13. Cannabinoids as antioxidant: An overview

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Abstract. Cannabinoids have been found to have antioxidant properties, unrelated to NMDA receptor antagonism. This new found property makes cannabinoids useful in the treatment and prophylaxis of wide variety of oxidation associated diseases, such as ischemic, age-related, inflammatory and autoimmune diseases. The cannabinoids are found to have particular application as neuroprotectants, for example in limiting neurological damage following ischemic insults, such as stroke and trauma, or in the treatment of neurodegenerative diseases, such as Alzheimer’s disease, Parkinson’s disease and HIV dementia. The aim of this chapter was to understand the mechanism of antioxidant action and therapeutic efficacy of cannabinoids.

1. Introduction

The recreational use of Cannabis Sativa preparations is known to most people [1]. However, the medicinal use of Cannabis also has a millenarian history that has been re-examined only very recently [2]. As early as 2600 BC, the Chinese emperor Huang Ti advised taking Cannabis for the relief of
cramps and rheumatic and menstrual pain [3]. This long history of Cannabis medical use has resulted in the development of pharmaceutical drugs, such as Dronabinol and Cesamet. These preparations is based on $\Delta^9$-tetrahydrocannabinol (THC, fig.1), which in 1964 was identified by Mechoulam and coworkers as the major psychoactive component of cannabis. They are prescribed in the United States as anti-emetic and appetite-stimulants to patients with cancer and AIDS. To date, some 60 plant terpenophenols more or less related to THC have been isolated and defined cannabinoids [4]. $\Delta^9$-tetrahydrocannabinol, for its potency and abundance in cannabis, is the most important.

![Figure 1. Chemical structure of $\Delta^9$-tetrahydrocannabinol.]

2. Cannabinoid receptors

Thus far, two cannabinoid-specific receptors have been cloned and characterized from mammalian tissues, the seven transmembrane G protein-coupled cannabinoid receptors type 1 (CB1 receptor), [5] and type 2 (CB2 receptor) [6]. Whereas the CB1 receptor expression is abundant in the central nervous system, the CB2 receptor is almost exclusively expressed in the immune system. The CB1 receptor is also expressed in peripheral nerve terminals and various extraneuronal sites such as the testis, uterus, eye, vascular endothelial, spleen and adipocytes [7-10]. Pharmacological evidence exists for the presence of other cannabinoid receptors, which, however, have not yet been cloned [11].

CB1 and CB2 receptors share only 44% overall identity and 68% within the transmembrane domains. Both cannabinoid receptors are coupled to G proteins, mostly of the $G_{i/o}$ type, through whose $\alpha$ subunit they inhibit the activity of adenylate cyclases and stimulate mitogen-activated protein kinases. However, additional studies established that cannabinoid receptors were also coupled to ion channels, resultant in the inhibition of Ca2+ influx through N type calcium channels [12]. CB1 receptors are also implicated in activation of both phospholipase C (via the $\beta_\gamma$ subunits of the G protein) and
PI-3-kinase. CB2 receptors, on the other hand, trigger a sustained activation of ceramide biosynthesis [13].

3. The endocannabinoid system

Several endogenous fatty-acid ligands, known as endocannabinoids, have been identified as having activity at the cannabinoid receptor. The first to discovered, in 1992, was arachidonoyl ethanolamide (anandamide, AEA) followed by 2-arachidonoylglycerol (2-AG). Both these compounds are derivates of arachidonic acid conjugated with ethanolamine or glycerol and are able to bind to CB1 and CB2 receptors, although with differences in affinities and activation efficacies [8]. During the last few years, several other bioactive lipid mediators have described; they appear to be active, through CB1 and/or CB2 receptors and confer specific pharmacological effects in vivo. Specifically, the compounds are 2-arachidonoyl-glyceryl-ether (noladin ether), o-arachidonoyl-ethanolamine (virodhamine), N- arachidonoyl-dopamine, and possibly oleamide [14; 10; 15; 16] (fig. 2).

Cannabinoid receptors, endocannabinoids and the whole apparatus appointed of their synthesis and degradation represent the elements of a novel

![Chemical structure of endogenous cannabinoid](image-url)

**Figure 2.** Chemical structure of endogenous cannabinoid.
endogenous signalling system (the endocannabinoid system) which is implicated in a overabundance of physiological functions \[17; 18\]. During the last few years a notable quantity of data has been reported to understand the biological roles of this system in more detail.

In general, endocannabinoid system serves several functions under physiological conditions. In the CNS, endocannabinoids intervene in the regulation of cognitive functions and emotions in neuronal circuits of the cortex, hippocampus and amygdale and to the reinforcement of substances of abuse in the mesolimbic system \[19\].

Endocannabinoids also modulate the control of movement and posture \[20\], the regulation of pain perception \[21\] and cardiovascular \[22\], gastrointestinal \[23\], respiratory and reproductive functions. CB2 receptors, instead, are involved in cellular and particularly humoral immune response, with possible implications for (neuro)inflammation and chronic pain \[25\].

Apart from the possible physiological functions of the endocannabinoid system briefly described above, endocannabinoid signalling undergoes dramatic tissue and blood changes under pathological conditions. Higher endocannabinoid levels are found in the case of experimental models of neurodegenerative disease, like Parkinson’s and Alzheimer’s disease and amyotropic lateral sclerosis, in gastrointestinal disorders like colon inflammation and in eating and metabolic disorders like anorexia nervosa, binge-eating disorders and obesity \[26\]. Finally, yet importantly, elevated levels of endocannabinoids have been observed in several types of cancer like glioblastoma \[27\], meningioma \[27\], colon \[28\] and prostate \[29\] carcinoma, colon polyps \[28\] and pituitary adenoma \[30\] as compared to their normal counterparts, suggesting a function of the endocannabinoid as potential tumor growth inhibitors.

4. Anti-oxidative and neuroprotective actions of cannabinoids

In the last years the number of studies related to anti-oxidative and neuroprotective actions of CBD are increased.

Hampson et al. \[31\] demonstrated that CBD reduced glutamate toxicity mediated by N-methyl-D-aspartate receptors (NMDAr), 2-amino-3-(4-butyl-3-hydroxyisoxazol-5-yl) propionic acid receptors (AMPA) or kainate receptors. The neuroprotection observed with cannabidiol was not affected by a cannabinoid receptor antagonist, indicating it is cannabinoid-receptor independent \[31\]. Previous studies had shown that glutamate toxicity may be prevented by antioxidants. In line with this, it was demonstrated that CBD can reduce hydroperoxide-induced oxidative damage as well as or better than other antioxidants. CBD was more protective against glutamate neurotoxicity
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than either ascorbate or a-tocopherol, indicating that this drug is a potent antioxidant [31].

Another study [32] showed that the anti-oxidative action of CBD may induce the neuroprotection in animal models of Parkinson's disease (PD). Daily administration of CBD during 2 weeks may produce a significant waning in the magnitude of toxic effects caused by a unilateral injection of 6-hydroxydopamine into the medial forebrain bundle [32] probably due to receptor-independent actions. In this model of PD, CBD led to an up-regulation of mRNA levels of Cu/Zn-superoxide dismutase, a key enzyme in endogenous defense against oxidative stress. The conclusion was that the antioxidant properties of CBD can provide neuroprotection against the progressive degeneration of nigrostriatal dopaminergic neurons that occur in PD [33]. This study was confirmed by a study showing that CBD reduced the striatal atrophy caused by 3-nitropropionic acid, in vivo, through mechanisms independent of the activation of cannabinoid, vanilloid TRPV1 and adenosine A2A receptors [34]. Also, the neuroprotective action of CBD in the human basal ganglia was supported by the strong relationship between N-acetylaspartate/total creatine ratio and CBD in the putamen/globus pallidum found in recreational cannabis users. This could reflect an enhancement of neuronal and axonal integrity in these regions by CBD [35].

Given the above preclinical evidences, for the first time, a clinical study evaluated the efficacy, tolerability and safety of CBD in PD patients with psychotic symptoms [36]. In an open-label pilot study, six consecutive outpatients with the diagnosis of PD and who also had psychosis for at least 3 months, have received a flexible-dose regimen of CBD administration (starting with an oral dose of 150 mg/day) for four weeks, in addition to their usual therapy. The psychotic symptoms significantly decreased along the CBD treatment, and the scale used to follow up the PD course exhibited a significant decrease of the total score. These preliminary data suggest that CBD may have a beneficial action in PD [37].

The possible neuroprotective actions of CBD was also considered in Alzheimer's disease (AD). Alzheimer's disease is widely held to be associated with oxidative stress due, in part, to the membrane action of β-amyloid peptide aggregates. Iuvone et al. [38] studied the effect of cannabidiol, a major non-psychoactive component of the marijuana plant (Cannabis sativa) on β-amyloid peptide-induced toxicity in cultured rat pheocromocytoma PC12 cells. Following exposure of cells to β-amyloid peptide (1 µg/mL), a marked reduction in cell survival was observed. This effect was associated with increased reactive oxygen species (ROS) production and lipid peroxidation, as well as caspase 3 (a key enzyme in the apoptosis cell-signalling cascade) appearance, DNA fragmentation and increased
intracellular calcium. Treatment of the cells with cannabidiol \((10^{-7} - 10^{-4} \text{m})\) prior to β-amyloid peptide exposure significantly elevated cell survival while it decreased ROS production, lipid peroxidation, caspase 3 levels, DNA fragmentation and intracellular calcium. These results indicate that cannabidiol exerts a combination of neuroprotective, anti-oxidative and anti-apoptotic effects against β-amyloid peptide toxicity, and that inhibition of caspase 3 appearance from its inactive precursor, pro-caspase 3, by cannabidiol is involved in the signalling pathway for this neuroprotection. A possible anti-inflammatory action may be involved in this CBD effect, since CBD inhibited both nitrite production and nitric oxide synthase (iNOS) protein expression induced by beta-A [39]. These results of in vitro studies were confirmed in vivo with a mouse model of AD-related neuroinflammation. Mice were inoculated with human beta-A into the right dorsal hippocampus, and treated daily with vehicle or CBD (2.5 or 10 mg kg, i.p.) for 7 days. In contrast to vehicle, CBD dose-dependent significantly inhibited mRNA for glial fibrillary acidic protein and the protein expression in beta-A injected animals. Moreover, under the same experimental conditions, CBD impaired iNOS and IL-1beta protein expression, and the related NO and IL-1beta release [40]. The possibility of CBD inhibiting beta-A-induced neurodegeneration is very promising to AD prevention.

Recently it has been suggested that CBD may protect neurons against the multiple molecular and cellular factors involved in the different steps of the neurodegenerative process, which takes place during prion infection [41]. Prion diseases are transmissible neurodegenerative disorders characterized by the accumulation in the CNS of the protease-resistant prion protein, a structurally misfolded isoform of its physiological counterpart [41].

The anti-oxidative and anti-inflammatory properties of CBD have led to the research of its possible activity in preventing damage caused by cerebral ischemia. CBD (1.25-20 mg/kg) was administered to freely-moving gerbils 5 min after bilateral carotid-artery occlusion for 10 minutes. Seven days after the ischemia, CBD antagonized electroencephalographic flattening, showing a dose-dependent bell-shaped curve. The best neuroprotective effect was observed at 5 mg/kg. Histological examination showed the complete survival of CA1 neurons in CBD-treated gerbils [42]. A similar effect has been reported by another research group in mice, after middle cerebral artery occlusion; the neuroprotective action of CBD being unaffected by CB1 receptor blockade [43]. The same research group has verified that this effect was inhibited by WAY100135, a serotonin 5-hydroxytriptamine 1A (5-HT_{1A}) receptor antagonist, but not by capsazepine, a vanilloid receptor antagonist, suggesting that the neuroprotective effect of CBD may be due to the increase in cerebral blood flow mediated by the serotonergic 5-HT_{1A} receptor [44].
Experimental evidence has suggested that beyond this action on the 5-HT$_{1A}$ receptor, the protective effect of CBD on ischemic injury is also secondary to its anti-inflammatory action [45]. In another study, the same research group reported that, while repeated treatment with delta9-THC leads to the development of tolerance for this neuroprotective effect, this phenomenon is not observed with CBD [46].

CBD has also been proven useful for possible complications of diabetes. The majority of diabetic complications are associated with pathophysiological alterations in the vasculature. Microvascular complications involve retinopathy and nephropathy while the atherosclerosis is the most common macrovascular complication of diabetes. The protective effects of CBD were studied in experimental diabetes induced by streptozotocin in rats. CBD treatment prevented retinal cell death and vascular hyperpermeability in the diabetic retina. In addition, it significantly reduced oxidative stress, decreased the levels of TNF-α, vascular endothelial growth factor, and intercellular adhesion-molecule [47]. It has also been suggested that CBD has significant therapeutic benefits against other diabetic complications and atherosclerosis, since it attenuated several effects of high glucose, including the disruption of the endothelial function [48].

5. Recent studies on mechanism of antioxidant action of cannabinoids

Dexanabinol, HU-211, a synthetic cannabinoid devoid of psychotropic effects, improves neurological outcome in models of brain trauma, ischemia and meningitis. Also, HU-211 was found to inhibit brain tumor necrosis factor (TNFalpha) production after head injury. Gallily et al. [49] demonstrated the ability of HU-211 to suppress TNFalpha production and to rescue mice and rats from endotoxic shock after LPS (Escherichia coli 055:B5) inoculation. In BALB/c mice, a dose of 10 mg/kg LPS, injected i.p., caused 57% and 100% mortality, at 24 and 48 hr, respectively. HU-211, administered i.p. 30 min before lipopolysaccharide (LPS), reduced lethality to 9 and 67% at these time points (P < .05). When coinjected with D-galactoseamine (i.p.), LPS was 100% lethal within 24 hr, whereas eight hourly injections of HU-211 caused mortality of C57BL/6 mice to drop to 10% (P < .001). Administration of LPS to Sprague-Dawley rats resulted in a 30% reduction in the mean arterial blood pressure within 30 min, which persisted for 3 hr. HU-211, given 2 to 3 min before LPS, completely abolished the typical hypotensive response. Furthermore, the drug also markedly suppressed in vitro TNFalpha production and nitric oxide
generation (by >90%) by both murine peritoneal macrophages and rat alveolar macrophage cell line exposed to LPS. HU-211 may, therefore, have therapeutic implications in the treatment of TNFalpha-mediated pathologies [49]. The results of the present in vivo studies, in two experimental models using two species, suggest that HU-211 may have important clinical implications. It is noteworthy that dexamabinol, HU-211, was tested in human volunteers in phase I clinical trial, and is now under phase II clinical trial for severe head injury. Thus, this novel drug appears to be a promising candidate for the treatment in the nontreatable and devastating TNFα-mediated diseases.

Interleukin-1 receptor antagonist (IL-1ra) is an important anti-inflammatory cytokine that blocks all known actions of IL-1 and markedly protects against experimentally induced ischemic, excitotoxic, and traumatic brain insults. Cannabinoids (CBs) also exert potent anti-inflammatory and neuroprotective effects, but the mechanisms of their actions are unknown. Molina-Holdago et al. [50] tested the hypothesis that the actions of CBs were mediated by endogenous IL-1ra. They reported for the first time that both CB₁ and CB₂ receptors modulate release of endogenous IL-1ra from primary cultured glial cells [50]. Activation of CB₁ or CB₂ receptors increased lipopolysaccharide-induced IL-1ra release, and specific CB₁ or CB₂ antagonists blocked lipopolysaccharide-induced production of IL-1ra from glial cells. Comparison of neuronal cultures from wild-type mice and mice lacking IL-1ra (knock-out) indicates that endogenous IL-1ra is essential for the neuro-protective effects of CBs against excessive activation of glutamate receptors (excitotoxicity) in response to S-AMPA or NMDA. Similarly, analysis of mixed glial cultures from IL-1ra knock-out mice indicates that endogenous IL-1ra is required for the CB-induced inhibition of nitric oxide production in response to bacterial lipopolysaccharide [50]. These data suggest a novel neuroprotective mechanism of action for CBs in response to inflammatory or excitotoxic insults that is mediated by both CB₁ and CB₂ receptor-dependent pathways. In summary, the results presented here support our hypothesis that endogenous IL-1ra mediates the neuroprotective and anti-inflammatory actions of CBs in primary neurons and glia. These effects appear to be mediated by both CB₁ and CB₂ receptors. CB-induced IL-1ra release may negatively regulate IL-1β actions in the brain, via IL-1ra blocking the IL-1 receptor (IL-1RI), after inflammatory or excitotoxic insults. It is tempting to speculate therefore that the neuroprotective and anti-inflammatory actions of CBs depend in part on modification of the balance between proinflammatory and anti-inflammatory cytokines. These findings have important implications for our understanding of the mechanisms of
action of CBs in diverse CNS disorders and for the development of new neuroprotective therapies.

CB1 cannabinoid receptors (CB1Rs) are involved in protecting the brain from ischemia and related disorders. However, the underlying protective mechanisms are incompletely understood. Kim et al. [51] investigated the effect of CB1R activation on oxidative injury, which has been implicated in neuronal death after cerebral ischemia and neurodegenerative disorders, in mouse cortical neuron cultures. The CB1R agonist Win 55212-2 [R-(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl)methanone mesylate] reduced neuronal death, measured by lactate dehydrogenase release, in cultures treated with 50 microM FeCl2, and its protective effect was attenuated by the CB1R antagonist SR141716A [N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-cichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride]. The endocannabinoid anandamide reproduced the effect of Win 55212-2, as did the antioxidant 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox). Neuronal injury was more severe after in vitro or in vivo administration of FeCl2 to CB1R-knockout compared with wild-type mice [51]. Win 55212-2 reduced the formation of reactive oxidative species in cortical neuron cultures treated with FeCl2, consistent with an antioxidant action [41]. Pertussis toxin reduced CB1R-mediated protection, which points to a protective mechanism that involves signaling through G(i/o) proteins. Since CB1R-activated G protein signaling inhibits protein kinase A but activates phosphatidylinositol 3-kinase (PI3K), Kim et al. [51] tested the involvement of these pathways in CB1R-mediated neuroprotection. Dibutyryl-cyclic adenosine monophosphate (dbcAMP) blocked protection by Win 55212-2, whereas the PI3K inhibitor wortmannin did not, and the effect of dbcAMP was inhibited by the protein kinase A inhibitor H89 [N-[2-((p-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide] (> or =10 nM). CB1R-induced, SR141716A-, pertussis toxin-, and dbcAMP-sensitive protection was also observed for two other oxidative insults, exposure to H2O2 or buthionine sulfoximine. Therefore, receptor-stimulated inhibition of protein kinase A seems to be required for the neuroprotective effect of CB1R activation against oxidative neuronal injury. These precedents will be helpful in guiding future studies on mechanisms of cannabinoid neuroprotection.

Alzheimer's disease (AD) is characterized by enhanced beta-amyloid peptide (betaA) deposition along with glial activation in senile plaques, selective neuronal loss, and cognitive deficits. Cannabinoids are neuroprotective agents against excitotoxicity in vitro and acute brain damage in vivo [52]. Ramirez et al. [52] have studied the localization, expression, and function of cannabinoid receptors in AD and the possible protective role of
cannabinoids after betaA treatment, both in vivo and in vitro. They showed that senile plaques in AD patients express cannabinoid receptors CB1 and CB2, together with markers of microglial activation, and that CB1-positive neurons, present in high numbers in control cases, are greatly reduced in areas of microglial activation [52]. In pharmacological experiments, they found that G-protein coupling and CB1 receptor protein expression are markedly decreased in AD brains. Additionally, in AD brains, protein nitration is increased, and, more specifically, CB1 and CB2 proteins show enhanced nitration [52]. Intracerebroventricular administration of the synthetic cannabinoid WIN55,212-2 to rats prevent betaA-induced microglial activation, cognitive impairment, and loss of neuronal markers [52]. Cannabinoids (HU-210, WIN55,212-2, and JWH-133) block betaA-induced activation of cultured microglial cells, as judged by mitochondrial activity, cell morphology, and tumor necrosis factor-alpha release; these effects are independent of the antioxidant action of cannabinoid compounds and are also exerted by a CB2-selective agonist [52]. Moreover, cannabinoids abrogate microglia-mediated neurotoxicity after betaA addition to rat cortical cocultures. These results indicate that cannabinoid receptors are important in the pathology of AD and that cannabinoids succeed in preventing the neurodegenerative process occurring in the disease.

The neuroprotective effects of Delta(9)-tetrahydrocannabinol (THC) were examined by Chen et al. [53] using an in vitro model in which the AF5 CNS cell line was exposed to toxic levels of N-methyl-d-aspartate (NMDA), an agonist of the NMDA glutamate receptor. NMDA toxicity was reduced by THC, but not by the more specific cannabinoid receptor agonist, WIN55,212-2. Addition of dibutyryl cAMP (dbcAMP) to the culture medium did not alter the neuroprotective effect of THC and did not unmask a neuroprotective effect of WIN55,212-2. The cannabinoid antagonist SR141716A did not inhibit the neuroprotection induced by THC or alter the response to WIN55,212-2, even in the presence of dbcAMP, indicating that the neuroprotective effect of THC was cannabinoid receptor-independent. On the other hand, both THC and WIN55,212-2 produced cellular toxicology at higher dosages, an effect which was blocked in part by SR141716A. Capsaicin, an antioxidant and vanilloid receptor agonist, also produced a protective effect against NMDA toxicology [53]. The protective effect of capsaicin was blocked by co-application of ruthenium red, but was not blocked by the specific vanilloid receptor antagonist capsazepine, and the transient receptor potential vanilloid type 1 (TRPV1) and ANKTM1 transcripts were not detected in AF5 cells. Thus, the neuroprotective effects of THC and capsaicin did not appear to be mediated by TRP ion channel family receptors. The antioxidant alpha-tocopherol prevented neurotoxicity in
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a dose-dependent manner. Therefore, THC may function as an antioxidant to increase cell survival in NMDA-induced neurotoxicity in the AF5 cell model, while higher dosages produce toxicity mediated by CB1 receptor stimulation [53].

Binge alcohol consumption in the rat induces substantial neurodegeneration in the hippocampus and entorhinal cortex. Oxidative stress and cytotoxic edema have both been shown to be involved in such neurotoxicity, whereas N-methyl-d-aspartate (NMDA) receptor activity has been implicated in alcohol withdrawal and excitotoxic injury. Because the nonpsychoactive cannabinoid cannabidiol (CBD) was previously shown in vitro to prevent glutamate toxicity through its ability to reduce oxidative stress, Hamelink et al. [54] evaluated CBD as a neuroprotectant in a rat binge ethanol model. When administered concurrently with binge ethanol exposure, CBD protected against hippocampal and entorhinal cortical neurodegeneration in a dose-dependent manner. Similarly, the common antioxidants butylated hydroxytoluene and alpha-tocopherol also afforded significant protection. In contrast, the NMDA receptor antagonists dizocilpine (MK-801) and memantine did not prevent cell death [54]. Of the diuretics tested, furosemide was protective, whereas the other two anion exchanger inhibitors, L-644,711 [(R)-(+)-(5,6-dichloro2,3,9,9a-tetrahydro 3-oxo-9a-propyl-1H-fluoren-7-yl)oxy acetic acid] and bumetanide, were ineffective [54]. In vitro comparison of these diuretics indicated that furosemide is also a potent antioxidant, whereas the nonprotective diuretics are not. The lack of efficacy of L-644,711 and bumetanide suggests that the antioxidant rather than the diuretic properties of furosemide contribute most critically to its efficacy in reversing ethanol-induced neurotoxicity in vitro, in our model. This study provides the first demonstration of CBD as an in vivo neuroprotectant and shows the efficacy of lipophilic antioxidants in preventing binge ethanol-induced brain injury [54].

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive motor neuron loss, paralysis and death within 2-5 years of diagnosis. Currently, no effective pharmacological agents exist for the treatment of this devastating disease. Neuroinflammation may accelerate the progression of ALS. Cannabinoids produce anti-inflammatory actions via cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2), and delay the progression of neuroinflammatory diseases [55]. Additionally, CB2 receptors, which normally exist primarily in the periphery, are dramatically up-regulated in inflamed neural tissues associated with CNS disorders. In G93A-SOD1 mutant mice, the most well-characterized animal model of ALS, endogenous cannabinoids are elevated in spinal cords of symptomatic mice [55]. Furthermore, treatment with non-selective cannabinoid partial
agonists prior to, or upon, symptom appearance minimally delays disease onset and prolongs survival through undefined mechanisms. Shoemaker et al. [55] demonstrated that mRNA, receptor binding and function of CB2, but not CB1, receptors are dramatically and selectively up-regulated in spinal cords of G93A-SOD1 mice in a temporal pattern paralleling disease progression. More importantly, daily injections of the selective CB2 agonist AM-1241, initiated at symptom onset, increase the survival interval after disease onset by 56% [55]. Therefore, CB2 agonists may slow motor neuron degeneration and preserve motor function, and represent a novel therapeutic modality for treatment of ALS.

Impaired endothelial activity and/or cell death play a critical role in the development of vascular dysfunction associated with congestive heart failure, diabetic complications, hypertension, coronary artery disease and atherosclerosis. Increasing evidence suggests that cannabinoid 1 (CB(1)) receptor inhibition is beneficial in atherosclerosis and cardiovascular inflammation both in experimental models, as well as in humans. Rajesh et al. [56] investigated the effects of CB(1) receptor activation with the endocannabinoid anandamide (AEA) or synthetic agonist HU210 on cell death and interrelated signal transduction pathways in human primary coronary artery endothelial cells (HCAECs). In HCAECs expressing CB(1) receptors (demonstrated by Western immunoblot and flow cytometry) AEA (5-15 microM) or HU210 (30-1000 nM) triggered concentration- and time-dependent activation of p38 and c-Jun NH(2)-terminal protein kinase (JNK)-mitogen-activated protein kinases (MAPKs), cell death and ROS generation [56]. The AEA- or HU210-induced cell death and MAPK activation were attenuated by CB(1) antagonists [SR141716 (rimonabant) and AM281], inhibitors of p38 and JNK-MAPKs or the antioxidant N-acetylcysteine. N-acetylcysteine alone prevented AEA- or HU210-induced ROS generation, but only partially attenuated MAPK activation and cell death. In contrast, in combination with CB(1) antagonists, N-acetylcysteine completely prevented these effects [56]. CB(1) receptor activation in endothelial cells may amplify the ROS-MAPK activation-cell death pathway in pathological conditions when the endocannabinoid synthetic or metabolic pathways are dysregulated by excessive inflammation and/or oxidative/nitrosative stress, thereby contributing to the development of endothelial dysfunction and pathophysiology of multiple cardiovascular diseases [56].

6. Recent developments in therapeutic efficacy of cannabinoids

There are currently a great number of studies which deal with the possible therapeutic applications of cannabinoids. Indeed, in the United
Kingdom and in several states of the United States doctors may prescribe THC or certain synthetic as appetite stimulants and vomit inhibitors in patients with AIDS or cancer treated chronically with chemotherapy. Among possible therapeutic uses of cannabinoids the following may be mentioned: (a) as analgesic agents they have been shown to be very effective in alleviating sharp and chronic pain; (b) as agents which reduce motor activity they are being tested nowadays for treatment of disorders associated to Parkinson's disease, Huntington's chorea and multiple sclerosis; (c) as anticonvulsive agents their use in treatment of epilepsy is being studied; (d) as agents which reduce intraocular pressure they could be used in treatment of glaucoma. One of the most intriguing and unexplored effects of cannabinoids is their ability to inhibit the growth of cells transformed in vitro. Thus, it has been shown that several cannabinoids inhibit the proliferation of breast tumor cells MCF-7, glioblastoma cells C6 and prostate tumor cells PC-3. However, these findings in culture cell systems have never been observed before in vivo, so that their biomedical significance is unknown.

Guzman et al. [57] studied a therapeutic use of cannabinoid compounds for treatment of brain tumors [57]. Currently employed therapies for these tumors (surgery, radiotherapy, chemotherapy, immunotherapy, gene therapy) are generally ineffective or at best palliative. The study implies a technically simple approach lacking appreciable side effects and highly effective in the treatment of brain tumors, including the most malign (glioblastomas).

The present study [57] makes a novel use of cannabinoids in the treatment of brain tumors, and is based on our original observations of cannabinoid-induced marked regressions (implying a longer life) and even eradication (implying curation) of glioblastomas in laboratory animals. This invention involves a technically simple therapy lacking any significant side effects, and more significantly very effective in the treatment of brain tumors, which as mentioned before cannot be satisfactorily treated nowadays by any other techniques or compounds.

The therapy with cannabinoids in the treatment of cerebral tumors involves (intracranial or systematic) administration of (natural or synthetic) cannabinoids to (human or non-human) mammals having cerebral tumors. Activation of the specific receptors of the cannabinoid leads to selective death of the transformed cells. Regression or eradication of the cerebral tumors is achieved without any significant side-effects [57].

Another study [58] relates to novel cannabinoid receptor modulators, in particular cannabimoid 1 (CB1) or cannabinoid 2 (CB2) receptor modulators, and uses thereof for treating diseases, conditions and/or disorders modulated by a cannabinoid receptor (such as cancer, pain, neurodegenerative disorders, eating disorders, weight loss or control, and obesity).
This study [58] provides compounds and pharmaceutical formulations thereof that are useful in the treatment, amelioration, and/or prevention of diseases, conditions and/or disorders modulated by a cannabinoid (CB) receptor, especially those modulated by the CB1 or CB2 receptor including those discussed below.

Cell growth related syndromes, disorders or diseases include, but are not limited to, dysregulated mammalian cell proliferation, breast cancer cell proliferation, prostate cancer cell proliferation and the like.

Pain related syndromes, disorders or diseases include, but are not limited to, central and peripheral pathway mediated pain, bone and joint pain, migraine headache associated pain, cancer pain, dental pain, menstrual cramps, labor pain, chronic pain of the inflammatory type, allergies, rheumatoid arthritis, dermatitis, immunodeficiency, chronic neuropathic pain, (e.g. pain associated with diabetic neuropathy, sciatica, non specific lower back pain, fibromyalgia; HIV-related neuropathy; post herpetic neuralgia, trigeminal neuralgia, and pain resulting from physical trauma, amputation, cancer, toxins or chronic inflammatory conditions), hodgkin's disease, myasthenia gravis, nephrotic syndrome, scleroderma, thyroiditis and the like.

Neurodegenerative related syndromes, disorders or diseases include, but are not limited to, Parkinson's disease, multiple sclerosis, epilepsy, ischemia or secondary biochemical injury collateral to traumatic head or brain injury, brain inflammation, eye injury or stroke and the like.

The compounds of this study [58] may also be used in conjunction with other pharmaceutical agents for the treatment of the diseases, conditions and/or disorders described herein.

This invention further provides a method of treating a disease, condition and/or disorder modulated by a cannabinoid receptor (CB), and in particular the CB1 or CB2 receptor, in a subject in need thereof by administering to the subject a therapeutically effective amount of a compound or a pharmaceutical composition of the present invention.

The psychoactive agent in Cannabis plant material is tetrahydrocannabinol (THC). Since THC is known to elicit various physiological effects (e.g., as an anti-inflammatory agent or analgesic) other than psychoactivity, various derivatives of THC that retain a favorable biochemical or pharmacological activity of THC without any potential for abuse or psychoactivity are beneficial and have been synthesized as potential drugs.

One of the activities associated with THC and some of its derivatives is inhibition of cell proliferation. However, this activity, as with psychoactivity, is dependent on binding to the cannabinoid receptor CB1. Thus, non-psychoactive derivatives of THC, which do not bind to the CB1 receptor are not expected to inhibit cell proliferation.
The study from Burstei et al. [59] is based on the discovery that non-psychoactive THC derivatives, such as THC acids, can decrease cell proliferation. Moreover, this effect is not dependent on an increase in the rate of apoptosis, which has been identified as a CB1 receptor-mediated activity of THC.

Accordingly, the invention features a method of decreasing cell proliferation in a mammal (e.g., a human) by identifying a mammal in which a decrease in cell proliferation is desirable, and administering to the mammal an amount of a compound of Formula I effective to decrease cell proliferation in the mammal [59].

The methods of the invention provide a new use for non-psychoactive cannabinoid as drugs for the treatment or prophylaxis of a condition or disease characterized by cell proliferations (e.g., cancer) [79]. Because of the low toxicity, non-psychoactive nature, and low abuse potential of such cannabinoids, the compounds can be used as a dietary supplement (e.g., like a daily vitamin pill) to prevent cancer. In addition, the compounds can be applied topically, e.g., to a skin lesion characterized by undesirable cell proliferation, such as in psoriasis [59].

Also, it has now been found that certain analogs of anandamide are potent inhibitors of transport of anandamide across cell membranes. The inventive analogs do not activate the cannabinoid receptors or inhibit anandamide hydrolysis per se but instead prevent anandamide reuptake thereby prolonging the level of the undegraded anandamide. Previously, cannabinoid drugs were targeted toward cannabinoid receptors and amidase enzymes. The anandamide transport inhibitor of the present invention targets activity of the anandamide transporter [80].

Some of the inventive analogs [60] and physiologically acceptable salts thereof, have high potential when administered in therapeutically effective amounts for providing a physiological effect useful to treat pain; peripheral pain; glaucoma; epilepsy; nausea such as associated with cancer chemotherapy; cancer. Thus, another aspect of the invention is the administration of a therapeutically effective amount of an inventive compound, or a physiologically acceptable salt thereof, to an individual or animal to provide a physiological effect.

Furthermore, novel tricyclic cannabinoid compounds are presented. Some of these compounds exhibit fluorescence properties. The fluorescent cannabinoid compounds are typically endogenously fluorescent. Some of these compounds, when administered in a therapeutically effective amount to an individual or animal, result in a sufficiently high level of that compound in the individual or animal to cause a physiological response. The physiological response useful to treat a number of physiological conditions [61].
This study relates generally to cannabinoid compounds. One embodiment of the present invention more particularly relates to cannabinoid compounds exhibiting fluorescence properties, particularly in the ultraviolet-visible wavelength ranges [61].

The inventive compounds [61], and physiologically acceptable salts thereof, have pharmacological properties when administered in therapeutically effective amounts for providing a physiological response useful to treat nausea associated with cancer chemotherapy.

The compound of this invention can be administered by a variety of known methods, including, for example, orally, rectally, or by parenteral routes (e.g., intramuscular, intravenous, subcutaneous, nasal or topical) [61]. The form in which the compounds are administered will be determined by the route of administration. Such forms include, but are not limited to, capsular and tablet formulations (for oral and rectal administration), liquid formulations (for oral, intravenous, intramuscular, subcutaneous, ocular, intranasal, inhalation-based and transdermal administration) and slow releasing microcarriers (for rectal, intramuscular or intravenous administration). The formulations can also contain a physiologically acceptable vehicle and optional adjuvants, flavorings, colorants and preservatives. Suitable physiologically acceptable vehicles include, for example, saline, sterile water, Ringer's solution and isotonic sodium chloride solutions. The specific dosage level of active ingredient will depend upon a number of factors, including, for example, biological activity of the particular preparation, age, body weight, sex and general health of the individual being treated [61].

Another study relates to the targeting of CB2 cannabinoid receptors as a novel therapy to treat malignant lymphoblastic disease, particularly by administration of active molecules possessing at least some effective CB2 receptor agonist activity to patients suffering from such disease [62].

Recently, anandamide was shown to inhibit the proliferation of human breast cancer cell lines MCF-7 and EFM-19 in vitro. Also, THC was shown to induce apoptosis in human prostate PC-3 cells and in C6 glioma cells in culture. THC-induced apoptosis involved cannabinoid receptor-dependent or -independent pathways. Such studies have triggered interest in targeting cannabinoid receptors in vivo to induce apoptosis in transformed cells. To this end, cannabinoids were shown recently to inhibit the growth of C6 glioma cells in vivo.

The inventors have noted that cells of the immune system express high levels of CB2 receptors which they considered might be implicated in induction of apoptosis in normal or transformed immune cells [62]. By using both murine and human leukemia and lymphoma lines as well as primary
acutelymphoblastic leukemia (ALL) cells they have demonstrated that ligation of CB2 receptors can induce apoptosis in a wide range of cancers of immune-cell origin. Furthermore, they demonstrate that TEC can inhibit the growth of murine lymphoma cells in vivo by inducing apoptosis and, in test experiments, completely cure approximately 25% of the mice bearing that tumor. Current data suggest that CB2 agonists that are devoid of psychotropic effects may constitute a novel and effective modality to treat malignancies of the immune system.

The inventors have particularly found that exposure of murine tumors EL-4, LSA, and P815 to delta-9-tetrahydrocannabinol (THC) in vitro led to a significant reduction in cell viability and an increase in apoptosis [62]. Exposure of BL-4 tumor cells to the synthetic cannabinoid HU-210 and the endogenous cannabinoid anandamide led to significant induction of apoptosis, whereas exposure to WIN55212 was not effective. Treatment of EL-4 tumor bearing mice with THC in vivo led to a significant reduction in tumor load, increase in tumor-cell apoptosis, and increase in survival of tumor-bearing mice.

The inventors have examined a number of human leukemia and lymphoma cell lines, including Jurkat, Molt-4, and Sup-T1, and have determined that they expressed CB2 but not CB1 receptors [82]. These human tumor cells were also susceptible to apoptosis induced by THC, HU-210, anandamide, and the CB2-selective agonist JWH-015. This effect was mediated at least in part through the CB2 receptors because pretreatment with the CB2 antagonist SR144528 partially reversed the THC-induced apoptosis. Culture of primary acute lymphoblastic leukemia cells with THC in vitro reduced cell viability and induced apoptosis. Thus CB2 cannabinoid receptors expressed on malignancies of the immune system are capable of serving as potential targets for the induction of apoptosis. CB2 agonists lack psychotropic effects, they can serve as novel anticancer agents to selectively target and kill tumors of immune origin. The present inventors have demonstrated that THC and other cannabinoids can induce apoptosis in murine and human leukemia and lymphoma cell lines as well as primary ALL cells. The human tumor-cell lines screened expressed CB2 but not CB1 receptors, whereas the murine tumors expressed both CB1 and CB2 receptors.

Ligation of CB2 receptors is sufficient to induce apoptosis inasmuch as CB2-selective agonists can induce apoptosis in tumor cells. THC-induced apoptosis in human tumor-cell lines is now shown to be reversed by CB2 antagonists. THC was effective not only in vitro but also in vivo, as demonstrated by its ability to induce apoptosis and decrease the tumor load. Moreover, THC treatment could cure approximately 25% of the mice bearing a syngeneic tumor. Thus targeting CB2 receptors on tumor cells of immune
origin provides a novel and relatively non-toxic approach to treating such cancers.

Finally, another study relates to methods and compositions for treating cancer [63]. More particularly, the invention provides cannabidiol derivatives and compositions thereof.

Previous studies demonstrated that the helix-loop-helix protein Id-1, an inhibitor of basic helix-loop-helix (bHLH) transcription factors, plays a crucial role during breast cancer progression. Id-1 stimulated proliferation, migration and invasion in breast cancer cells. Moreover, targeting Id-1 expression partially in breast cancer cells reduced invasion and breast cancer metastasis in vitro and in preclinical animal models. The disclosure shows that Id-1 is a target for therapy approaches, and that inhibiting Id-1 expression and/or activity provides a mechanism for treating patients with breast cancer. This approach may be highly effective and safe in advanced breast cancer patients, given (1) the relationship between high Id-1 expression levels and aggressive breast cancer cell behaviors; (2) partial reduction in Id-1 activity can achieve significant outcomes; and (3) Id-1 expression is low in normal adult tissues, thereby eliminating unwanted toxicities generally associated with currently available therapeutic modalities.

Id-1 protein plays a key role in the malignant progression of many aggressive and invasive human cancer such as: leukemia, melanoma, hepatocellular carcinoma, colorectal adenocarcinoma, pancreatic cancer, lung cancer, kidney cancer, medullary thyroid cancer, papillary thyroid cancer, astrocytic tumor, neuroblastoma, Ewing's sarcoma, ovarian tumor, cervical cancer, endometrial carcinoma, breast cancer, prostate cancer, malignant seminoma, and squamous cell carcinomas, such as esophageal cancer, and head and neck cancer. Accordingly, Id-1 associated cell proliferative disorders include, but are not limited to, Leukemia, Melanoma, Squamous cell carcinoma (SCC) (e.g., head and neck, esophageal, and oral cavity), Hepatocellular carcinoma, Colorectal adenocarcinoma, Pancreatic cancer, Lung cancer, Kidney cancer, Medullary thyroid cancer, Papillary thyroid cancer, Astrocytic tumor, Neuroblastoma, Ewing's sarcoma, Ovarian tumor, Cervical cancer, Endometrial carcinoma, Breast cancer, Prostate cancer, and Malignant seminoma.

Approaches for targeting Id-1 expression include gene therapy using antisense oligonucleotide, siRNA, non-viral or viral plasmid-based strategies. In addition, the development of new strategies to modulate Id-1 expression/functional activity include the identification of small molecules that modulate the activity of Id-1. A range of small molecules that target the molecular pathology of cancer are now being developed, and a significant number of them are being tested in ongoing human clinical trials. The disclosure demonstrates that cannabidiol (CBD) and CBD derivatives are inhibitors of
Id-1. The use of CBD, and derivatives thereof, represents a novel strategy for the treatment of cancer.

As used herein, the term “CBD” and “CBD derivatives” includes cannabinoids and derivatives thereof such as cannabidiol. cannabinoids are a group of terpenophenolic compounds present in Cannabis sativa. The term “cannabinoids” generally refers to a group of substances that are structurally related to tetrahydrocannabinol (THC) or that bind to cannabinoid receptors. Plant cannabinoids are stable compounds with low toxicity profiles that are well tolerated by animals and humans during chronic administration. A variety of chemical classes of cannabinoids are useful in the methods provided herein including cannabinoids structurally related to THC, aminoalkylindoles, the eicosanoids related to the endocannabinoids, 1,5-diarylpyrazoles, quinolines and arylsulphonamides and additional compounds that do not fall into these standard classes but bind to cannabinoid receptors.

Data provided herein indicates that CBD and derivatives thereof that act as Id-1 inhibitor effectively inhibit genotypic and phenotypic changes that allow aggressive breast cancers to proliferate, invade and metastasize [63]. Since CBD inhibits Id-1 expression in aggressive breast cancer, the disclosure also provides a rational drug design strategy and compounds obtained there from as potent and efficacious analogs. The disclosure demonstrates that the opened tetrahydropyran ring in CBD and aliphatic side chain of CBD are key pharmacophores involved in the inhibition of Id-1, alterations of these functional groups allow one to improve both the potency and efficacy of the parent compound, CBD [63]. Moreover, reducing Id-1 expression with cannabinoids provides a therapeutic strategy for the treatment of additional aggressive cancers since Id-1 expression was found to be up-regulated during the progression of almost all types of solid tumors investigated.

Accordingly, provided herein are methods for modulating the activity of a metastatic cell by regulating the activity of a target Id-1 using a CBD or CBD derivative. Methods can also include “regulating the activity of a target Id-1” includes: 1) mechanisms for modulating endogenous nucleic acid sequences that encode a target Id-1 such that Id-1 polypeptide levels are decreased in a cell; 2) introducing exogenous nucleic acid sequences that inhibit Id-1 expression in a cell; 3) increasing the turnover rate of endogenous Id-1 polypeptides such that Id-1 polypeptide levels are decreased in a cell [63].

7. Conclusions

In conclusion, the present paper showed that cannabinoids possess antioxidant and neuroprotective effects. In addition to directly salvaging
neurons affected by several disorders, cannabinoids also have anti-inflammatory effects and promote the birth of new neurons (neurogenesis) in the adult brain, either of which may contribute to improving neurological outcome. The antioxidative properties of cannabinoids suggest a therapeutic use as neuroprotective agents, and the particular properties of cannabidiol make it a good candidate for such development.

8. References

Cannabinoids as antioxidant


