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

Gaurav Bedse, Adele Romano, Angelo M. Lavecchia, Tommaso Cassano, and Silvana Gaetani

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 a 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, anandamide, 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 with moderate-severe AD. The use of memantine is associated with a modest delay in disease progression, but substantial variability in treatment response. No treatment has been shown to slow disease progression or prevent dementia. In addition, numerous therapies targeting various aspects of AD pathogenesis have been developed, but none have reached widespread approval for use in clinical practice. These include agents that target β-amyloid (Aβ) deposition, tau pathology, and neuroinflammation. Despite the extensive research, no therapy has been shown to modify the course of AD. Several studies have shown that cannabinoids may play a role in the treatment of AD due to their anti-inflammatory and neuroprotective effects. In this review, we will discuss 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.
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 (Δ9-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 aggregates [17]. NFTs consist of paired helical tau aggregation 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 B (NF-B) 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-1 β (IL-1β) and tissue tumor necrosis factor-alpha...
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 Aβ42 monomers, which under pathological conditions, assemble into oligomers. Aβ42 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 released from activated microglia activate MAPK, NFκB, glycogen synthase kinase-3β (GSK-3β), and caspase-3 pathways. Aβ42, through MAPK and NFκB pathways, negatively modulates long-term potentiation by controlling NMDA and mGlu receptor expression, and ultimately causing memory impairment. Moreover, Aβ42, through the activation/release of kinases, mitogen-activated protein kinase (MAPK) pathways, 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 (anandamide; AEA and 2-arachidonoylglycerol; 2-AG), which through cannabinoid receptors (CB1, CB2) 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 CB1 receptors inhibit the release of cell apoptotic factors and Ca2+ influx in response to reactive oxygen species. Thus activation of endocannabinoid signaling exerts antioxidant, anti-inflammatory and anti-apoptotic effects. NAPE, N-acyl-phosphatidylethanolamine; 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 domains-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.
phospholipase D (PLD) [42, 43]. 2-AG synthesis of N-acyl-phosphatidyl-ethanolamine (NAPE) by phospholipids. AEA is synthesized from hydrolysis in a transient or long-lasting manner at both receptors in the CNS, eCBs suppress neurotransmitter release into the synapse [38]. CB1 receptors are also expressed in periphery organs [51]. Following CB1 receptor identification, peripheral CB-receptor was identified and designated as CB2 receptor [52]. CB2 receptors are widely distributed in cells and tissues of immune system. Recently, it has been discovered that CB2 is also expressed within the CNS and its expression occurs at various stages of inflammation [53–56]. This expression of CB2 was primarily localized in the microglia and astrocytes [57–59]. Interestingly, CB2 receptor expression can be detected in these cells in CNS only after various insults, whereas it cannot be detected in resting microglia [60]. The CB2 receptor signaling 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 TRPV1 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 amid hydrolase (FAAH) [64] expressed mostly by postsynaptic neurons. FAAH metabolizes also other N-acyl ethanolamines, like palmitoylethanolamide (PEA) and oleoylethanolamide (OEA).
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 non-retrograde 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 adipocytes [49]. It has been recently shown that the majority of CB1 receptors does not reach the cell surface but instead shows intracellular localization. A significant part of intracellular CB1 receptor is present on endosomes [69, 70]. Moreover, it has been revealed that CB1 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 CB1 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 CB1 receptors and AD is sparse and often contradictory in the literature. Westlake and colleagues evaluated the CB1 mRNA expression and [3H]CP-55,940 binding density in postmortem AD human brains [78]. [3H]CP-55,940 binding was reduced but no alterations in CB1 expression levels were observed in AD brains compared to aged-matched controls. Though [3H]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 CB1 receptor levels were unaltered in patients suffering from AD [79–83]. In contrast, significant decrease in CB1 receptor expression has been reported in the cortex of AD patients [82, 83]. CB1 expression was greatly reduced and CB1 protein nitration was enhanced in the areas of microglial activation in AD brains [82]. However, reduced CB1 levels were correlated to hypophagia but not with any AD molecular marker or cognitive status [83]. Furthermore, CB1 receptor selective radioligand study revealed that CB1 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 CB1 protein expression in transgenic mice models of AD. However, we found that in aged triple transgenic mice of AD (3 × Tg-AD) CB1 mRNA was significantly increased in limbic brain areas. Though we did not find a direct correlation between CB1 mRNA and CB1 protein, an inverse correlation between CB1 protein levels and Aβ protein were observed in hippocampus and basolateral amygdala [85]. The reduced CB1 expression in AβPPswt/PS1ΔE9 mice was associated with astroglial proliferation and elevated expression of cytokines, iNOS and TNF-α [86]. Similarly, pretreatment with Aβ42 in rats and C6 rat astroglia cells can cause a down-regulation of CB1 receptor [87]. Furthermore, Ahmad and colleagues investigated the availability of CB1 receptor in AD patients by positron emission tomography. This study neither found any difference in CB1 receptor availability between AD and healthy volunteers nor found a correlation between CB1 receptor and Aβ deposition [88]. Even though CB1 receptors were unchanged, it has been proposed that the coupling between receptor and G protein could underlie the reduced signaling of CB1 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
Table 1

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Tissue</th>
<th>Component of eCB system</th>
<th>Observation</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human AD patient</td>
<td>Cortex, Hippocampus, Striatum, Anterior cingulate gyrus, Caudate nucleus</td>
<td>CB₁ protein and binding</td>
<td>Unchanged</td>
<td>[79–81, 88]</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Hippocampus, Neocortex, Basal ganglia, Brainstem</td>
<td>CB₁ mRNA CB₁ binding</td>
<td>CB₁ mRNA- Unchanged</td>
<td>[78]</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Hippocampus, BLA, Prefrontal cortex</td>
<td>CB₁ mRNA and protein</td>
<td>Decreased</td>
<td>[78, 83]</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Prefrontal cortex</td>
<td>CB₁ binding</td>
<td>CB₁ mRNA-altered</td>
<td>[85]</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Prefrontal cortex, Hippocampus</td>
<td>CB₁ receptor-dependent Gi protein activation</td>
<td>Increased CB1 receptor activity during the initial stages of AD might indicate neuroprotective action mediated by eCBs in response to initial neuronal damage. Differently from CB₁ receptor, the relationship between CB₂ receptor and FAAH in AD pathology is well documented in the literature. In fact, postmortem brains from patients with AD revealed that CB₂ 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 enhanced in Aβ₁₋₄₂ plaques and surrounding areas [79, 93]. Increased FAAH activity may contribute to inflamma-</td>
<td>[84]</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Prefrontal cortex, Hippocampus</td>
<td>CB₁ protein</td>
<td>Increased F AAH activity may contribute to inflammation in AD pathology.</td>
<td>[90]</td>
</tr>
<tr>
<td>AβPPa/wt/PS1ΔE9 mice</td>
<td>Hippocampus</td>
<td>CB₁ protein</td>
<td>Decreased</td>
<td>[86]</td>
</tr>
<tr>
<td>AβPPa/wt/PS1ΔE9 mice</td>
<td>Hippocampus, Cortex</td>
<td>CB₁ receptor-dependent Gi protein activation</td>
<td>Increased CB1 receptor activity during the initial stages of AD might indicate neuroprotective action mediated by eCBs in response to initial neuronal damage. Differently from CB₁ receptor, the relationship between CB₂ receptor and FAAH in AD pathology is well documented in the literature. In fact, postmortem brains from patients with AD revealed that CB₂ 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 enhanced in Aβ₁₋₄₂ plaques and surrounding areas [79, 93]. Increased FAAH activity may contribute to inflamma-</td>
<td>[91]</td>
</tr>
<tr>
<td>AβPPa/wt/Neuro-2a cells</td>
<td>Neuro-2a cells</td>
<td>FAAH</td>
<td>Increased F AAH activity may contribute to inflammation in AD pathology.</td>
<td>[93]</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Cortex, blood</td>
<td>FAAH protein, mRNA and activity</td>
<td>Increased F AAH activity may contribute to inflammation in AD pathology.</td>
<td>[79, 192]</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Cortex</td>
<td>AEA and NaPPE</td>
<td>Decreased</td>
<td>[93]</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Plasma</td>
<td>AEA and 2-AG</td>
<td>Unchanged</td>
<td>[93]</td>
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<tr>
<td>PS1/AβPPa/wt mice</td>
<td>Whole-brain</td>
<td>AEA and 2-AG</td>
<td>Increased</td>
<td>[99]</td>
</tr>
<tr>
<td>Rats (Aβ42 insult)</td>
<td>C6 glioma cells, Hippocampus</td>
<td>AEA and 2-AG</td>
<td>2-AG-Increased</td>
<td>[87, 101]</td>
</tr>
<tr>
<td>AβPPa/wt/PS1ΔE9 mice</td>
<td>Frontal cortex, Hippocampus and Striatium</td>
<td>AEA, 2-AG, PEA and OEA</td>
<td>AEA- decreased</td>
<td>[193]</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Hippocampus</td>
<td>MAGL, ABHD6</td>
<td>MAGL- increased</td>
<td>[80]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ABHD6- abolished</td>
<td>MAGL- decreased</td>
<td>[80]</td>
</tr>
</tbody>
</table>

CB₁ and CB₂, cannabinoid receptors; BLA, basolateral amygdala; DS, Down’s syndrome; FAAH, fatty acid amide hydrolase; NAPE, N-arachidonoyl-2-docosahexaenoyl-sn-glycerophosphoethanolamine; AEA, anandamide; 2-AG, 2-arachidonoylglycerol; DAGL, diacylglycerol lipase; MAGL, monoacylglycerol lipase; ABHD6, serine hydrolase α6/ hydrolase 6.
matory 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 CB2 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 CB2 expression have been observed [95]. Moreover, increased levels of CB2 receptors were positively correlated with Aβ1-42 and senile plaque score [83]. Apart from human studies, transgenic model of AD has also revealed overexpression of CB2 receptors in brain areas affected by the AD-pathology [96]. Increased CB2 mRNA in peripheral blood has been suggested as a peripheral biomarker for the early diagnosis of AD [97]. Pretreatment with Aβ1-42 to rats and C6 rat astroglia cells also increases CB2 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 Aβ1-42; 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 PS1AβPP 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 Aβ1-42 have also shown the increase of DAG lipase and 2-AG levels [87, 101].

**BENEFICIAL EFFECTS OF CANNABINOIDS IN TREATMENT OF ALZHEIMER’S DISEASE**

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]. CB2 activation is known to reduce microglia activity and inflammatory cytokines productions [107]. So it can be hypothesized that CB2 agonist could lower Aβ plaque load by increasing Aβ clearance. In line with this hypothesis, it has been shown that *in vitro* activation of CB2 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 CB2 agonist was able to induce a prompt Aβ clearance in Aβ-induced animal model of AD [109]. The mechanism underlying CB2 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 plaque load [107]. The role of CB2 receptors in lowering Aβ plaques was further confirmed by a study where CB2 receptors were deleted in AβPP mutant
Table 2
Beneficial effects of modulators of the endocannabinoid system and their molecular mechanisms in AD

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Treatment</th>
<th>Effects and mechanism involved</th>
<th>Ref.</th>
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</thead>
<tbody>
<tr>
<td><strong>Endocannabinoids</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ntera 2/D1 neurons (Aβ insult)</td>
<td>AEA</td>
<td>↓ Aβ toxicity</td>
<td>[149]</td>
</tr>
<tr>
<td>Wistar rats (Aβ insult)</td>
<td>Noladin ether</td>
<td>MAPK pathway activation</td>
<td></td>
</tr>
<tr>
<td>PC12 cells</td>
<td>AEA</td>
<td>↑ cell viability</td>
<td>[150]</td>
</tr>
<tr>
<td>SH-SY5Y cells (Aβ40 and peroxide insult)</td>
<td>2-AG</td>
<td>♦ Aβ clearance</td>
<td>[112]</td>
</tr>
<tr>
<td><strong>vitro model of the BBB</strong></td>
<td>2-AG</td>
<td>♦ expression of LRPI</td>
<td></td>
</tr>
<tr>
<td>Primary hippocampal neurons (Aβ1-42, Aβ40 insult)</td>
<td>JZL185</td>
<td>↓ neurodegeneration</td>
<td>[145]</td>
</tr>
<tr>
<td>JZL 195</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary cortical neurons (Aβ treatment)</td>
<td>AEA, PEA and OEA</td>
<td>↓ lysosomal membrane permeabilization</td>
<td>[137]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ arachidonic acid, PGE2, PGD2, TXB2</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>↓ GFAP, CD11b, TNF-α, IL-1β, IL-6, Aβ42, Aβ40</td>
<td>[99]</td>
</tr>
<tr>
<td>Mouse astrocytes (Aβ treatment)</td>
<td>JZL184</td>
<td>↓ BACE1 expression</td>
<td>[115]</td>
</tr>
<tr>
<td>eCB degradation enzyme inhibitors</td>
<td></td>
<td>↓ levels</td>
<td></td>
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<td></td>
<td></td>
<td>↓ neuroinflammation</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>improved learning and memory</td>
<td></td>
</tr>
<tr>
<td>Primary cortical neurons (Aβ treatment)</td>
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<td></td>
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<tr>
<td>AβPP/PS1 AD mouse</td>
<td>Genetic/pharmaceutical inactivation of MAGL</td>
<td>↓ microglia-mediated neurotoxicity</td>
<td>[82]</td>
</tr>
<tr>
<td>5 × FAD AβPP transgenic mice</td>
<td>JZL184</td>
<td>↓ inflammation</td>
<td></td>
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<td><strong>Cannabinoid agonists</strong></td>
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<tr>
<td>microglial cells (Aβ insult)</td>
<td>HU210, WIN55,212-2, and JWH-133</td>
<td>↓ microglia-mediated neurotoxicity</td>
<td>[121]</td>
</tr>
<tr>
<td>Human fetal astrocytes (IL-1β insults)</td>
<td>WIN55,212-2 (mixed CB1/CB2 agonist)</td>
<td>↓ production of inflammatory mediators</td>
<td>[134]</td>
</tr>
<tr>
<td>rat glomus cells (Aβ insult)</td>
<td>WIN 55,212-2</td>
<td>↓ iNOS expression</td>
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<td>SD rats brain slices</td>
<td>WIN 55212-2</td>
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<td>WIN-55212-2</td>
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<td>↓ BBB integrity</td>
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<td>↑ learning impairment ↓ soluble Aβ, tau, and p-tau levels ↓ astrogliosis, microgliosis, and inflammatory-related molecules</td>
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mice (PDGFB-AβPPSwInd). Results from this study revealed that soluble Aβ and plaque deposition were significantly increased in AβPP/CB2−/− mice compared to AβPP/CB2+/− mice [110]. The exact role of CB1 receptor is not yet clear in same context. Effect of cannabinoid treatment on Aβ fibril and aggregate formation was recently reported. Biochemical and morphological assessment showed that Δ9-THC, among other cannabinoids (eCBs, CB1, and CB2 agonist), significantly reduced fibril and aggregate formation [109]. However, CB1 receptor deletion from AβPP23 transgenic mouse
model of AD resulted in reduced amount of AβPP, reduced Aβ plaques load and less inflammation [110]. AβPP35/CB1−/− mice showed lower body weight and most of the animals died before typical AD associated changes could become apparent [111]. Though the AβPP35/CB1−/− study questioned the beneficial role of CB1 receptors in the Aβ generation and clearance, another study by Bachmeier and colleagues [112] supported the hypothesis that CB1 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 CB1 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 (β-site AβPP cleaving enzyme 1; BACE1), an enzyme involved in the production of Aβ [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 CB1 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 Δ2-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 pro-inflammatory precursor molecules.

Regulation of glial cell activity

As discussed earlier in this review, CB2 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 CB2 receptor in AD is a defensive mechanism to limit inflammation and to clear plaques from the affected brain region [79, 110, 129]. CB2 receptors are coupled to Gβγ inhibitory proteins so that their activation is associated with inhibition of adenyl cyclase and cAMP/protein kinases A (PKA) dependent pathway [130]. CB2 receptor activation could provide beneficial effects at various levels. In particular, CB2 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
inflammatory components, such as cytokines (TNF-\(\kappa\)) and various anti-inflammatory proteins, such as inhibitor of NF-\(\kappa\)B, have been demonstrated in AD [134]. In another report, PP AR-\(\gamma\) was able to exert anti-inflammatory effects in AD [135, 136]. It has been also shown that cannabinoids treatment, in activated astrocytes, inhibits synthesis of inflammatory chemokines and NO release [133]. PP AR-\(\gamma\) activated murine astrocytes [137] and CD40-mediated inhibition of microglia. Furthermore, mechanistic insight of beneficial effects provided by CB\(_1\) receptor stimulation in AD was demonstrated. Stimulation of CB\(_1\) receptor significantly attenuated CD40-mediated inhibition of microglial phagocytosis of A\(_{B}\) peptide [107]. Cannabidiol dose dependently reduced A\(_{B}\)-induced neuroinflammation by suppressing microglial activation, IL-1\(\beta\) and iNOS expression [132].

It has been also shown that cannabinoids treatment, in activated astrocytes, inhibits synthesis of inflammatory chemokines and NO release [133]. Win55,212-2, an agonist of CB\(_1\) and CB\(_2\) receptors, inhibited inducible NO synthase (iNOS) and corresponding NO production in astrocytes activated by IL-1\(\beta\) [134]. Win55,212-2 treatment also inhibited production of chemokines (CXCL10, CCL2, and CCL5) and TNF-\(\alpha\). 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 PP AR-\(\gamma\) dependent manner, A\(_{B}\)-induced reactive gliosis by reducing proinflammatory molecules and cytokine release [133]. PP AR-\(\gamma\) activation could inhibit NF\(_\kappa\)B 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 A\(_{B}\)-activated murine astrocytes [137]. Previous studies have shown that N-acylethanolamines activate anti-inflammatory nuclease receptor PPAR-\(\alpha\) that causes formation of a multiprotein complex along with variable set of protein co-activators [138]. With this multiprotein complex, PPAR-\(\alpha\) binds to responsive elements on DNA and enhances the transcription of various anti-inflammatory proteins, such as inhibitor of NF\(_\kappa\)B (I\(\kappa\)B), that suppress the gene expression of pro-inflammatory components, such as cytokines (TNF-\(\alpha\), IL-1\(\beta\)) including iNOS and COX-2 (see Fig. 1) [138, 139]. Anti-inflammatory effects of cannabinoids have been also demonstrated in A\(_{B}\)-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 A\(_{B}\) 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 A\(_{B}\) plaques and the function of BACE1, the enzyme involved in making toxic A\(_{B}\) 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.

MAGL 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 CB1 but not by CB2 or TRPV1 receptors [145]. CB1 receptor activation by 2-AG suppresses phosphorylation of ERK1/2/p38MAPK/NF\(_\kappa\)B 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-\(\gamma\), mediates 2-AG-induced inhibition of NF\(_\kappa\)B phosphorylation and COX-2 expression in response to pro-inflammatory IL-1\(\beta\). Moreover, 2-AG is able to restore IL-1\(\beta\)-induced reduction of PPAR-\(\gamma\)-expression in CB1 dependent mechanism [146]. Inflammation activates the transcription factor NF\(_\kappa\)B, for which \(\beta\) secretase (BACE1) promoter harbors a highly conserved binding site that is functional [125]. Thus NF\(_\kappa\)B activates BACE1 promoter, expression, and enzymatic activity leading to increased A\(_{B}\) production. The prostaglandin PG\(_E2\) after production stimulates the generation of A\(_{B}\) through both EP2 and EP4 receptors (PG\(_E2\) receptors). Activation of the EP4 receptor...
Fig. 2. Modulation of 2-AG signaling provides anti-inflammatory effects in AD. Through a CB\(_1\)-dependent mechanism, 2-AG increases PPAR-\(\gamma\)/H9253 expression, which is suppressed by A/H9252 in AD. 2-AG directly, through CB\(_1\) and PPAR-\(\gamma\) 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 \(\beta\)- and \(\gamma\)-secretase resulting in increased A/H9252 production. Inflammation activates the transcription factor NF\(\kappa\)B, for which \(\beta\)-secretase (BACE1) promoter harbors a highly conserved binding site that is functional. Thus, NF\(\kappa\)B activates BACE1 promoter, expression, and enzymatic activity leading to increased A/H9252 production. Prostaglandin PGE2 stimulates the generation of A/H9252 through both EP2 and EP4 receptors (PGE2 receptors). Activation of the EP4 receptor stimulates A/H9252 production through the endocytosis and the activation of \(\gamma\)-secretase. The inhibition of prostaglandins synthesis by MAGL inhibitors could suppress all these mechanisms.

**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 caspase-3 [145, 151]. CB\(_1\) knock-out studies indicated that lack of CB\(_1\) is associated with increased 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 Aβ is critical for oxidative stress (for more details, see [154]), NfxB, 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, NfxB 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 phyto cannabinoids have anti-oxidant properties [158]. Cannabidiol is a well studied cannabinoid in this context. It has been shown that cannabidiol significantly decreases glutamate toxicity, Ca2+ toxicity, iNOS expression, and NO production [131, 158]. Cannabidiol mediates these effects through inhibition of p38 MAPK and NfxB pathways probably through involvement of the PPAR-γ receptor [132, 133, 135]. Moreover, CB1 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]. CB1 receptors are also expressed on mitochondria and regulate its activity [71]. Activation of mitochondrial CB1 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 Ca2+-induced cytochrome c release from mitochondria (Fig. 1) [162–164].

Memory and learning impairments

CB1-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 CB1 receptor deficiency on development of AD pathology were studied by knocking out CB1 receptor in AβPP23 mice of AD. AβPP23/CB1−/− mice showed worsen cognitive deficits than AβPP23 mice, thus suggesting that CB1 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 CB1, but not CB2 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 NfxB. Antagonist experiment confirmed that these neuroprotective effects of Win55,212-2 were partially mediated by CB1 and CB2 receptors [140]. Through CB2 receptor, Win55,212-2 increased PPAR-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 CB2 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\textsubscript{2} receptor, TNF-\textalpha{} and COX-2 suggesting a critical role of CB\textsubscript{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 AJ\textsubscript{PP}/PS1 mouse model of AD [170]. The authors further revealed that these effects were not associated with decreased A\beta{} 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 \textDelta{}\textsubscript{9}-THC reduced learning impairment, decreased soluble A\beta{}\textsubscript{1-42} 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 AJ\textsubscript{PP}/PS1 double transgenic mice did not improve water maze performance or a contextual fear conditioning task [172]. HU-210 neither altered AJ\textsubscript{PP} processing and neuritic plaque formation nor enhanced hippocampal neurogenesis in AJ\textsubscript{PP}/PS1 transgenic mice. It has been reported that CB\textsubscript{1} blockade by rimonabant improved A\beta{}-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, \textDelta{}\textsubscript{9}-THC, has been demonstrated to competitively inhibit ACHe and to thus increase ACh levels [175]. Moreover, \textDelta{}\textsubscript{9}-THC prevented ACHe-induced aggregation of A\beta{} which can reduce plaques formation [175]. In addition to \textDelta{}\textsubscript{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\textsuperscript{2+} influx. In similar way, HU-211 (synthetic cannabinoid devoid of CB\textsubscript{1} and CB\textsubscript{2} agonist activity) protects neurons from excitotoxicity by antagonizing NMDA receptors [177–179].

Moreover, recently it was demonstrated that eCBs can modulate A\beta{}-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 A\beta{} negatively regulates Notch-1 signaling by increasing expression of Numb, the endogenous negative regulator of Notch-1 cleavage [183]. Interestingly, AEA, through CB\textsubscript{1} receptors, was able to reverse this effect by increasing expression of Notch-1 signaling components like nicastrene, Notch intracellular domain, Hes1 and Hes5 (see Fig. 1). Moreover, AEA and 2-AG were also able to inhibit A\beta{}-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\textsubscript{1} receptor activation can elicit vasodilatation [184]. Moreover, as discussed earlier, cannabinoids can increase A\beta{} clearance at blood brain barrier [112]. CB\textsubscript{1} 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, CB\textsubscript{1} receptor activation may precipitate episodes of psychosis and panic while its inhibition may lead to depression and anxiety-related disorders (for more details, see [190]). Furthermore, CB\textsubscript{1} agonists may worsen AD by inhibiting acetylcholine release in the brain [7]. CB\textsubscript{2} 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.
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.

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 mechanisms 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 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 CB2 expression and hydrolyzing FAAH activity is well
documented in human AD patients as well as animal models of AD, a combination therapy of CB2 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 [337].

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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