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Review

The Role of Endocannabinoid Signaling in the Molecular Mechanisms of Neurodegeneration in Alzheimer's Disease

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Abstract. Alzheimer's disease (AD) is the most common form of progressive neurodegenerative disease characterized by cognitive impairment and mental disorders. The actual cause and cascade of events in the progression of this pathology is not fully determined. AD is multifaceted in nature and is linked to different multiple mechanisms in the brain. This aspect is related to the lack of efficacious therapies that could slow down or hinder the disease onset/progression. The ideal treatment for AD should be able to modulate the disease through multiple mechanisms rather than targeting a single dysregulated pathway. Recently, the endocannabinoid system emerged as novel potential therapeutic target to treat AD. In fact, exogenous and endogenous cannabinoids seem to be able to modulate multiple processes in AD, although the mechanisms that are involved are not fully elucidated. This review provides an update of this area. In this review, we recapitulate the role of endocannabinoid signaling in AD and the probable mechanisms through which modulators of the endocannabinoid system provide their effects, thus highlighting how this target might provide more advantages over other therapeutic targets.

Keywords: 2-AG, Alzheimer's disease, amyloid-β, anadamide, cannabinoids, CB1, CB2, FAAH, MAGL, tau

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia. About 35.6 million people worldwide are now suffering from AD, and disease prevalence is expected to affect 115 million by 2050 [1]. AD was discovered 100 years ago but the insight into symptoms, etiology, disease progression, pathological mechanism, and treatment has gained a significant progress over last 30 years. Although we have known about this disease for over a century, to date there is no curative treatment available. Three acetylcholinesterase (AChE) inhibitors (donepezil, rivastigmine, and galantamine), and a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, memantine, are the only drugs available and approved by the United States Food and Drug Administration (FDA) for the treatment of AD [2]. The latest (2011) guidance from the National Institute for Health and Clinical Excellence recommends that the three AChE inhibitors are available for managing mild-to-moderate AD, whereas memantine is recommended as an option for treating people

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with moderate AD who are intolerant to or have a contraindication to AChE inhibitors treatment or with severe AD symptoms.

However, all present pharmacological therapies for AD do not reverse the disease progression and are accompanied by several side effects. Moreover, most AD cases are diagnosed when the disease is already progressed to an advanced level, and this might be due to the lack of early blood-based biomarkers of the disease. Interestingly, a recent study discovered and validated a set of ten lipids from peripheral blood that are proposed to be early biomarkers of AD [3].

Today, worldwide efforts are underway to find new compounds to treat the disease, delay its onset, and prevent it from developing. Unfortunately, not a single new drug has been approved for AD treatment in more than a decade. Therefore, it is necessary to explore novel potential therapeutic targets.

The endocannabinoid (eCB) system appears to be a promising therapeutic target as it has the ability to modulate a range of aspects of AD pathology. At a first glance, it is striking that cannabinoids like delta-9-tetrahydrocannabinol (Δ^9-THC), known to impair memory, could be beneficial in AD [4]. However, augmentation of eCB signaling could reduce excitotoxicity, oxidative stress, and neuroinflammation and thus could alleviate symptoms of AD [5]. Previous reviews have highlighted the beneficial effects of cannabinoids in AD treatment [5–10], but none of them have focused on the molecular mechanisms through which eCBs exert their beneficial effects. Thus, the present review will extensively cover recent findings on the dysregulation of eCB signaling and the molecular mechanisms involved in beneficial effects of cannabinoids in AD.

ALZHEIMER'S DISEASE PATHOPHYSIOLOGY

AD is a progressive, degenerative, and irreversible neurological disorder that causes deterioration of memory, judgment, and reasoning in the elderly [11]. Patients suffering from AD exhibit cognitive impairment, memory loss, and behavioral changes [11]. The neurodegeneration in AD is characterized by neuronal loss and synaptic injury [12]. Moreover, AD is associated with extracellular insoluble plaques [13], intracellular neurofibrillary tangles (NFTs) [14], astrogliosis [15], and microglial cell proliferation [16]. Extracellular senile plaques are mainly composed of amyloid-β (Aβ) protein. The deposition of Aβ is the first event in the pathogenesis of AD that precedes the formation of phosphorylated tau aggregation [17]. NFTs consist of paired helical filaments resulting from hyperphosphorylation of the microtubule-binding protein tau [11]. Tau plays an important role in the maintenance of microtubule stability. In AD, tau is aberrantly hyperphosphorylated and proteolyzed resulting in impairment of normal functions of tau [11].

AD may be classified in two types based on genetic endowment. The first type is inherited via an autosomal dominant pattern, i.e., familial AD, and the second type is sporadic AD. Familial AD displays early disease onset, whereas sporadic AD cases mostly develop the disorder at an older age [18]. Etiology of AD is multifactorial with genetic, environmental, and developmental components playing a role [2]. A large body of evidence supports the notion that AD pathogenesis is related to a progressive accumulation of Aβ protein due to an imbalance between Aβ production, aggregation, and clearance [11, 19]. Aβ is formed following sequential cleavage of amyloid-β protein precursor (AβPP) by two proteases termed β- and γ-secretases (see Fig. 1). After excessive generation, Aβ self aggregates into Aβ oligomer and then it further aggregates into insoluble extracellular senile plaques. Most of the evidence suggests that Aβ oligomers instead of fibrils are responsible for neurotoxic effects of Aβ [20–23].

Besides plaques and NFTs, AD is also characterized by neuroinflammation. It is widely accepted that the deposition of Aβ is one of the main features of AD and seems to trigger a cascade of neuroinflammatory events that ultimately leads to neurodegeneration [24, 25]. Brain inflammation is mediated by the activation of glial cells, microglia, and astrocytes, and expression of inflammatory mediators and neurotoxic free radicals [26]. Microglial cells are the central nervous system (CNS) resident phagocytes of the immune system and produce a wide range of cytokines, such as interleukins [27]. Activated microglia accumulates at the site of Aβ deposition and, as expected, actively engulfs and clears Aβ deposits [28]. Aβ is able to stimulate Src family kinases and Syk tyrosin kinases [29], which further can activate mitogen-activated protein kinase (MAPK) and nuclear factor κB (NFκB) cascades that are required for proinflammatory cytokine and reactive oxygen species (ROS) production (see Fig. 1) [27]. It has been also reported that Aβ can directly activate MAPK and extracellular signal regulated kinase (ERK) pathways [30]. Transient activation of these signaling pathways after Aβ binding to microglia results in upregulation of proinflammatory cytokines such as interleukin-1β (IL-1β) and tissue tumor necrosis factor-alpha

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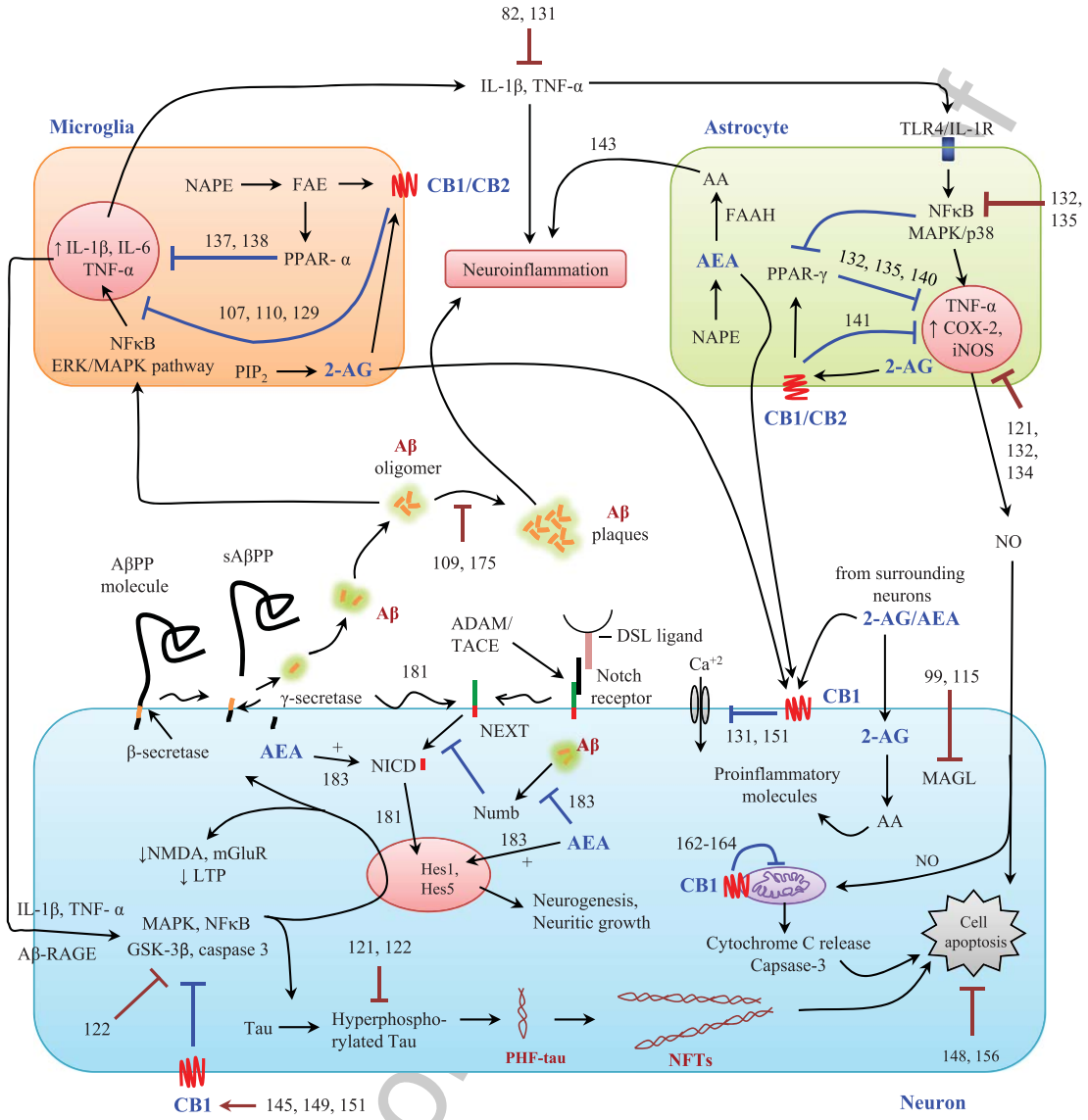

Fig. 1. Endocannabinoid signaling and molecular mechanisms of neurodegeneration in AD. Proteolytic cleavage of amyloid-β protein precursor (AβPP) by β- and γ-secretase results in generation of $A\beta_{42}$ monomers, which under pathological conditions, assembles into oligomers. $A\beta_{42}$ oligomers activate microglia and astrocytes. Activated microglia produces inflammatory cytokines through nuclear factor κB (NFκB) and mitogen-activated protein kinase (MAPK) pathways. Cytokines released from microglia integrate inflammation process in surrounding astrocytes and neurons through various signaling pathways. Cytokines and $A\beta_{42}$ through various mechanisms, activate MAPK, NFκB, glycogen synthase kinase-3β (GSK-3β), and caspase-3 pathways. $A\beta_{42}$ through MAPK and NFκB pathways negatively modulates long-term potentiation by controlling NMDA and mGlu receptor expression, and ultimately causing memory impairment. Moreover, $A\beta_{42}$ through the activation/release of kinases, nitric oxide (NO), and caspase-3 increases phosphorylation of tau, which ends in the formation of neurofibrillary tangles (NFTs) in neurons. Under inflammatory conditions both microglia and astrocytes synthesize endocannabinoids (anadamide; AEA and 2-arachidonoylglycerol; 2AG), which through cannabinoid receptors (CB_1/CB_2) and peroxisome proliferator-activated receptors (PPAR) suppress production of cytokines, iNOS and COX-2 expression. Moreover, AEA augments Notch-1 signaling, which is important in neuronal development, neurogenesis, and neuritic growth. Mitochondrial CB_1 receptors inhibit the release of cell apoptotic factors and Ca^{+2} influx in response to reactive oxygen species. Thus activation of endocannabinoid signaling exerts antioxidant, anti-inflammatory and anti-apoptotic effects. NAPE, N-acyl-phosphatidyl-ethanolamine; FAE, fatty acid ethanolamides; ERK, extracellular signal regulated kinase; PIP2, phosphatidylinositol-4,5-bisphosphate; AA, arachidonic acid; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; TLR-4, toll-like receptor-4; ADAM, metalloproteinase domain-containing protein; TACE, tumor necrosis factor-converting enzyme; DSL, Delta/Serrate/LAG-2; NICD, notch intracellular domain; NEXT, notch extracellular truncation; RAGE, receptor for advanced glycation end-products

(TNF-α) [27]. IL-1β and TNF-α are considered as primary cytokines responsible for chronic inflammation in AD [31]. Furthermore, IL-1β released from glia activates MAPK and NFκB signaling cascades in astrocytes and neurons, resulting in excessive inflammation and tau phosphorylation [27, 31] (Fig. 1). Additionally Aβ oligomers can induce production of inducible nitric oxide synthase (iNOS), nitric oxide (NO), and TNF-α in astrocytes [32]. Activation of toll-like receptor (TLR; e.g., TLR-4), fundamental receptors involved in pathogen recognition and activation of innate immunity, can also activate MAPK and NFκB pathways [33, 34]. Activation of these signaling cascades in neurons could inhibit synaptic plasticity. p38-MAPK cascade has been recognized as one of the signal transducer downstream of NMDA and metabotropic (mGlu) glutamate receptors and its activation contributes to the inhibition of long term-potentiation (LTP) [35, 36]. Moreover, MAPK is rapidly activated after interaction of Aβ with the receptor for advanced glycation end-products, leading to inhibition of LTP and tau phosphorylation (Fig. 1) [27].

THE ENDOCANNABINOID SYSTEM

eCBs are highly lipophilic molecules which are synthesized from lipid membrane precursors and have been shown to modulate neuronal activities [37]. These are elements of the eCB system that also includes the enzymes required for their synthesis and metabolism and the cannabinoid (CB) receptors that serve as their molecular targets. Unlike classical neurotransmitters, eCBs are synthesized and immediately released "on demand" upon neuronal activation and act retrogradely through the synaptic cleft to activate CB receptors located pre-synaptically [37, 38]. By activating CB receptors in the CNS, eCBs suppress neurotransmitter release in a transient or long-lasting manner at both excitatory and inhibitory synapses [38].

The first identified eCB was anadamide (arachidonoylethanolamine; AEA) [39], which is the derivative of ethanolamine and arachidonic acid (AA). The existence of a second eCB was postulated and soon identified as 2-arachidonoylglycerol (2-AG) [40, 41]. 2-AG is an ester derivative of AA and glycerol. The synthesis of AEA and 2-AG is believed to be driven by the cleavage of membrane-associated phospholipids. AEA is synthesized from hydrolysis of N-acyl-phosphatidyl-ethanolamine (NAPE) by phospholipase D (PLD) [42, 43]. 2-AG synthesis derives from the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) and is mediated by the generation of diacylglycerol (DAG), via the actions of either phospholipase C (PLC) or phospholipase D (PLD) [44]. DAG is subsequently converted to 2-AG by DAG lipase [44]. eCBs are produced by a variety of cell types including endothelial cells, adipocytes, glial cells, and macrophages [45–47]. 2-AG is more abundant than AEA in the brain and behaves as a full agonist for CB_1 and CB_2 receptors, while AEA acts as a partial agonist for CB_1 receptors [48]. In addition to CB_1 receptors, AEA can also activate peroxisome proliferator-activated-alpha receptors (PPAR-α) and transient receptor potential vannilloid-1 (TRPV1) channels [49].

CB_1 receptors are widely expressed throughout the brain [50], predominantly in cerebellum, cortex, hippocampus, and basal ganglia [38]. They are mostly found on axon terminals of a variety of neuronal populations and their activation results in inhibition of adenylate cyclase activity and calcium influx into the axon terminal; thus, CB_1 receptor signaling functions to suppress neurotransmitter release into the synapse [38]. CB_1 receptors are also expressed in periphery organs [51]. Following CB_1 receptor identification, peripheral CB-receptor was identified and designated as CB_2 receptor [52]. CB_2 receptors are widely distributed in cells and tissues of immune system. Recently, it has been discovered that CB_2 is also expressed within the CNS and its expression occurs at various stages of inflammation [53–56]. This expression of CB_2 was primarily localized in the microglia and astrocytes [57–59]. Interestingly, CB_2 receptor expression can be detected in these cells in CNS only after various insults, whereas it cannot be detected in resting microglia [60]. The CB_2 exerts its effects through initiation of phospholipase C (PLC) and inositol 1, 4, 5-triphosphate (IP3) signaling pathways that results in increased levels of intracellular calcium [59]. There is also evidence on other putative CB-receptor subtypes [61], but no new receptor has been fully characterized or cloned yet. Moreover, it has been proposed that G-protein coupled receptor GPR55 may be a novel cannabinoid receptor [62]. Another suggested putative novel CB-receptor is the TRVP1 receptor, a ligand-gated ion channel [63].

eCBs after their actions are rapidly eliminated by cellular uptake and enzymatic hydrolysis. After cellular re-uptake AEA is metabolized by the fatty acid amide hydrolase (FAAH) [64] expressed mostly by postsynaptic neurons. FAAH metabolizes also other N-acyl ethanolamines, like palmitoylethanolamide (PEA) and oleoylethanolamide

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(OEA). N-acyl ethanolamine hydrolyzing acid amidase (NAAA) has been identified to take also part in the metabolism of AEA [65]. 2-AG is mainly metabolized by monoacylglycerol lipase (MAGL) in presynaptic neurons [66]. At lesser extent 2-AG is also metabolized by FAAH, serine hydrolase α/β hydrolase 6 (ABDH6), serine hydrolase α/β hydrolase 12 (ABDH12), and cyclooxygenase-2 (COX-2) [65].

The understanding of the eCB system is constantly evolving as new discoveries are progressing. Previously it was thought that retrograde signaling was the principal mode by which eCBs mediate short- and long-term forms of plasticity at both excitatory and inhibitory synapses. However, increasing evidence suggests that eCBs can also signal in a nonretrograde manner [67]. The general physiological actions of nonretrograde signaling eCBs are mediated by TRPV1 in the CNS [68]. The concept of on demand synthesis of eCBs is also challenged now as recent studies have demonstrated intracellular storage of AEA in adiposomes [49]. It has been recently shown that the majority of CB_1 receptors does not reach the cell surface but instead shows intracellular localization. A significant part of intracellular CB_1 receptor is present on endosomes [69, 70]. Moreover, it has been revealed that CB_1 receptors are also present on mitochondrial membranes and regulate activity of mitochondria [71].

ENDOCANNABINOID SIGNALING IN ALZHEIMER'S DISEASE

Multiple data are available showing that the eCB system is implicated in AD progression. Cortex and hippocampus, key structures for learning and memory functions, are the two brain regions that are affected by AD pathology [72], and they express high levels of CB_1 receptors as well as other components of the eCB system [73]. Evidence suggests that microglia and astrocytes also express the enzymes involved in the synthesis and degradation of the eCBs and that the activation of cannabinoid receptors expressed by activated microglia controls immune-related function [59]. Moreover, eCBs are known to exert anti-inflammatory, antioxidant, and neuroprotective effects [7, 74–77].

Therefore, it is not surprising that eCB signaling plays a crucial role in AD. Table 1 compiles all reports addressing the expression levels of eCB signaling components in AD in humans as well as in *in vitro* and *in vivo* preclinical models. The major implications of dysregulated eCB signaling in AD are briefly discussed below.

The relationship of CB_1 receptors and AD is sparse and often contradictory in the literature. Westlake and colleagues evaluated the CB_1 mRNA expression and [^{3}H]CP-55,940 (CB_1 and CB_2 agonist) binding density in postmortem AD human brains [78]. [^{3}H]CP-55,940 binding was reduced but no alterations in CB_1 expression levels were observed in AD brains compared to aged-matched controls. Though [^{3}H]CP-55,940 binding was reduced, it was not selectively associated with the AD-pathology. In accordance to this report, other research groups found that CB_1 receptor levels were unaltered in patients suffering from AD [79–81]. In contrast, significant decrease in CB_1 receptor expression has been reported in the cortex of AD patients [82, 83]. CB_1 expression was greatly reduced and CB_1 protein nitration was enhanced in the areas of microglial activation in AD brains [82]. However, reduced CB_1 levels were correlated to hypophagia but not with any AD molecular marker or cognitive status [83]. Furthermore, CB1 receptor selective radioligand study revealed that CB_1 receptor density increases in early AD and decreases during later disease stages [84]. In line with these results, two recent papers by, our group [85] and by Kalifa and his colleagues [86] reported a decrease in CB_1 protein expression in transgenic mice models of AD. However, we found that in aged triple transgenic mice of AD ($3 \times$ Tg-AD) CB_1 mRNA was significantly increased in limbic brain areas. Though we did not find a direct correlation between CB_1 mRNA and CB_1 protein, an inverse correlation between CB_1 protein levels and Aβ protein were observed in hippocampus and basolateral amygdala [85]. The reduced CB_1 expression in AβPPswe/PS1ΔE9 mice was associated with astroglial proliferation and elevated expression of cytokines, iNOS and TNF-α [86]. Similarly, pretreatment with $A\beta_{42}$ in rats and C6 rat astroglioma cells can cause a down-regulation of CB_1 receptor [87]. Furthermore, Ahmad and colleagues investigated the availability of CB_1 receptor in AD patients by positron emission tomography. This study neither found any difference in CB_1 receptor availability between AD and healthy volunteers nor found a correlation between CB_1 receptor and Aβ deposition [88]. Even though CB_1 receptors were unchanged, it has been proposed that the coupling between receptor and Gi protein could underlie the reduced signaling of CB_1 receptor [89]. A recent study further showed that CB1 receptor activity depends on the AD stages. CB1 activity was found higher at earlier AD stages in limited hippocampal areas and internal layers of frontal cortex, but a decrease was observed at the advanced stages [90]. The

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Table 1
Altered eCB signaling in AD

Subjects	Tissue	Component of eCB system	Observation	Ref.
Human AD patient	Cortex, Hippocampus, Striatum, Anterior cingulate gyrus, Caudate nucleus	CB_1 protein and binding	Unchanged	[79–81, 88]
Human AD patient	Hippocampus, Neocortex, Basal ganglia, Brainstem	CB1 mRNA CB1 binding	CB1 mRNA- Unchanged CB1 binding-reduced in hippocampus, substantia nigra, globus pallidus	[78]
Human AD patient	Cortex	CB_1 protein	Decreased	[82, 83]
Human AD patient	Blood	CB_1 mRNA	Increased	[97]
3 × Tg-AD mice	Hippocampus, BLA, Prefrontal cortex	CB_1 mRNA and protein	CB_1 mRNA-altered CB_1 protein- reduced in dorsal hippocampus and BLA	[85]
Human AD patient	Prefrontal cortex	CB1 binding	CB1 density increases in early AD followed by decreases during later disease stages	[84]
Human AD patient	Prefrontal cortex, Hippocampus	CB_1 -receptor-dependent Gi protein activation	CB1 activity increased at earlier AD stages and decreased at advance stages	[90]
AβPPswe/PS1ΔE9 mice	Hippocampus	CB_1 protein	Decreased	[86]
AβPPswe/PS1ΔE9 mice	Hippocampus, Cortex	CB_1 -receptor-dependent Gi protein activation	Unchanged	[191]
Rat ($A\beta_{42}$ insult)	Brain/Cells	CB_1 and CB_2 mRNA/protein	CB_1 -decreased CB_2-increased	[87]
Human AD patient	Cortex, Hippocampus, Blood	CB2 protein and mRNA	Increased	[79, 82, 83, 91, 92, 97]
Human DS patient	Cortex	CB_2 protein and FAAH protein	Increased	[95]
AβPPswe/PS1ΔE9 mice	Cortex	CB_2 binding	Increased	[96]
$A\beta PP_{SWE}$ / Neuro-2a cells	Neuro-2a cells	FAAH	Increased activity and expression	[93]
Human AD patient	Cortex, blood	FAAH protein, mRNA and activity	Increased	[79, 192]
Human AD patient	Cortex	AEA and NarPE	Decreased	[93]
Human AD patient	Plasma	AEA and 2-AG	Unchanged	[98]
PS1/AβPP mice	Whole brain	AEA and 2-AG	Increased	[99]
Rats (Aβ42 insult)	C6 glioma cells, Hippocampus	AEA and 2-AG	2-AG-Increased AEA- decreased	[87, 101]
AβPPswe/PS1ΔE9 mice	Frontal cortex, Hippocampus and Striatum	AEA, 2-AG, PEA and OEA	Decreased only in striatum	[193]
Human AD patient	Hippocampus	DAGL, MAGL, ABHD6	DAGL- increased MAGL- decreased ABHD6- abolished	[80]

CB1and CB2, cannabinoid receptors; BLA, basolateral amygdala; DS, Down's syndrome; FAAH, fatty acid amide hydrolase; NarPE, 2-docosahexaenoyl-sn-glycerophosphoethanolamine-N-arachidonoyl; AEA, anandamide;, 2-AG, 2-arachidonoylglycerol; DAGL, diacylglycerol lipase; MAGL, monoacylglycerol lipase; ABHD6, serine hydrolase α/β hydrolase 6.

increased CB1 receptor activity during the initial stages of AD might indicate neuroprotective action medicated by eCBs in response to initial neuronal damage.

Differently from CB_1 receptor, the relationship between CB_2 receptor and FAAH in AD pathology is well documented in the literature. In fact, postmortem brains from patients with AD revealed that CB_2 receptors and FAAH are selectively overexpressed in cells that are associated to Aβ-enriched neuritic plaques [79, 80, 82, 83, 91, 92]. The hydrolytic activity of FAAH is

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enhanced in $A\beta_{42}$ plaques and surrounding areas [79, 93]. Increased FAAH activity may contribute to inflammatory processes by increasing AA (precursor for proinflammatory molecules) through increased AEA metabolism in astrocyte cells surrounding plaques. Moreover, FAAH is selectively overexpressed in reactive astrocytes and CB_2 receptors are overexpressed in activated microglial cells in AD [79, 94, 95]. Similarly, in Down's syndrome, characterized by Aβ deposition, increased FAAH activity and CB_2 expression have been observed [95]. Moreover, increased levels of CB_2 receptors were positively correlated with $A\beta_{42}$ and senile plaque score [83]. Apart from human studies, transgenic model of AD has also revealed overexpression of CB_2 receptors in brain areas affected by the AD-pathology [96]. Increased CB_2 mRNA in peripheral blood has been suggested as a peripheral biomarker for the early diagnosis of AD [97]. Pretreatment with $A\beta_{42}$ to rats and C6 rat astroglioma cells also increases CB_2 receptor expression [87].

Since AEA and, to a lesser extent, 2-AG are the substrates of FAAH, reduction in AEA and/or 2-AG can be expected in brain areas severely affected by AD pathology. In line with this, Jung and colleagues reported that AEA and its precursor 1-stearoyl, 2-docosahexaenoyl-sn-glycerophosphoethanolamine-N-arachidonoyl (NarPE) levels, but not 2-AG, were significantly reduced in cortex of AD patients [93]. However, AEA and 2-AG plasma levels were unchanged in AD patients compared to healthy volunteers [98]. Moreover, AEA and NarPE levels in cortex were positively correlated to cognitive impairment and inversely correlated to $A\beta_{42}$; however, no correlation was found with plasma eCBs and cognitive performance [93, 98]. Conversely, AEA and 2-AG levels were found to be increased in brains of the PS1/AβPP transgenic mice of AD [99]. Mulder and colleagues found that 2-AG signaling is altered in postmortem AD brains. The expression of 2-AG synthesizing enzyme, i.e., DAG lipase, was significantly and selectively increased in microglia surrounding senile plaques [80, 100]. The activity of 2-AG degrading enzymes, MAGL and ABHD6, was differentially altered in hippocampal neurons. ABHD6 expression was completely abolished and MAGL expression was lowered in NFT-bearing pyramidal neurons. This study demonstrated that AD progression slows down the termination of 2-AG signaling and that could contribute to synapse silencing particularly around senile plaques [80]. Apart from postmortem analyses and transgenic models of AD, studies on animal models of AD induced by acute administration of $A\beta_{42}$ have also shown the increase of DAG lipase and 2-AG levels [87, 101].

BENEFICIAL EFFECTS OF CANNABINOIDS IN TREATMENT OF ALZHEIMER'S DIEASE

Increasing evidence suggests that the eCB system could be a potential target for the treatment of AD. During the last decade, an ample number of interesting studies allowed for a new perspective into the prevention and/or treatment of AD focusing on the eCB system (for review, see [5–10, 74–76, 102–104]). Cannabinoids could exert neuroprotective, antioxidant, anti-apoptosis, and anti-inflammatory effects [77]. Cannabinoids play a neuroprotective role, through the CB-receptor activation, by preventing excitotoxicity, calcium efflux, and inflammation as well as by modulating other signaling pathways [105]. Most of the initial reports on the effects of cannabinoids in AD were investigated in *in vitro* models of Aβ-induced neuronal toxicity. Later, these investigations were extended to animal models of Aβ-induced toxicity and to transgenic murine models expressing plaques and/or tangles pathology. Table 2 compiles the *in vitro* and *in vivo* experimental evidence of beneficial effects of cannabinoids in AD treatment. Figure 1 summarizes the probable molecular and cellular mechanisms underlying these beneficial effects. In the following section the effects of cannabinoids on various pathological processes of AD will be discussed.

Aβ generation and clearance

Microglia plays an important role in phagocytosis of Aβ, and there is an inverse relationship between cytokine production and Aβ clearance [26, 106]. CB_2 activation is known to reduce microglia activity and inflammatory cytokines productions [107]. So it can be hypothesized that CB_2 agonist could lower Aβ plaque load by increasing Aβ clearance. In line with this hypothesis, it has been shown that *in vitro* activation of CB_2 receptor facilitates the removal of native Aβ from human frozen tissue sections as well as the removal of synthetic pathogenic peptide by a human macrophage cell line [108]. Moreover, a CB_2 agonist was able to induce a prompt Aβ clearance in Aβ-induced animal model of AD [109]. The mechanism underlying CB_2 mediated decrease in Aβ plaque load is not clear yet. However, it was suggested that it might be link to a lower the production of inflammatory cytokines and increase of Aβ phagocytosis that might decrease Aβ

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Table 2
Beneficial effects of modulators of the endocannabinoid system and their molecular mechanisms in AD

Subjects	Treatment	Effects and mechanism involved	Ref.
Endocannabinoids			
Ntera 2/cl-D1 neurons (Aβ insult)	AEA Noladin ether	↓ Aβ toxicity MAPK pathway activation	[149]
Wistar rats ($A\beta_{42}$ insult)	VDM-11	Reversed hippocampal damage Improved memory retention	[101]
PC12 cells SH-SY5Y cells ($A\beta_{40}$ and peroxide insult)	AEA	↑ cell viability CB_1 mediated effect	[150]
vitro model of the BBB	2-AG JZL185 JZL 195	↑ Aβ clearance ↑ expression of LRP1	[112]
Primary hippocampal neurons ($A\beta_{25\text{-}35,}$ $A\beta_{42}$ insult)	2-AG URB602 JZL184	↓ neurodegeneration ↓ apoptosis ↓ capsase-3 cleavage CB_1 mediated effect ↓ ERK1/2 and NFκB phosphorylation ↓ COX2	[145]
Mouse astrocytes (Aβ treatment)	AEA, PEA and OEA	↓ inflammation	[137]
eCB degradation enzyme inhibitors			
Primary cortical neurons (Aβ treatment)	AEA, 2-AG, URB597	↓ Apoptosis ↓ lysosomal membrane permeabilization	[148]
AβPP/PS1 AD mouse	Genetic/pharmacological inactivation of MAGL	↓ arachidonic acid, PGE2, PGD2, TXB2 ↓ GFAP, CD11b, TNF-α, IL- 1β, IL-6, $A\beta_{42,}$ $A\beta_{40}$	[99]
5×FAD AβPP transgenic mice	JZL184	↓ BACE1 expression ↓ Aβ levels ↓ neuroinflammation Improved learning and memory	[115]
Cannabinoid agonists			
microglial cells (Aβ insult)	HU-210, WIN55,212-2, and JWH-133	↓ microglia-mediated neurotoxicity	[82]
Human fetal astrocytes (IL-1β insults)	WIN55,212-2 (mixed CB_1 / CB_2 agonist)	↓ production of inflammatory mediators	[134]
C6 rat glioma cells (Aβ insult)	WIN 55,212-2	↓ iNOS expression ↓ NO production	[121]
SD rats brain slices	WIN 55212-2	↓ acetylcholine release	[194]
Wistar rats ($A\beta_{42}$ insult)	ACEA (CB_1 agonist) WIN-55212-2	↓ caspase 3 Improved memory deficits ↓ Ca^{+2} currents in CA1 neurons	[151]
Rats ($A\beta_{42}$ treatment)	Win55,212-2	↓ inflammation CB_1, CB_2 and PPAR-γ mediated	[140]
AβPP23/PS45 double transgenic mouse model of AD	HU210 (mixed CB_1 / CB_2 agonist)	Unchanged AβPP and Aβ levels No effect on learning and memory	[172]
microglial cells (Aβ insult)	JWH-015 (CB_2 agonist)	↓ microglial activation ↓ phosphorylation of JAK/STAT1 ↑ phagocytosis of $A\beta_{42}$	[107]
human brain microvascular endothelial cells, mice	JWH133 (CB_2 agonist)	↓ intercellular adhesion molecule-1 ↓ vascular cell adhesion molecule-1 ↑ BBB integrity	[185]
Rats ($A\beta_{40}$ insult)	MDA7 (CB_2 agonist)	↓ CD11b expression ↓ GFAP expression ↓ interlukin-1β ↑ Aβ clearance restored cognition and memory	[129]

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Table 2
(Continued)

Subjects	Treatment	Effects and mechanism involved	Ref.
Tg AβPP mice	JWH-133 (CB_2 agonist)	Improves cognitive performance ↓ lba-1, COX-2, TNF-α ↓ $Aβ_{40}$, $Aβ_{42}$ and CB_2 ↓ GSK3-β tau phosphorylation kinase	[141]
Pharmacological or genetic inhibition of cannabinoid receptors			
swiss mice ($Aβ_{25\text{-}35,42}$ insult)	Rimonabant (CB_1 antagonist)	improves Aβ-induced amnesia	[173]
AβPP23/ $CB_1{}^{-/-}$ mice		↓ AβPP levels, plaque load ↓ neuroinflammation Impaired learning Memory deficits	[111]
AβPP/ $CB_2{}^{-/-}$ mice		↑ soluble $Aβ_{42}$ ↑ $Aβ_{42}$plaque ↑ microglia activation ↓ soluble tau	[110]
Phytocannabinoids			
Rat cortical neuron culture (glutamate insult)	Cannabidiol Δ^9-THC	↓ glutamate toxicity -Antioxidant effect	[158]
microglial cells C57/Bl6 Mice ($Aβ_{40}$ insult)	Cannabidiol	↓ ATP induced Ca^{+2}	[131]
	WIN 55,212-2 JWH-133	↑ microglia migration ↓ NO, TNF-α, IL-6 ↓ cognitive impairment	
C57BL/6J mice ($Aβ_{42}$ insult)	cannabidiol	↓ GFAP ↓ iNOS and IL-1b	[132]
AChE from Electrophorus electricus	Δ^9-THC	inhibits AChE ↓ AChE-induced Aβ aggregation	[175]
N2a/AβPPswe cells	Δ^9-THC	↓ Aβ levels ↓ Aβ aggregation ↓ GSK-3β and p-GSK-3β	[124]
PC12 neuronal cells ($Aβ_{42}$ insult)	cannabidiol	↓ tau hyperphosphorylation ↓ p-GSK-3β ↑ β-catenin	[122]
PC12 cells ($Aβ_{42}$ insult)	cannabidiol	↓ iNOS, NO ↓ p38 MAP kinase ↓ NFκB	[135]
Primary cultured astrocytes Rats ($Aβ_{42}$ insult)	cannabidiol	↓ NO, TNF-α, IL-6, S1OOB ↓ reactive gliosis ↑ neurogenesis Mediated through PPAR-γ	[133]
Neuroblastoma cells ($Aβ_{42}$ insult)	ACEA (CB_1 agonist)	↓ Aβ fibrils	[109]
microglial	JWH-015 (CB_2 agonist)	↑ neuronal cell viability	
BV-2 cells (LPS insult)	Δ^9-THC, cannabidiol, 2-AG, AEA	neuroprotective action	
PC12 cells (Aβ insult)	Cannabidiol	neuroprotective, anti-oxidative anti-apoptotic	[156]
AβPP/PS1 mice	Cannabidiol	Inhibits development of social recognition memory deficits ↑ dietary phytosterols	[170]
AβPP/PS1 mice	Cannabidiol + Δ^9-THC	↓ learning impairment ↓soluble Aβ42 peptide levels ↓astrogliosis, microgliosis, and inflammatory-related molecules	[171]

plaque load [107]. The role of CB_2 receptors in lowering Aβ plaques was further confirmed by a study where CB_2 receptors were deleted in AβPP mutant mice (PDGFB-AβPPSwInd). Results from this study revealed that soluble Aβ and plaque deposition were significantly increased in AβPP/$CB_2{}^{-/-}$ mice compared to AβPP/$CB_2{}^{+/+}$ mice [110].

The exact role of CB_1 receptor is not yet clear in same context. Effect of cannabinoid treatment on Aβ fibril and aggregate formation was recently

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reported. Biochemical and morphological assessment showed that Δ^9-THC, among other cannabinoids (eCBs, CB_1 and CB_2 agonist), significantly reduced fibril and aggregate formation [109]. However, CB_1 receptor deletion from AβPP23 transgenic mouse model of AD resulted in reduced amount of AβPP, reduced Aβ plaque load and less inflammation [110]. AβPP23/$CB_1^{-/-}$ mice showed lower body weight and most of the animals died before typical AD associated changes could become apparent [111]. Though the AβPP23/$CB_1^{-/-}$ study questioned the beneficial role of CB_1 receptors in the Aβ generation and clearance, another study by Bachmeier and colleagues [112] supported the hypothesis that CB_1 agonist could increase Aβ clearance from the brain. In fact, this study showed that CB receptor agonist or pharmacological elevation of eCBs significantly enhanced Aβ clearance from the brain [112]. eCBs increased Aβ clearance across the blood-brain barrier by increasing the expression of Aβ transport protein, lipoprotein receptor protein 1 (LRP1). Moreover, this study suggests that eCBs could decrease the Aβ brain burden not only due to changes in Aβ synthesis or release but also due to increase in Aβ transport from brain to periphery by the way of blood-brain barrier. It has been proposed that eCBs, through CB_1 receptor, activate PPAR-γ receptor, which has been shown to stimulate expression of LRP1 [113, 114]. Furthermore, MAGL inactivation reduced Aβ plaque load and also suppressed the expression of β-secretase (beta-site AβPP cleaving enzyme 1; BACE1), an enzyme involved in the production of $Aβ_{42}$ [115].

Tau hyperphosphorylation

Abnormal hyperphosphorylation of tau prompts an accumulation of NFTs in axons of neurons, can impair normal axonal transport, disrupt synaptic plasticity, and finally induce cell loss [116]. The link connecting Aβ plaques and tau pathologies has remained elusive. Evidence suggests that abnormal activation of kinases like glycogen synthase kinase-3β (GSK-3 β), MAPK family members as well as caspases may be responsible for hyperphosphorylation of tau [117, 118], and Aβ might be involved in the activation of these enzymes [119]. Along with various kinases, NO secreted from astrocytes induces tau hyperphosphorylation in neurons [120]. It has been shown that arachidonoyl-2´-chloroethylamide (ACEA), a selective CB_1 agonist, down regulates iNOS protein expression and NO production in astrocytes, and that leads to a significant inhibition of NO-dependent tau hyperphosphorylation in neurons [121]. In another report [122], it has been demonstrated that cannabidiol (a non psychoactive component of marijuana) inhibits hyperphosphorylation of tau protein in Aβ-stimulated neuronal cells. The effect of cannabidiol was mediated through the Wnt/β-catenin pathway [122]. Wnt activation leads to inhibition of GSK-3β, which is also known as tau protein kinase, responsible for a massive tau protein hyperphosphorylation and relative NFT formation observed in brains of AD patients [123]. A recent report also demonstrated that Δ^9-THC treatment inhibits activation of GSK-3β in N2a-variant AβPP cells [124].

Neuroinflammation

Besides plaques and NFTs, neuroinflammation plays a major role in neurodegeneration and activation of various apoptosis pathways. The notion that Aβ is a pathological molecule is slowly changing and it seems that it represents a cellular adaptive strategy to oxidative stress [125]. Aβ is a proinflammatory molecule, which can induce its own production by increasing the expression of its synthesizing enzymes such as β-secretase (BACE1) and through various inflammatory pathways [125]. In particular, it has been recognized that Aβ is able to initiate an inflammatory response, which in turn activates microglia and recruits astrocytes, and therefore the release of inflammatory mediators (IL-1β, TNF-α, and IL-6), reactive oxygen species (NO), and neurotoxic products that have been involved in neuronal and synaptic damage [31]. Neuroprotective effects of eCBs against brain injury and inflammation is associated with reduction of cytokines, ROS, and prostaglandins [126–128]. eCB modulators can reduce neuroinflammation in AD by inhibiting glial cell activation and generation of proinflammatory precursor molecules.

Regulation of glial cell activity

As discussed earlier in this review, CB_2 and FAAH expression is upregulated in microglia and astrocytes, respectively, in surrounding areas of neuritic plaques in AD brains. This notion suggests that both microglia and astrocytes play an important role in eCB signaling in AD pathology. It seems that upregulation of CB_2 receptor in AD is a defensive mechanism to limit inflammation and to clear plaques from the affected brain region [79, 110, 129]. CB_2 receptors are coupled to $G_{i/o}$ inhibitory proteins so that their activation is associated with inhibition of adenyl cyclase and the cAMP/protein kinases A (PKA) dependent pathway [130]. CB_2 receptor activation could provide

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beneficial effects at various levels. In particular, CB_2 activation could 1) suppress activation of microglia, 2) reduce production of inflammatory molecules like IL-1β, IL-6, TNF-α, NO, etc., 3) enhance microglial proliferation, and 4) enhance microglial phagocytic activity [59, 82, 107, 108, 131].

The effects of non selective cannabinoid agonists on microglial activation were demonstrated by Ramirez and colleagues [82]. In their study authors investigated the effects of non selective cannabinoids and selective CB_2 agonists in Aβ-induced microglial cells [82]. As expected, Aβ peptide activated microglial cells with increased mitochondrial activity, TNF-α release, and cellular morphological changes. Cannabinoid treatment prevented the enhancement of TNF-α release and counteracted Aβ-mediated activation of microglia. Furthermore, mechanistic insight of beneficial effects provided by CB_2 receptor stimulation in AD was demonstrated. Stimulation of CB_2 receptor significantly attenuated CD40-mediated inhibition of microglial phagocytosis of $A\beta_{42}$ peptide [107]. Cannabidiol dose dependently reduced Aβ-induced neuroinflammation by suppressing microglial activation, IL-1β and iNOS expression [132].

It has been also shown that cannabinoid treatment, in activated astrocytes, inhibits synthesis of inflammatory chemokines and NO release [133]. Win55,212-2, an agonist of CB1 and CB2 receptors, inhibited inducible NO synthase (iNOS) and corresponding NO production in astrocytes activated by IL-1β [134]. Win55,1212-2 treatment also inhibited production of chemokines (CXCL10, CCL2, and CCL5) and TNF-α. Both selective CB_1 and CB_2 antagonists partially blocked these effects suggesting the involvement of both receptors [134]. Cannabidiol markedly down-regulates, in a PPAR-γ dependently manner, Aβ-induced reactive gliosis by reducing proinflammatory molecules and cytokine release [133]. PPAR-γ activation could inhibit NFκB pathway, which is involved in the synthesis of inflammatory cytokines [135, 136]. In another report, different N-acylethanolamides (AEA, PEA, and OEA) were able to exert anti-inflammatory effects in Aβ-activated murine astrocytes [137]. Previous studies have shown that N-acylethanolamines activate anti-inflammatory nuclease receptor PPAR-α that causes formation of a multiprotein complex along with variable set of protein co-activators [138]. With this multiprotein complex, PPAR-α binds to responsive elements on DNA and enhances the transcription of various anti-inflammatory proteins, such as inhibitor of κB-α (IκB-α), that suppress the gene expression of pro-inflammatory components, such as cytokines (TNF-α, IL-1β) including iNOS and COX-2 (see Fig. 1) [138, 139]. Anti-inflammatory effects of cannabinoids have been also demonstrated in Aβ-induced *in vivo* AD models [129, 140] and transgenic mice models of AD [141].

Regulation of pro-inflammatory precursors

Phospholipase A2 (PLA2) enzymes are considered the primary source of AA for COX–mediated biosynthesis of prostaglandins [142]. Recently, Nomura and colleagues [143] have shown that MAGL-mediated hydrolysis of 2-AG can act as a distinct pathway to generate AA in the brain [143]. In line with this report, two independent research teams [99, 115] reported that the inactivation of MAGL reduced neuroinflammation, neurodegeneration, and the production and accumulation of Aβ plaques in the transgenic mice of AD. These effects were not mediated by CB_1 and/or CB_2 receptors but were caused by reduced production of AA [99, 136]. The inhibition of MAGL also improved the neuronal plasticity and learning and memory deficits [99, 115]. Inactivation of MAGL for eight weeks was sufficient to decrease production and deposition of Aβ plaques and the function of BACE1, the enzyme involved in making toxic Aβ in the brain (Fig. 2) [115]. These results suggest that MAGL contributes to the cause and development of AD and that the inhibition of MAGL might represent a promising potential therapeutic target.

MGL inhibition can cause an elevation of 2-AG endogenous levels. In turn, 2-AG, by activating CB1 receptor is able to suppress COX-2 elevation in response to inflammatory insult like lipopolysaccharide [144]. Furthermore, it was revealed that the neuroprotective effects of 2-AG were mediated by CB_1 but not by CB_2 or TRPV1 receptors [145]. CB_1 receptor activation by 2-AG suppresses phosphorylation of ERK1/2/p38MAPK/NFκB in neurons, which further suppresses COX-2 expression (Fig. 2) [144, 145]. COX-2 plays an important role in production of prostaglandins, which are crucial in neuroinflammation [142]. Further research in this field revealed that PPAR-γ, mediates 2-AG-induced inhibition of NFκB phosphorylation and COX-2 expression in response to pro-inflammatory IL-1β. Moreover, 2-AG is able to restore IL-1β-induced reduction of PPAR-γ expression in CB_1 dependent mechanism [146]. Inflammation activates the transcription factor NFκB, for which β-secretase (BACE1) promoter harbors a highly conserved binding site that is functional [125]. Thus NFκB activates BACE1 promoter, expression, and

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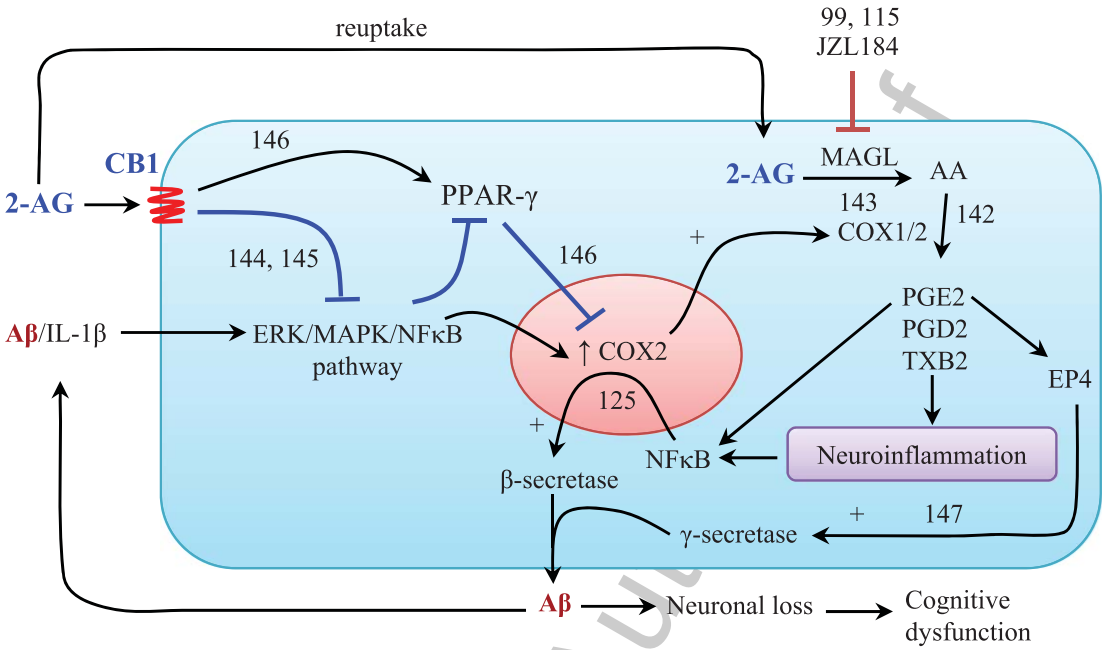

Fig. 2. Modulation of 2-AG signaling provides anti-inflammatory effects in AD. Through a CB_1-dependent mechanism, 2-AG increases PPAR-γ expression, which is suppressed by $A\beta_{42}$ in AD. 2-AG directly, through CB_1 and PPAR-γ receptors, inhibits the expression of COX-2 and the synthesis of inflammatory cytokines. COX-2 plays a major role in the synthesis of proinflammatory prostaglandins from arachidonic acid (AA), which is a degradation product of 2-AG. Proinflammatory prostaglandins can increase neuroinflammation as well as the expression and activity of β- and γ-secretase resulting in increased Aβ production. Inflammation activates the transcription factor NFκB, for which β-secretase (BACE1) promoter harbors a highly conserved binding site that is functional. Thus, NFκB activates BACE1 promoter, expression, and enzymatic activity leading to increased Aβ production. Prostaglandin PGE2 stimulates the generation of Aβ through both EP2 and EP4 receptors (PGE2 receptors). Activation of the EP4 receptor stimulates Aβ production through the endocytosis and the activation of γ-secretase. The inhibition of prostaglandin synthesis by MAGL inhibitors could suppress all these mechanisms.

enzymatic activity leading to increased Aβ production. The prostaglandin PGE_2 after production stimulates the generation of Aβ through both EP2 and EP4 receptors (PGE_2 receptors). Activation of the EP4 receptor stimulates Aβ production through endocytosis and activation of γ-secretase [147].

Neurodegeneration

Aβ has been shown to induce cell apoptosis in neuronal cells through a variety of mechanisms that include activation of caspase-3, lysosomal cathepsins, and lysosomal membrane permeabilization [17, 118]. Cannabinoids at physiological concentrations increase lysosomal stability and integrity [148]. Noonan and colleagues showed that eCBs can stabilize lysosomes against Aβ permeabilization and can increase cell survival. eCBs prevented upregulation of tumor suppressor protein, p53, and reduced its interaction with lysosomal membrane [148]. Moreover, 2-AG and AEA prevented Aβ-induced increase in DNA fragmentation and caspase-3 activation [101]. Acute *in vivo* administration of Aβ increases 2-AG release in the brain suggesting that endogenous 2-AG plays an important role in protecting neurons from Aβ-induced toxicity [101].

Milton and colleagues [149] showed the neuroprotective effects of eCBs (AEA and nodaline ether) on Aβ-induced neurotoxicity. These effects were mediated by CB_1 receptors and the MAPK pathway activation as suggested by the finding that CB_1 antagonist and MAPK inhibitor blocked their neuroprotective effects. Another study confirmed the neuroprotective effect of AEA on Aβ-evoked neurotoxicity via a pathway unrelated to CB_1 and CB_2 [150]. In fact, selective CB_1 and CB_2 agonists were unable to protect neurons against Aβ challenge [150]. Further research revealed that increasing endogenous levels of 2-AG by MAGL inhibitor was able to protect hippocampal neurons from Aβ-induced neurodegeneration and apoptosis [145]. Active caspase-3 levels are increased in AD [118]. CB_1 agonist was also able to inhibit Aβ-induced activation of capsase-3 [145, 151]. CB_1 knock-out studies indicated that lack of CB_1 is associated with increased

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caspase activation and greater loss and/or alterations of myelin and axonal/neuronal proteins [152].

Oxidative damage and mitochondrial dysfunction

Enhanced oxidative stress in brain generally correlates with cognitive decline and with enhanced risk for development of neurodegenerative diseases. Among the different pro-inflammatory proteins produced in response to Aβ-induced oxidative stress, iNOS and its enzymatic product NO [105, 153] are considered the most important neurotoxic effectors during AD. In particular, methionine-35 of $A\beta_{42}$ is critical for oxidative stress (for more details, see [154]). NFκB, a redox-sensitive transcription factor that is activated by a family of stress activated kinases (SAPK) including p38 MAP kinase [122], regulates the expression of different genes involved in cell differentiation, proliferation, and apoptosis, as well as in oxidative, inflammatory, and immune response [155]. As it is well known, NFκB activation is of primarily importance to induce iNOS protein transcription [156] both in Aβ-stimulated neuronal cells [156] and in postmortem AD brains [157]. It is well known that phytocannabinoids have anti-oxidant properties [158]. Cannabidiol is a well studied cannabinoid in this context. It has been shown that cannabidiol significantly decreases glutamate toxicity, Ca^{+2} toxicity, iNOS expression, and NO production [131, 158]. Cannabidiol mediates these effects through inhibition of p38 MAPK and NFκB pathways probably through involvement of the PPAR-γ receptor [132, 133, 135]. Moreover, CB_1 agonists were also shown to decrease iNOS and NO production [121, 131]. In another study, cannabidiol treatment significantly decreased ROS, lipid peroxidation, capsase-3 levels, DNA fragmentation and intracellular calcium [156].

CB_1 receptors are also expressed on mitochondria and regulate its activity [71]. Activation of mitochondrial CB_1 receptors can decrease oxidative metabolism, oxygen consumption, ROS production, and oxidative phosphorylation [71, 159–161]. In oxidative stress conditions, cannabinoids have shown protective actions against mitochondrial damage and have decreased Ca^{+2}-induced cytochrome c release from mitochondria (Fig. 1) [162–164].

Memory and learning impairments

CB_1-mediated effects of cannabinoids on learning and memory have been reported for many years [165]. eCBs are involved in modulation of long-term plasticity such as LTP [166], a cellular model of learning and memory. Activation of CB1 receptors on the GABAergic neurons leads to a decrease in GABA release [166] and thus to formation of the depolarization-induced suppression of GABAergic inhibition (DSI). Importantly, DSI temporarily removes GABAergic inhibitory tone and facilitates LTP of pyramidal neurons. It has been reported that Aβ strongly suppresses LTP in hippocampal synapses and this is one of the cause for observed learning and memory deficits in AD [167]. Recently, Orr and colleagues demonstrated a possible role of eCB signaling in Aβ-induced reduction in LTP and excitatory postsynaptic potential-spike coupling (E-S) potentiation [168]. In this study, authors showed that Aβ inhibits E-S potentiation through suppression of CB1-dependent synaptic disinhibition. This effect is not a direct effect on excitatory synapses but rather it is an indirect effect, which involves the reduction of eCB mediated GABAergic disinhibition. In another study, it has been shown that deletion of CB1 receptors from the forebrain GABAergic, but not glutamatergic neurons, led to a neuronal loss and increased neuroinflammation in the hippocampus as observed in brain aging [169]. The same authors suggested that CB1 receptor activity on hippocampal GABAergic neurons protects against age-dependent cognitive decline by reducing pyramidal cell degeneration and neuroinflammation [169].

Moreover, the consequences of CB_1 receptor deficiency on development of AD pathology were studied by knocking out CB_1 receptor in AβPP23 mice of AD. AβPP23/$CB_1{}^{-/-}$ mice showed worsen cognitive deficits than AβPP23 mice, thus suggesting that CB_1 deficiency can worsen AD-related learning and memory deficits [111]. Moreover, an eCB re-uptake inhibitor, VDM-11, reversed Aβ-induced hippocampal damage and memory impairment in passive avoidance test [101]. Further research in this field revealed that cannabinoid treatment was able to prevent Aβ-induced memory impairments in rats and that CB_1, but not CB_2, receptors may be directly involved in improving Aβ-induced memory impairments and intrinsic electrophysiological properties of hippocampal pyramidal neurons [151]. Fakhfouri and colleagues [140] showed that administration of the synthetic cannabinoid agonist, Win55,212-2, significantly improved memory functions and decreased the elevated levels of neuroinflammatory markers like TNF-α, active caspase-3, and nuclear NFκB. Antagonist experiment confirmed that these neuroprotective effects of Win55,212-2 were partially mediated by CB_1 and CB_2 receptors [140]. Through CB_1 receptor, Win55,212-2 increased PPAR-

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γ pathway by increasing its transcription activity and provided neuroprotection [140]. Furthermore, the effects of cannabinoids were studied in transgenic murine models of AD. Prolonged oral treatment of CB_2 receptor agonist (JWH-133) was able to improve cognitive impairments and decrease microglial activation in Tg2576 mice, while Win55,212-2 was ineffective [141]. Moreover, both cannabinoids significantly reduced the expression of CB_2 receptor, TNF-α and COX-2 suggesting a critical role of CB_2 in inflammatory processes in AD [141]. Recently, it has been shown that long-term treatment with cannabidiol was able to prevent the development of social recognition deficits in the AβPP/PS1 mouse model of AD [170]. The authors further revealed that these effects were not associated with decreased Aβ plaque load or oxidative changes while they noticed subtle effects induced by cannabidiol on neuroinflammation and cholesterol levels [170]. Moreover, a different study conducted on the same model showed that a combined treatment with cannabidiol and Δ^9-THC reduced learning impairment, decreased soluble $A\beta_{42}$ peptide levels and caused a change in plaques composition [171].

However, there are few reports that do not support beneficial effects of cannabinoids in AD treatment. Chen and colleagues found that chronic administration of the cannabinoid agonist HU-210 to AβPP23/PS45 double transgenic mice did not improve water maze performance or a contextual fear conditioning task [172]. HU-210 neither altered AβPP processing and neuritic plaque formation nor enhanced hippocampal neurogenesis in AβPP23/PS45 transgenic mice. It has been reported that CB_1 blockade by rimonabant improved Aβ-induced memory impairments in mice tested in a passive avoidance paradigm. The authors suggested that such memory improvement might be due to the increased acetylcholine release in the brain [173].

Additional effects of cannabinoids

Apart from aforementioned mechanisms, few cannabinoids exert their therapeutic effects in similar way of currently US-FDA approved drugs for AD treatment. Most of the drugs currently used in AD treatment (donepezil, rivastigmine, and galantamine) are inhibitors of AChE. AChE is involved in degradation of neurotransmitter acetylcholine (ACh), which is reduced in AD [174]. Active component of marijuana, Δ^9-THC, has been demonstrated to competitively inhibit AChE and to thus increase ACh levels [175]. Moreover, Δ^9-THC prevented AChE-induced aggregation of Aβ which can reduce plaques formation [175]. In addition to Δ^9-THC, other CB agonists also showed to have AChE and butyrylcholinesterase inhibition properties [176]. Alternative strategies based on multiple targets such as CB receptors and cholinesterase with single compound is gaining acceptance for treatment of AD.

Besides AChE inhibitors, current AD treatment includes memantine, a NMDA receptor antagonist, which reduces excitotoxicity by inhibiting Ca^{+2} influx. In similar way, HU-211 (synthetic cannabinoid devoid of CB_1 and CB_2 agonist activity) protects neurons from excitotoxicity by antagonizing NMDA receptors [177–179].

Moreover, recently it was demonstrated that eCBs can modulate Aβ-induced alterations in Notch signaling. Notch signaling plays a pivotal role in neurodevelopment, and it is also involved in control of neurogenesis, neuritic growth, synaptic plasticity, and long term memory [180, 181]. In advance neurodegeneration, Notch signaling is reduced [180]. Long term spatial deficits were observed in Notch mutant mice [182]. It has been shown that Aβ negatively regulates Notch-1 signaling by increasing expression of Numb, the endogenous negative regulator of Notch-1 cleavage [183]. Interestingly, AEA, through CB_1 receptors, was able to reverse this effect by increasing expression of Notch-1 signaling components like nicastrine, Notch intracellular domain, Hes1 and Hes5 (see Fig. 1). Moreover, AEA and 2-AG were also able to inhibit Aβ-induced expression of Numb [183].

Furthermore, cannabinoids could provide beneficial effects by modulating cerebral blood flow functions. AD is characterized by a decreased regional cerebral blood flow that could result in decrease brain supply of oxygen, glucose, and nutrients. Cannabinoids can improve blood flow to the brain as CB_1 receptor activation can elicit vasodilatation [184]. Moreover, as discussed earlier, cannabinoids can increase Aβ clearance at blood brain barrier [112]. CB_2 receptor activation has been shown to improve blood-brain barrier integrity by decreasing adhesion of leukocytes to endothelial cells under inflammatory conditions [185], which may reduce further exaggeration of inflammation.

However, besides beneficial effects, cannabinoids (especially at high doses) may exert unwanted cannabimimetic and psychiatric side effects such as hypolocomotion, hypothermia, aversion, and anxiety-related behaviors [186–189]. Moreover, CB1 receptor activation may precipitate episodes of psychosis and panic while its inhibition may lead to depression

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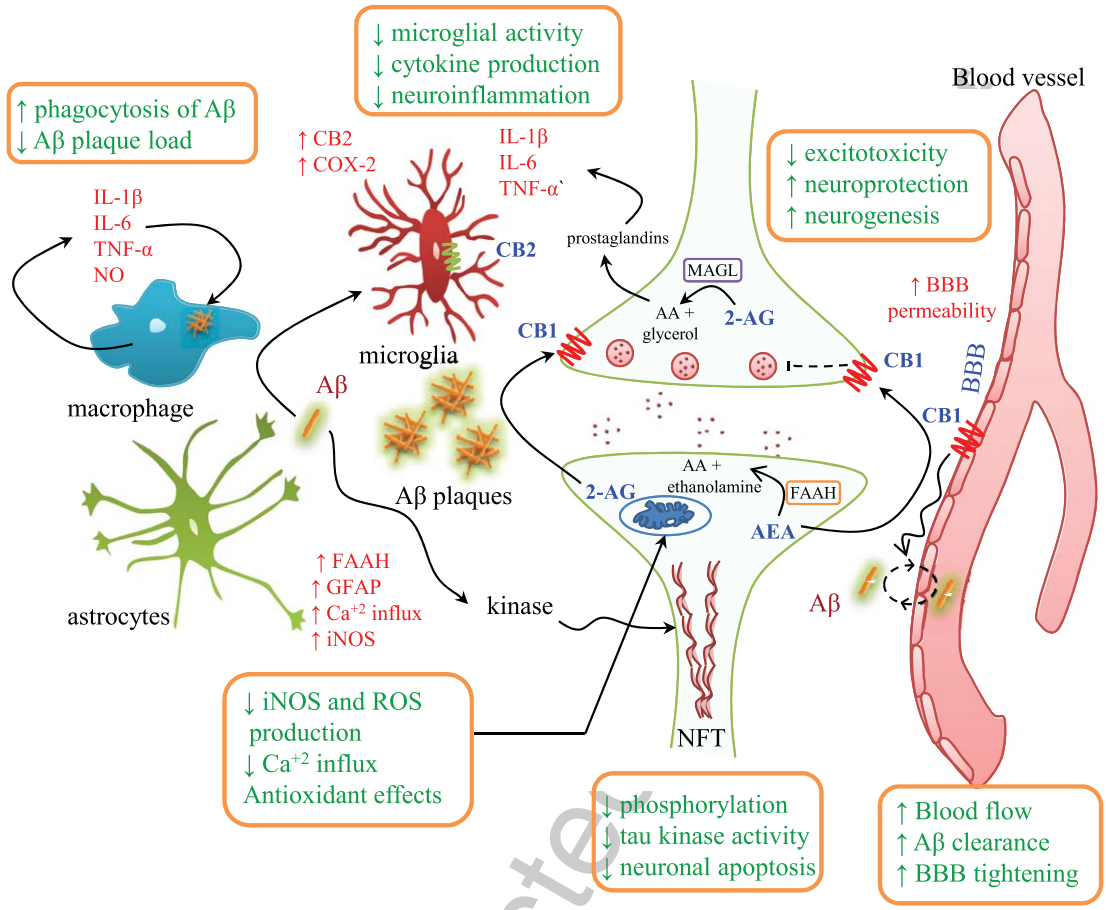

Fig. 3. Schematic diagram showing the beneficial effects of cannabinoid treatment in AD. Cannabinoid treatment can modulate multiple disease processes, which could reduce Aβ and phosphorylated tau deposition, neuroinflammation, oxidative damage, microglial activation, and excitotoxicity. Moreover, it can provide beneficial effects by increasing Aβ clearance, neurogenesis, neuroprotection and cerebral blood flow.

and anxiety-related disorders (for more details, see [190]). Furthermore, CB1 agonists may worsen AD by inhibiting acetylcholine release in the brain [7]. CB2 agonist and inhibitors of endocannabinoid deactivating enzymes seems to be devoid of such side effects. Therefore, much attention has been focused on this kind of compounds as potentially useful for the AD treatment.

CONCLUSIONS

The advances in AD research in the last decade have revealed that this disease is multifaceted in nature and is linked to different multiple mechanisms in the brain. A novel, more effective therapeutic approach for AD treatment should target multiple mechanism of disease progression. A large body of evidence suggested the involvement of the eCB system in the neurodegenerative process associated with AD. Aβ deposition in the brain is linked to significant changes in the expression pattern of CB2 receptors and FAAH enzyme. CB2 receptors and FAAH are selectively and abundantly overexpressed in microglia and astrocytes, respectively, in vicinity of Aβ neuritic plaques. AEA and its precursor NarPE levels are decreased in frontal cortex. In contrast, 2-AG degrading enzymes MAGL and ABHD6 activity is reduced in plaques and surrounding area. Over all AEA signaling is lowered and 2-AG signaling is increased in the vicinity of plaques. CB1 receptors expression in AD is still controversial and brain region specific. Although results of different groups are sometimes conflicting, a decline in the eCB system activity in AD is probable.

This review proposes cannabinoids as potential therapeutics, which can target simultaneously neurodegeneration, neuroinflammation, oxidative damage, cognitive impairments, and clearance of Aβ from the brain. Figure 3 summarizes the beneficial effects

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of cannabinoids in AD treatment. Elevation of CB receptor activity either by pharmacological blockade of enzymes responsible for eCBs degradation or by direct receptor agonist could be a promising strategy for slowing down the progression of AD and alleviating its symptoms. Although increased CB_2 expression and hydrolyzing FAAH activity is well documented in human AD patients as well as animal models of AD, a combination therapy of CB_2 agonist and FAAH inhibitor did not receive much research attention. This combination therapy could potentially lead to more effective treatment for AD, as they would target the altered eCB signaling in AD patients and could thereby reduce neuro-inflammation through reduced pro-inflammatory eicosanoids production and microglial activation. However, treatment with FAAH inhibitors should be done with caution as FAAH knockout astrocytes showed exaggerated inflammation [137].

Endogenous or exogenous cannabinoids, through cannabinoid receptors and/or PPAR control the activity of various signaling pathways like MAPK, NFκB, Notch-1, and Wnt/β-catenin pathways. Through these pathways, cannabinoids could reduce inflammation, generation of Aβ plaques, and NFTs resulting in improvement of synaptic structure, synaptic plasticity, and learning and memory deficits. However, the pharmacological modulation of eCB signaling should be done considering the disease stage.

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