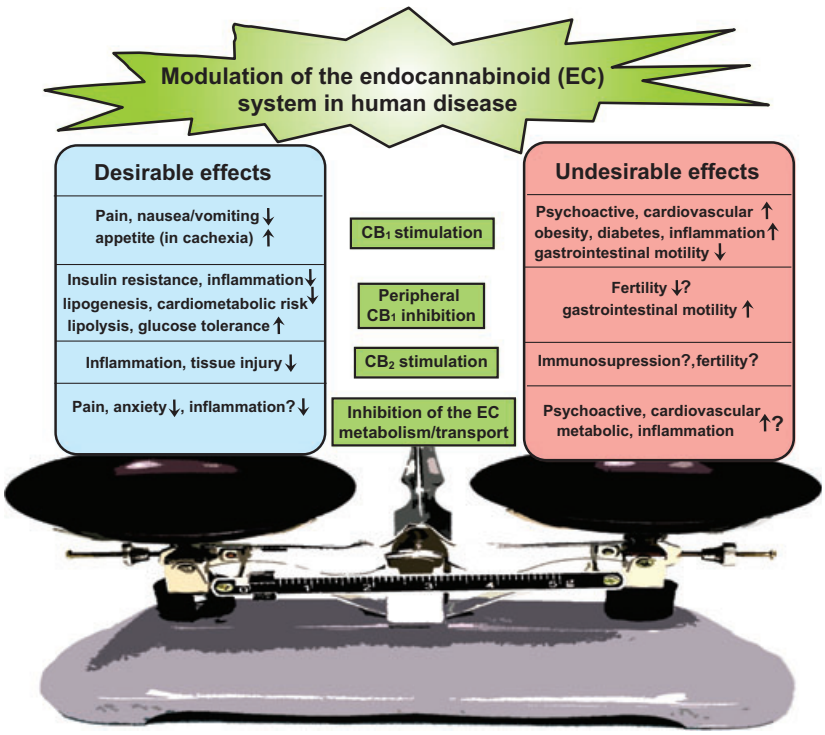

Fig. 1. Cannabinoid therapeutics: finding the right balance.

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, nonsteroid 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? Nonpsychoactive constituents of marijuana, such as CBD or their analogues, may have therapeutic utility in certain forms of acute tissue injury, inflammatory disorders, diabetes and diabetic complications	In the 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?
Peripherally restricted or global CB_2 agonists (peripheral CB_2)	Re-evaluation 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 anti-inflammatory effects are confirmed in humans	Most likely related to effects on immune and haematopoietic 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 signalling)	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.) Re-evaluation of human indications based on previous failures of trials with FAAH inhibitors in pain Search for new indications, better and more selective ligands	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 cyclooxygenase 2 inhibitors (e.g. cardiovascular). Pro-inflammatory effects in certain cases?

minireview. However, it is clear that, for the successful translation of preclinical findings to clinical practice, a better understanding of the pathological role of the ECS in various diseases, of the 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 (Fig. 1 and Table 2).

Future studies should focus on a 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 also aim to identify potential novel targets/mechanisms of action of THC and other plant-derived cannabinoids, coupled with the identification of nonpsychoactive constituents in cannabis extracts with potential therapeutic effects. Novel highly selective, orally available nontoxic 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. The development of specific novel antibodies for $CB_{1/2}$ receptors and endocannabinoid metabolic enzymes (FAAH, MAGL, diacylglycerol lipase α/β) validated by using positive and negative controls is essential for accurately assessing the time-dependent changes in $CB_{1/2}$ receptors and metabolic enzyme expression in diseased animal and human tissues, with the aim of understanding the human relevance of these changes. Our limited knowledge should be expanded to enable an understanding of $CB_{1/2}$ receptor trafficking, signalling and their interspecies differences. The development of reliable radioligands suitable for human imaging studies and research could contribute to a better understanding of the role of ECS in human health and disease.

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