

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 , 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

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

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

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

60 The endocannabinoid (eCB) system appears to be
61 a promising therapeutic target as it has the ability to
62 modulate a range of aspects of AD pathology. At a
63 first glance, it is striking that cannabinoids like delta-
64 9-tetrahydrocannabinol (Δ^9 -THC), known to impair
65 memory, could be beneficial in AD [4]. However,
66 augmentation of eCB signaling could reduce exci-
67 totoxicity, oxidative stress, and neuroinflammation
68 and thus could alleviate symptoms of AD [5]. Previ-
69 ous reviews have highlighted the beneficial effects of
70 cannabinoids in AD treatment [5–10], but none of them
71 have focused on the molecular mechanisms through
72 which eCBs exert their beneficial effects. Thus, the
73 present review will extensively cover recent findings on
74 the dysregulation of eCB signaling and the molecular
75 mechanisms involved in beneficial effects of cannabi-
76 noids in AD.

77 ALZHEIMER'S DISEASE 78 PATHOPHYSIOLOGY

79 AD is a progressive, degenerative, and irreversible
80 neurological disorder that causes deterioration of
81 memory, judgment, and reasoning in the elderly [11].
82 Patients suffering from AD exhibit cognitive impair-
83 ment, memory loss, and behavioral changes [11].
84 The neurodegeneration in AD is characterized by
85 neuronal loss and synaptic injury [12]. Moreover,
86 AD is associated with extracellular insoluble plaques
87 [13], intracellular neurofibrillary tangles (NFTs) [14],
88 astrogliosis [15], and microglial cell proliferation [16].
89 Extracellular senile plaques are mainly composed of
90 amyloid- β (A β) protein. The deposition of A β is the

91 first event in the pathogenesis of AD that precedes
92 the formation of phosphorylated tau aggregation [17].
93 NFTs consist of paired helical filaments resulting from
94 hyperphosphorylation of the microtubule-binding pro-
95 tein tau [11]. Tau plays an important role in the
96 maintenance of microtubule stability. In AD, tau
97 is aberrantly hyperphosphorylated and proteolyzed
98 resulting in impairment of normal functions of tau [11].

99 AD may be classified in two types based on genetic
100 endowment. The first type is inherited via an autosomal
101 dominant pattern, i.e., familial AD, and the second
102 type is sporadic AD. Familial AD displays early dis-
103 ease onset, whereas sporadic AD cases mostly develop
104 the disorder at an older age [18]. Etiology of AD is
105 multifactorial with genetic, environmental, and devel-
106 opmental components playing a role [2]. A large body
107 of evidence supports the notion that AD pathogenesis
108 is related to a progressive accumulation of A β protein
109 due to an imbalance between A β production, aggrega-
110 tion, and clearance [11, 19]. A β is formed following
111 sequential cleavage of amyloid- β protein precursor
112 (A β PP) by two proteases termed β - and γ -secretases
113 (see Fig. 1). After excessive generation, A β self aggre-
114 gates into A β oligomer and then it further aggregates
115 into insoluble extracellular senile plaques. Most of the
116 evidence suggests that A β oligomers instead of fibrils
117 are responsible for neurotoxic effects of A β [20–23].

118 Besides plaques and NFTs, AD is also character-
119 ized by neuroinflammation. It is widely accepted that
120 the deposition of A β is one of the main features of AD
121 and seems to trigger a cascade of neuroinflammatory
122 events that ultimately leads to neurodegeneration [24,
123 25]. Brain inflammation is mediated by the activation
124 of glial cells, microglia, and astrocytes, and expression
125 of inflammatory mediators and neurotoxic free radicals
126 [26]. Microglial cells are the central nervous system
127 (CNS) resident phagocytes of the immune system and
128 produce a wide range of cytokines, such as interleukins
129 [27]. Activated microglia accumulates at the site of A β
130 deposition and, as expected, actively engulfs and clears
131 A β deposits [28]. A β is able to stimulate Src family
132 kinases and Syk tyrosin kinases [29], which further can
133 activate mitogen-activated protein kinase (MAPK) and
134 nuclear factor κ B (NF κ B) cascades that are required for
135 proinflammatory cytokine and reactive oxygen species
136 (ROS) production (see Fig. 1) [27]. It has been also
137 reported that A β can directly activate MAPK and
138 extracellular signal regulated kinase (ERK) pathways
139 [30]. Transient activation of these signaling pathways
140 after A β binding to microglia results in upregulation
141 of proinflammatory cytokines such as interleukin-
142 1 β (IL-1 β) and tissue tumor necrosis factor-alpha

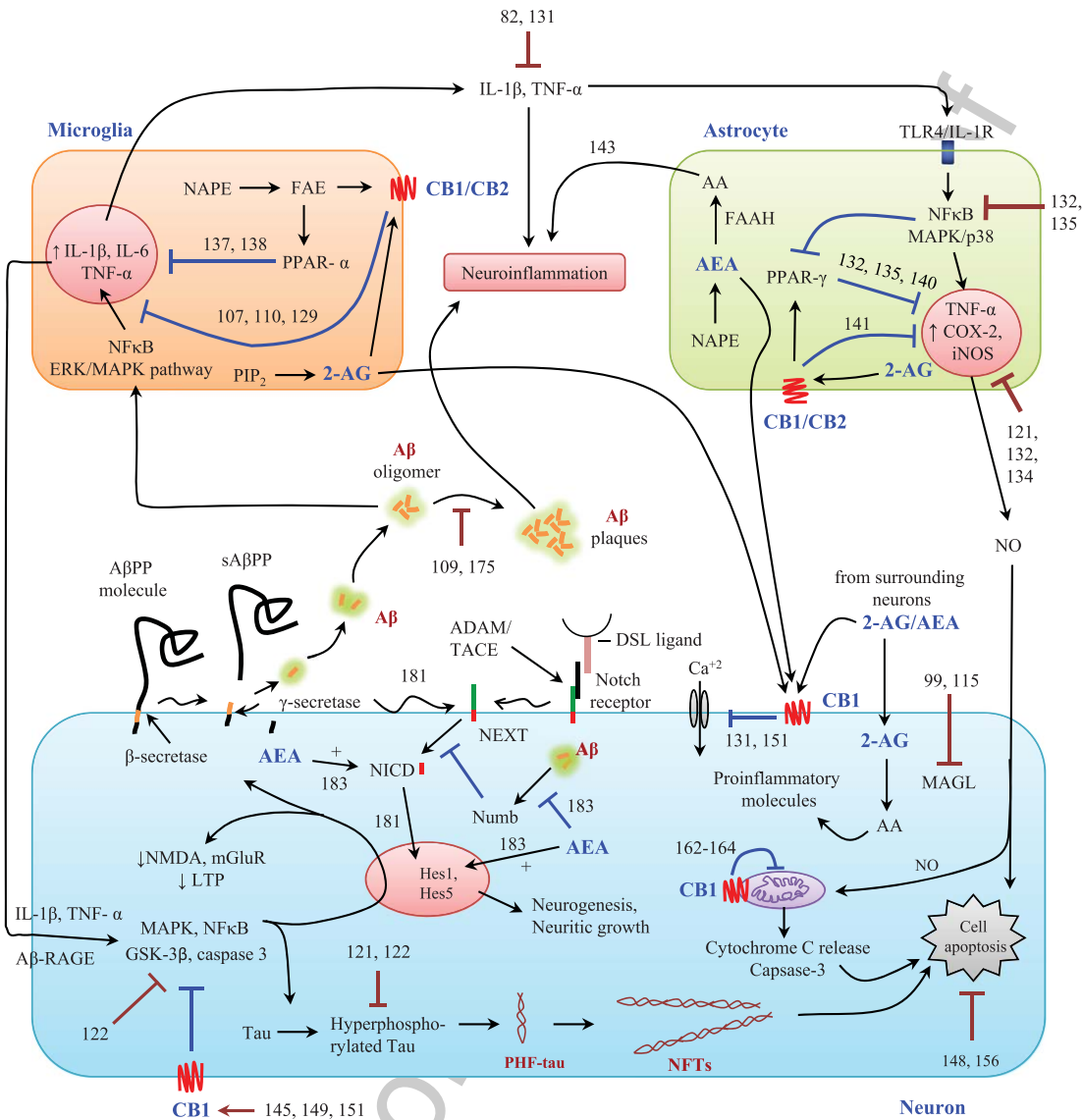


Fig. 1. Endocannabinoid signaling and molecular mechanisms of neurodegeneration in AD. Proteolytic cleavage of amyloid- β protein precursor (A β PP) by β - and γ -secretase results in generation of A β_{42} monomers, which under pathological conditions, assembles into oligomers. A β_{42} oligomers activate microglia and astrocytes. Activated microglia produces inflammatory cytokines through nuclear factor κ B (NF κ B) 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 β_{42} , through various mechanisms, activate MAPK, NF κ B, glycogen synthase kinase-3 β (GSK-3 β), and caspase-3 pathways. A β_{42} through MAPK and NF κ B pathways negatively modulates long-term potentiation by controlling NMDA and mGluR receptor expression, and ultimately causing memory impairment. Moreover, A β_{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 (anandamide; 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; PIP $_2$, 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-1 β and TNF- α are considered as primary cytokines responsible for chronic inflammation in AD [31]. Furthermore, IL-1 β released from glia activates MAPK and NF κ B signaling cascades in astrocytes and neurons, resulting in excessive inflammation and tau phosphorylation [27, 31] (Fig. 1). Additionally A β 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 NF κ B 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-potential (LTP) [35, 36]. Moreover, MAPK is rapidly activated after interaction of A β 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 anandamide (arachidonylethanolamine; 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 (PIP₂) 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 CB₁ and CB₂ receptors, while AEA acts as a partial agonist for CB₁ receptors [48]. In addition to CB₁ receptors, AEA can also activate peroxisome proliferator-activated- α receptors (PPAR- α) and transient receptor potential vanilloid-1 (TRPV1) channels [49].

CB₁ 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, CB₁ receptor signaling functions to 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 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

(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 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 adiposomes [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 (CB₁ and CB₂ agonist) 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 [79–81]. 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 A β protein were observed in hippocampus and basolateral amygdala [85]. The reduced CB₁ expression in A β PPswe/PS1 Δ E9 mice was associated with astroglial proliferation and elevated expression of cytokines, iNOS and TNF- α [86]. Similarly, pretreatment with A β ₄₂ in rats and C6 rat astrogloma 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 A β deposition [88]. Even though CB₁ receptors were unchanged, it has been proposed that the coupling between receptor and G_i 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
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 | CB ₁ mRNA CB ₁ binding | CB ₁ mRNA- Unchanged CB ₁ 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 | [85] |
| Human AD patient | Prefrontal cortex | CB ₁ binding | CB ₁ protein- reduced in dorsal hippocampus and BLA CB ₁ 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 | CB ₁ activity increased at earlier AD stages and decreased at advance stages | [90] |
| AβPP _{Swe} /PS1ΔE9 mice | Hippocampus | CB ₁ protein | Decreased | [86] |
| AβPP _{Swe} /PS1ΔE9 mice | Hippocampus, Cortex | CB ₁ -receptor-dependent Gi protein activation | Unchanged | [191] |
| Rat (Aβ ₄₂ insult) | Brain/Cells | CB ₁ and CB ₂ mRNA/protein | CB ₁ -decreased CB ₂ -increased | [87] |
| Human AD patient | Cortex, Hippocampus, Blood | CB ₂ protein and mRNA | Increased | [79, 82, 83, 91, 92, 97] |
| Human DS patient | Cortex | CB ₂ protein and FAAH protein | Increased | [95] |
| AβPP _{Swe} /PS1ΔE9 mice | Cortex | CB ₂ binding | Increased | [96] |
| AβPP _{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 (Aβ ₄₂ insult) | C6 glioma cells, Hippocampus | AEA and 2-AG | 2-AG-Increased | [87, 101] |
| AβPP _{Swe} /PS1ΔE9 mice | Frontal cortex, Hippocampus and Striatum | AEA, 2-AG, PEA and OEA | AEA- decreased Decreased only in striatum | [193] |
| Human AD patient | Hippocampus | DAGL, MAGL, ABHD6 | DAGL- increased MAGL- decreased ABHD6- abolished | [80] |

CB₁ and 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.

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

348 Differently from CB₁ receptor, the relationship
349 between CB₂ receptor and FAAH in AD pathology is

well documented in the literature. In fact, postmortem
350 brains from patients with AD revealed that CB₂ recep-
351 tors and FAAH are selectively overexpressed in cells
352 that are associated to Aβ-enriched neuritic plaques [79,
353 80, 82, 83, 91, 92]. The hydrolytic activity of FAAH is
354

enhanced in A β 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 CB₂ receptors are overexpressed in activated microglial cells in AD [79, 94, 95]. Similarly, in Down's syndrome, characterized by A β deposition, increased FAAH activity and CB₂ expression have been observed [95]. Moreover, increased levels of CB₂ receptors were positively correlated with A β 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 A β ₄₂ to rats and C6 rat astrogloma 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 A β 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/A β PP 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 β 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 A β -induced neuronal toxicity. Later, these investigations were extended to animal models of A β -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.

A β generation and clearance

Microglia plays an important role in phagocytosis of A β , and there is an inverse relationship between cytokine production and A β 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 A β plaque load by increasing A β clearance. In line with this hypothesis, it has been shown that *in vitro* activation of CB₂ receptor facilitates the removal of native A β 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 A β clearance in A β -induced animal model of AD [109]. The mechanism underlying CB₂ mediated decrease in A β 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 A β phagocytosis that might decrease A β

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 (A β insult) | AEA | ↓ A β toxicity | [149] |
| Wistar rats (A β ₄₂ insult) | Noladin ether | MAPK pathway activation | [101] |
| | VDM-11 | Reversed hippocampal damage Improved memory retention | |
| PC12 cells | AEA | ↑ cell viability | [150] |
| SH-SY5Y cells (A β ₄₀ and peroxide insult) | | CB ₁ mediated effect | |
| vitro model of the BBB | 2-AG | ↑ A β clearance | [112] |
| | JZL185 | ↑ expression of LRP1 | |
| | JZL 195 | | |
| Primary hippocampal neurons (A β ₂₅₋₃₅ , A β ₄₂ insult) | 2-AG | ↓ neurodegeneration | [145] |
| | URB602 JZL184 | ↓ apoptosis ↓ capsase-3 cleavage CB ₁ mediated effect ↓ ERK1/2 and NF κ B phosphorylation ↓ COX2 ↓ inflammation | |
| Mouse astrocytes (A β treatment) | AEA, PEA and OEA | | [137] |
| eCB degradation enzyme inhibitors | | | |
| Primary cortical neurons (A β treatment) | AEA, 2-AG, URB597 | ↓ Apoptosis | [148] |
| A β PP/PS1 AD mouse | Genetic/pharmacological inactivation of MAGL | ↓ lysosomal membrane permeabilization ↓ arachidonic acid, PGE2, PGD2, TXB2 | [99] |
| | | ↓ GFAP, CD11b, TNF- α , IL-1 β , IL-6, A β ₄₂ , A β ₄₀ | |
| 5 \times FAD A β PP transgenic mice | JZL184 | ↓ BACE1 expression ↓ A β levels ↓ neuroinflammation Improved learning and memory | [115] |
| Cannabinoid agonists | | | |
| microglial cells (A β insult) | HU-210, WIN55,212-2, and JWH-133 | ↓ microglia-mediated neurotoxicity | [82] |
| Human fetal astrocytes (IL-1 β insults) | WIN55,212-2 (mixed CB ₁ / CB ₂ agonist) | ↓ production of inflammatory mediators | [134] |
| C6 rat glioma cells (A β insult) | WIN 55,212-2 | ↓ iNOS expression ↓ NO production | [121] |
| SD rats brain slices | WIN 55212-2 | ↓ acetylcholine release | [194] |
| Wistar rats (A β ₄₂ insult) | ACEA (CB ₁ agonist) | ↓ caspase 3 | [151] |
| | WIN-55212-2 | Improved memory deficits | |
| Rats (A β ₄₂ treatment) | Win55,212-2 | ↓ Ca ⁺² currents in CA1 neurons ↓ inflammation CB ₁ , CB ₂ and PPAR- γ mediated | [140] |
| A β PP23/PS45 double transgenic mouse model of AD | HU210 (mixed CB ₁ / CB ₂ agonist) | Unchanged A β PP and A β levels | [172] |
| microglial cells (A β insult) | JWH-015 (CB ₂ agonist) | No effect on learning and memory | [107] |
| | | ↓ microglial activation ↓ phosphorylation of JAK/STAT1 ↑ phagocytosis of A β ₄₂ | |
| human brain microvascular endothelial cells, mice | JWH133 (CB ₂ agonist) | ↓ intercellular adhesion molecule-1 | [185] |
| | | ↓ vascular cell adhesion molecule-1 ↑ BBB integrity | |
| Rats (A β ₄₀ insult) | MDA7 (CB ₂ agonist) | ↓ CD11b expression ↓ GFAP expression ↓ interleukin-1 β ↑ A β clearance restored cognition and memory | [129] |

Table 2
(Continued)

| Subjects | Treatment | Effects and mechanism involved | Ref. |
|---|---|--|----------------|
| Tg A β PP mice | JWH-133 (CB ₂ agonist) | Improves cognitive performance ↓ Iba-1, COX-2, TNF- α ↓ A β ₄₀ , A β ₄₂ and CB ₂ ↓ GSK3- β tau phosphorylation kinase | [141] |
| Pharmacological or genetic inhibition of cannabinoid receptors | | | |
| swiss mice (A β _{25-35,42} insult) | Rimonabant (CB ₁ antagonist) | improves A β -induced amnesia | [173] |
| A β PP23/ CB ₁ ^{-/-} mice | | ↓ A β PP levels, plaque load ↓ neuroinflammation Impaired learning Memory deficits | [111] |
| A β PP/ CB ₂ ^{-/-} mice | | ↑ soluble A β ₄₂ ↑ A β ₄₂ plaque ↑ microglia activation ↓ soluble tau | [110] |
| Phytocannabinoids | | | |
| Rat cortical neuron culture (glutamate insult) | Cannabidiol Δ^9 -THC | ↓ glutamate toxicity | [158] |
| microglial cells C57/B16 Mice (A β ₄₀ insult) | Cannabidiol | -Antioxidant effect ↓ ATP induced Ca ⁺² | [131] |
| | WIN 55,212-2 JWH-133 | ↑ microglia migration ↓ NO, TNF- α , IL-6 ↓ cognitive impairment | |
| C57BL/6J mice (A β ₄₂ insult) | cannabidiol | ↓ GFAP ↓ iNOS and IL-1b | [132] |
| AChE from Electrophorus electricus | Δ^9 -THC | inhibits AChE | [175] |
| N2a/A β PPsw cells | Δ^9 -THC | ↓ AChE-induced A β aggregation ↓ A β levels ↓ A β aggregation | [124] |
| PC12 neuronal cells (A β ₄₂ insult) | cannabidiol | ↓ GSK-3 β and p-GSK-3 β ↓ tau hyperphosphorylation ↓ p-GSK-3 β ↑ β -catenin | [122] |
| PC12 cells (A β ₄₂ insult) | cannabidiol | ↓ iNOS, NO ↓ p38 MAP kinase ↓ NF κ B | [135] |
| Primary cultured astrocytes Rats (A β ₄₂ insult) | cannabidiol | ↓ NO, TNF- α , IL-6, S100B ↓ reactive gliosis ↑ neurogenesis | [133] |
| Neuroblastoma cells (A β ₄₂ insult) microglial | ACEA (CB ₁ agonist) | Mediated through PPAR- γ ↓ A β fibrils | [109] |
| BV-2 cells (LPS insult) | JWH-015 (CB ₂ agonist) Δ^9 -THC, cannabidiol, 2-AG, AEA | ↑ neuronal cell viability neuroprotective action | |
| PC12 cells (A β insult) A β PP/PS1 mice | Cannabidiol Cannabidiol | neuroprotective, anti-oxidative anti-apoptotic Inhibits development of social recognition memory deficits | [156] [170] |
| A β PP/PS1 mice | Cannabidiol + Δ^9 -THC | ↑ dietary phytosterols ↓ learning impairment ↓ soluble A β ₄₂ peptide levels ↓ astrogliosis, microgliosis, and inflammatory-related molecules | [171] |

455 plaque load [107]. The role of CB₂ receptors in low-
456 ering A β plaques was further confirmed by a study
457 where CB₂ receptors were deleted in A β PP mutant
458 mice (PDGFB-A β PPswInd). Results from this study
459 revealed that soluble A β and plaque deposition were

significantly increased in A β PP/CB₂^{-/-} mice compared to A β PP/CB₂^{+/+} mice [110].

The exact role of CB₁ receptor is not yet clear in same context. Effect of cannabinoid treatment on A β fibril and aggregate formation was recently

460
461
462
463
464

465 reported. Biochemical and morphological assessment
 466 showed that Δ^9 -THC, among other cannabinoids
 467 (eCBs, CB₁ and CB₂ agonist), significantly reduced
 468 fibril and aggregate formation [109]. However, CB₁
 469 receptor deletion from A β PP23 transgenic mouse
 470 model of AD resulted in reduced amount of A β PP,
 471 reduced A β plaque load and less inflammation [110].
 472 A β PP23/CB₁^{-/-} mice showed lower body weight and
 473 most of the animals died before typical AD associ-
 474 ated changes could become apparent [111]. Though the
 475 A β PP23/CB₁^{-/-} study questioned the beneficial role
 476 of CB₁ receptors in the A β generation and clearance,
 477 another study by Bachmeier and colleagues [112] sup-
 478 ported the hypothesis that CB₁ agonist could increase
 479 A β clearance from the brain. In fact, this study showed
 480 that CB receptor agonist or pharmacological eleva-
 481 tion of eCBs significantly enhanced A β clearance from
 482 the brain [112]. eCBs increased A β clearance across
 483 the blood-brain barrier by increasing the expression
 484 of A β transport protein, lipoprotein receptor protein
 485 1 (LRP1). Moreover, this study suggests that eCBs
 486 could decrease the A β brain burden not only due to
 487 changes in A β synthesis or release but also due to
 488 increase in A β transport from brain to periphery by the
 489 way of blood-brain barrier. It has been proposed that
 490 eCBs, through CB₁ receptor, activate PPAR- γ receptor,
 491 which has been shown to stimulate expression of LRP1
 492 [113, 114]. Furthermore, MAGL inactivation reduced
 493 A β plaque load and also suppressed the expression
 494 of β -secretase (beta-site A β PP cleaving enzyme 1;
 495 BACE1), an enzyme involved in the production of
 496 A β ₄₂ [115].

497 *Tau hyperphosphorylation*

498 Abnormal hyperphosphorylation of tau prompts an
 499 accumulation of NFTs in axons of neurons, can impair
 500 normal axonal transport, disrupt synaptic plasticity,
 501 and finally induce cell loss [116]. The link connecting
 502 A β plaques and tau pathologies has remained elusive.
 503 Evidence suggests that abnormal activation of kinases
 504 like glycogen synthase kinase-3 β (GSK-3 β), MAPK
 505 family members as well as caspases may be responsi-
 506 ble for hyperphosphorylation of tau [117, 118], and A β
 507 might be involved in the activation of these enzymes
 508 [119]. Along with various kinases, NO secreted
 509 from astrocytes induces tau hyperphosphorylation in
 510 neurons [120]. It has been shown that arachidonoyl-
 511 2'-chloroethylamide (ACEA), a selective CB₁ agonist,
 512 down regulates iNOS protein expression and NO pro-
 513 duction in astrocytes, and that leads to a significant
 514 inhibition of NO-dependent tau hyperphosphorylation

515 in neurons [121]. In another report [122], it has been
 516 demonstrated that cannabidiol (a non psychoactive
 517 component of marijuana) inhibits hyperphosphoryla-
 518 tion of tau protein in A β -stimulated neuronal cells.
 519 The effect of cannabidiol was mediated through the
 520 Wnt/ β -catenin pathway [122]. Wnt activation leads
 521 to inhibition of GSK-3 β , which is also known as
 522 tau protein kinase, responsible for a massive tau pro-
 523 tein hyperphosphorylation and relative NFT formation
 524 observed in brains of AD patients [123]. A recent report
 525 also demonstrated that Δ^9 -THC treatment inhibits acti-
 526 vation of GSK-3 β in N2a-variant A β PP cells [124].

527 *Neuroinflammation*

528 Besides plaques and NFTs, neuroinflammation
 529 plays a major role in neurodegeneration and activa-
 530 tion of various apoptosis pathways. The notion that
 531 A β is a pathological molecule is slowly changing and
 532 it seems that it represents a cellular adaptive strat-
 533 egy to oxidative stress [125]. A β is a proinflammatory
 534 molecule, which can induce its own production by
 535 increasing the expression of its synthesizing enzymes
 536 such as β -secretase (BACE1) and through various
 537 inflammatory pathways [125]. In particular, it has been
 538 recognized that A β is able to initiate an inflamma-
 539 tory response, which in turn activates microglia and
 540 recruits astrocytes, and therefore the release of inflam-
 541 matory mediators (IL-1 β , TNF- α , and IL-6), reactive
 542 oxygen species (NO), and neurotoxic products that
 543 have been involved in neuronal and synaptic damage
 544 [31]. Neuroprotective effects of eCBs against brain
 545 injury and inflammation is associated with reduction of
 546 cytokines, ROS, and prostaglandins [126–128]. eCB
 547 modulators can reduce neuroinflammation in AD by
 548 inhibiting glial cell activation and generation of pro-
 549 inflammatory precursor molecules.

550 *Regulation of glial cell activity*

551 As discussed earlier in this review, CB₂ and FAAH
 552 expression is upregulated in microglia and astrocytes,
 553 respectively, in surrounding areas of neuritic plaques
 554 in AD brains. This notion suggests that both microglia
 555 and astrocytes play an important role in eCB sig-
 556 naling in AD pathology. It seems that upregulation
 557 of CB₂ receptor in AD is a defensive mechanism
 558 to limit inflammation and to clear plaques from the
 559 affected brain region [79, 110, 129]. CB₂ receptors are
 560 coupled to G_{i/o} inhibitory proteins so that their acti-
 561 vation is associated with inhibition of adenylyl cyclase
 562 and the cAMP/protein kinases A (PKA) dependent
 563 pathway [130]. CB₂ receptor activation could provide

564 beneficial effects at various levels. In particular, CB₂
565 activation could 1) suppress activation of microglia,
566 2) reduce production of inflammatory molecules like
567 IL-1 β , IL-6, TNF- α , NO, etc., 3) enhance microglial
568 proliferation, and 4) enhance microglial phagocytic
569 activity [59, 82, 107, 108, 131].

570 The effects of non selective cannabinoid agonists on
571 microglial activation were demonstrated by Ramirez
572 and colleagues [82]. In their study authors investi-
573 gated the effects of non selective cannabinoids and
574 selective CB₂ agonists in A β -induced microglial cells
575 [82]. As expected, A β peptide activated microglial
576 cells with increased mitochondrial activity, TNF- α
577 release, and cellular morphological changes. Cannabi-
578 noid treatment prevented the enhancement of TNF- α
579 release and counteracted A β -mediated activation of
580 microglia. Furthermore, mechanistic insight of ben-
581 efiticial effects provided by CB₂ receptor stimulation
582 in AD was demonstrated. Stimulation of CB₂ recep-
583 tor significantly attenuated CD40-mediated inhibition
584 of microglial phagocytosis of A β ₄₂ peptide [107].
585 Cannabidiol dose dependently reduced A β -induced
586 neuroinflammation by suppressing microglial activa-
587 tion, IL-1 β and iNOS expression [132].

588 It has been also shown that cannabinoid treat-
589 ment, in activated astrocytes, inhibits synthesis of
590 inflammatory chemokines and NO release [133].
591 Win55,212-2, an agonist of CB₁ and CB₂ recep-
592 tors, inhibited inducible NO synthase (iNOS) and
593 corresponding NO production in astrocytes activated
594 by IL-1 β [134]. Win55,212-2 treatment also inhib-
595 ited production of chemokines (CXCL10, CCL2,
596 and CCL5) and TNF- α . Both selective CB₁ and
597 CB₂ antagonists partially blocked these effects sug-
598 gesting the involvement of both receptors [134].
599 Cannabidiol markedly down-regulates, in a PPAR- γ
600 dependently manner, A β -induced reactive gliosis
601 by reducing proinflammatory molecules and cytokine
602 release [133]. PPAR- γ activation could inhibit NF κ B
603 pathway, which is involved in the synthesis of
604 inflammatory cytokines [135, 136]. In another report,
605 different N-acylethanolamides (AEA, PEA, and OEA)
606 were able to exert anti-inflammatory effects in A β -
607 activated murine astrocytes [137]. Previous studies
608 have shown that N-acylethanolamines activate anti-
609 inflammatory nucleic acid receptor PPAR- α that causes
610 formation of a multiprotein complex along with vari-
611 able set of protein co-activators [138]. With this
612 multiprotein complex, PPAR- α binds to responsive
613 elements on DNA and enhances the transcription of
614 various anti-inflammatory proteins, such as inhibitor of
615 κ B- α (I κ B- α), that suppress the gene expression of pro-

616 inflammatory components, such as cytokines (TNF- α ,
617 IL-1 β) including iNOS and COX-2 (see Fig. 1) [138,
618 139]. Anti-inflammatory effects of cannabinoids have
619 been also demonstrated in A β -induced *in vivo* AD
620 models [129, 140] and transgenic mice models of AD
621 [141].

622 Regulation of pro-inflammatory precursors

623 Phospholipase A2 (PLA2) enzymes are considered
624 the primary source of AA for COX-mediated biosyn-
625 thesis of prostaglandins [142]. Recently, Nomura and
626 colleagues [143] have shown that MAGL-mediated
627 hydrolysis of 2-AG can act as a distinct pathway to
628 generate AA in the brain [143]. In line with this report,
629 two independent research teams [99, 115] reported that
630 the inactivation of MAGL reduced neuroinflammation,
631 neurodegeneration, and the production and accumula-
632 tion of A β plaques in the transgenic mice of AD. These
633 effects were not mediated by CB₁ and/or CB₂ recep-
634 tors but were caused by reduced production of AA
635 [99, 136]. The inhibition of MAGL also improved the
636 neuronal plasticity and learning and memory deficits
637 [99, 115]. Inactivation of MAGL for eight weeks was
638 sufficient to decrease production and deposition of
639 A β plaques and the function of BACE1, the enzyme
640 involved in making toxic A β in the brain (Fig. 2) [115].
641 These results suggest that MAGL contributes to the
642 cause and development of AD and that the inhibi-
643 tion of MAGL might represent a promising potential
644 therapeutic target.

645 MGL inhibition can cause an elevation of 2-
646 AG endogenous levels. In turn, 2-AG, by activating
647 CB₁ receptor is able to suppress COX-2 elevation
648 in response to inflammatory insult like lipopolysac-
649 charide [144]. Furthermore, it was revealed that the
650 neuroprotective effects of 2-AG were mediated by
651 CB₁ but not by CB₂ or TRPV1 receptors [145]. CB₁
652 receptor activation by 2-AG suppresses phosphoryla-
653 tion of ERK1/2/p38MAPK/NF κ B in neurons, which
654 further suppresses COX-2 expression (Fig. 2) [144,
655 145]. COX-2 plays an important role in production of
656 prostaglandins, which are crucial in neuroinflamma-
657 tion [142]. Further research in this field revealed that
658 PPAR- γ , mediates 2-AG-induced inhibition of NF κ B
659 phosphorylation and COX-2 expression in response to
660 pro-inflammatory IL-1 β . Moreover, 2-AG is able to
661 restore IL-1 β -induced reduction of PPAR- γ expression
662 in CB₁ dependent mechanism [146]. Inflammation
663 activates the transcription factor NF κ B, for which
664 β -secretase (BACE1) promoter harbors a highly con-
665 served binding site that is functional [125]. Thus
666 NF κ B activates BACE1 promoter, expression, and

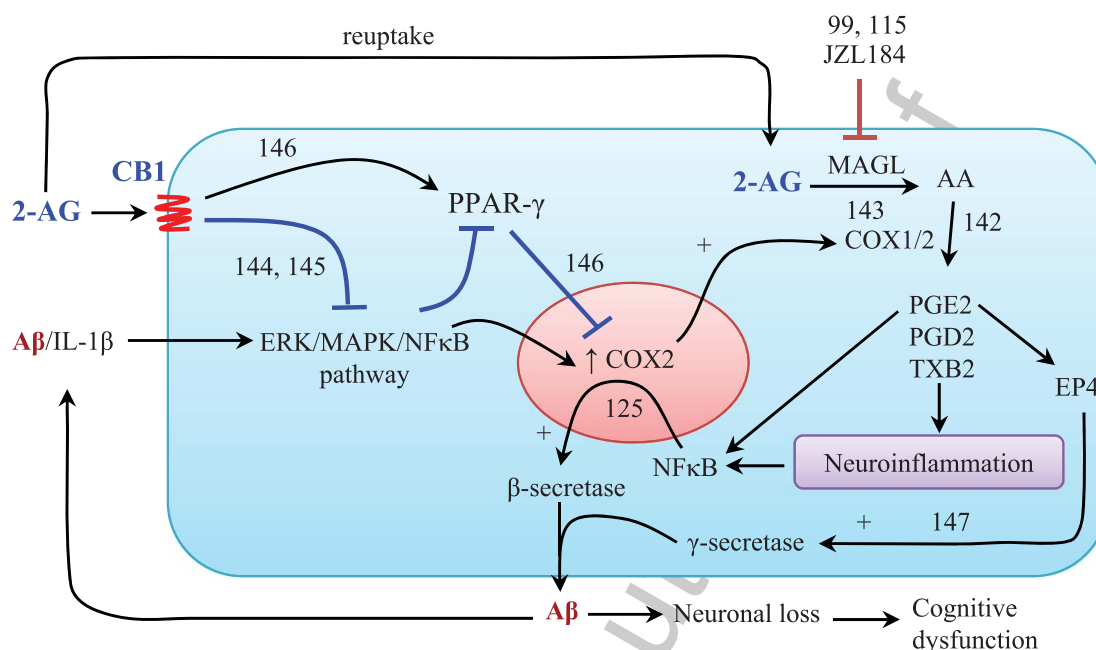


Fig. 2. Modulation of 2-AG signaling provides anti-inflammatory effects in AD. Through a CB₁-dependent mechanism, 2-AG increases PPAR- γ expression, which is suppressed by A β ₄₂ in AD. 2-AG directly, through CB₁ and PPAR- γ receptors, inhibits the expression of COX-2 and the synthesis of inflammatory 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 A β production. Inflammation activates the transcription factor NF κ B, for which β -secretase (BACE1) promoter harbors a highly conserved binding site that is functional. Thus, NF κ B activates BACE1 promoter, expression, and enzymatic activity leading to increased A β production. Prostaglandin PGE₂ stimulates the generation of A β through both EP2 and EP4 receptors (PGE₂ receptors). Activation of the EP4 receptor stimulates A β production through the endocytosis and the activation of γ -secretase. The inhibition of prostaglandin synthesis by MAGL inhibitors could suppress all these mechanisms.

667 enzymatic activity leading to increased A β production.
 668 The prostaglandin PGE₂ after production stimulates
 669 the generation of A β through both EP2 and EP4 receptors
 670 (PGE₂ receptors). Activation of the EP4 receptor
 671 stimulates A β production through endocytosis and
 672 activation of γ -secretase [147].

673 Neurodegeneration

674 A β has been shown to induce cell apoptosis in
 675 neuronal cells through a variety of mechanisms that
 676 include activation of caspase-3, lysosomal cathepsins,
 677 and lysosomal membrane permeabilization [17, 118].
 678 Cannabinoids at physiological concentrations increase
 679 lysosomal stability and integrity [148]. Noonan and
 680 colleagues showed that eCBs can stabilize lysosomes
 681 against A β permeabilization and can increase cell
 682 survival. eCBs prevented upregulation of tumor sup-
 683 pressor protein, p53, and reduced its interaction with
 684 lysosomal membrane [148]. Moreover, 2-AG and AEA
 685 prevented A β -induced increase in DNA fragmentation
 686 and caspase-3 activation [101]. Acute *in vivo* admin-

687 istration of A β increases 2-AG release in the brain
 688 suggesting that endogenous 2-AG plays an important
 689 role in protecting neurons from A β -induced toxicity
 690 [101].

691 Milton and colleagues [149] showed the neuropro-
 692 tective effects of eCBs (AEA and nodaline ether) on
 693 A β -induced neurotoxicity. These effects were medi-
 694 ated by CB₁ receptors and the MAPK pathway
 695 activation as suggested by the finding that CB₁ antago-
 696 nist and MAPK inhibitor blocked their neuroprotective
 697 effects. Another study confirmed the neuroprotective
 698 effect of AEA on A β -evoked neurotoxicity via a path-
 699 way unrelated to CB₁ and CB₂ [150]. In fact, selective
 700 CB₁ and CB₂ agonists were unable to protect neurons
 701 against A β challenge [150]. Further research revealed
 702 that increasing endogenous levels of 2-AG by MAGL
 703 inhibitor was able to protect hippocampal neurons from
 704 A β -induced neurodegeneration and apoptosis [145].
 705 Active caspase-3 levels are increased in AD [118]. CB₁
 706 agonist was also able to inhibit A β -induced activation
 707 of caspase-3 [145, 151]. CB₁ knock-out studies indi-
 708 cated that lack of CB₁ is associated with increased

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

711 *Oxidative damage and mitochondrial dysfunction*

712 Enhanced oxidative stress in brain generally corre-
713 lates with cognitive decline and with enhanced risk for
714 development of neurodegenerative diseases. Among
715 the different pro-inflammatory proteins produced in
716 response to A β -induced oxidative stress, iNOS and
717 its enzymatic product NO [105, 153] are considered
718 the most important neurotoxic effectors during AD.
719 In particular, methionine-35 of A β ₄₂ is critical for
720 oxidative stress (for more details, see [154]). NF κ B,
721 a redox-sensitive transcription factor that is activated
722 by a family of stress activated kinases (SAPK) includ-
723 ing p38 MAP kinase [122], regulates the expression
724 of different genes involved in cell differentiation,
725 proliferation, and apoptosis, as well as in oxidative,
726 inflammatory, and immune response [155]. As it is well
727 known, NF κ B activation is of primary importance
728 to induce iNOS protein transcription [156] both in
729 A β -stimulated neuronal cells [156] and in postmortem
730 AD brains [157]. It is well known that phytocannabi-
731 noids have anti-oxidant properties [158]. Cannabidiol
732 is a well studied cannabinoid in this context. It has
733 been shown that cannabidiol significantly decreases
734 glutamate toxicity, Ca⁺² toxicity, iNOS expression,
735 and NO production [131, 158]. Cannabidiol medi-
736 ates these effects through inhibition of p38 MAPK
737 and NF κ B pathways probably through involvement of
738 the PPAR- γ receptor [132, 133, 135]. Moreover, CB₁
739 agonists were also shown to decrease iNOS and NO
740 production [121, 131]. In another study, cannabidiol
741 treatment significantly decreased ROS, lipid perox-
742 idation, caspase-3 levels, DNA fragmentation and
743 intracellular calcium [156].

744 CB₁ receptors are also expressed on mitochon-
745 dria and regulate its activity [71]. Activation of
746 mitochondrial CB₁ receptors can decrease oxidative
747 metabolism, oxygen consumption, ROS production,
748 and oxidative phosphorylation [71, 159–161]. In
749 oxidative stress conditions, cannabinoids have shown
750 protective actions against mitochondrial damage and
751 have decreased Ca⁺²-induced cytochrome c release
752 from mitochondria (Fig. 1) [162–164].

753 *Memory and learning impairments*

754 CB₁-mediated effects of cannabinoids on learning
755 and memory have been reported for many years [165].
756 eCBs are involved in modulation of long-term plastic-

757 ity such as LTP [166], a cellular model of learning and
758 memory. Activation of CB₁ receptors on the GABAer-
759 gic neurons leads to a decrease in GABA release
760 [166] and thus to formation of the depolarization-
761 induced suppression of GABAergic inhibition (DSI).
762 Importantly, DSI temporarily removes GABAergic
763 inhibitory tone and facilitates LTP of pyramidal neu-
764 rons. It has been reported that A β strongly suppresses
765 LTP in hippocampal synapses and this is one of the
766 cause for observed learning and memory deficits in
767 AD [167]. Recently, Orr and colleagues demonstrated
768 a possible role of eCB signaling in A β -induced reduc-
769 tion in LTP and excitatory postsynaptic potential-spike
770 coupling (E-S) potentiation [168]. In this study, authors
771 showed that A β inhibits E-S potentiation through
772 suppression of CB₁-dependent synaptic disinhibition.
773 This effect is not a direct effect on excitatory synapses
774 but rather it is an indirect effect, which involves the
775 reduction of eCB mediated GABAergic disinhibition.
776 In another study, it has been shown that deletion of CB₁
777 receptors from the forebrain GABAergic, but not gluta-
778 matergic neurons, led to a neuronal loss and increased
779 neuroinflammation in the hippocampus as observed
780 in brain aging [169]. The same authors suggested
781 that CB₁ receptor activity on hippocampal GABAer-
782 gic neurons protects against age-dependent cognitive
783 decline by reducing pyramidal cell degeneration and
784 neuroinflammation [169].

785 Moreover, the consequences of CB₁ receptor defi-
786 ciency on development of AD pathology were studied
787 by knocking out CB₁ receptor in A β PP23 mice of
788 AD. A β PP23/CB₁^{-/-} mice showed worsen cogni-
789 tive deficits than A β PP23 mice, thus suggesting that
790 CB₁ deficiency can worsen AD-related learning and
791 memory deficits [111]. Moreover, an eCB re-uptake
792 inhibitor, VDM-11, reversed A β -induced hippocampal
793 damage and memory impairment in passive avoidance
794 test [101]. Further research in this field revealed that
795 cannabinoid treatment was able to prevent A β -induced
796 memory impairments in rats and that CB₁, but not
797 CB₂, receptors may be directly involved in improving
798 A β -induced memory impairments and intrinsic elec-
799 trophysiological properties of hippocampal pyramidal
800 neurons [151]. Fakhfour and colleagues [140] showed
801 that administration of the synthetic cannabinoid ago-
802 nist, Win55,212-2, significantly improved memory
803 functions and decreased the elevated levels of neu-
804 roinflammatory markers like TNF- α , active caspase-3,
805 and nuclear NF κ B. Antagonist experiment confirmed
806 that these neuroprotective effects of Win55,212-2 were
807 partially mediated by CB₁ and CB₂ receptors [140].
808 Through CB₁ receptor, Win55,212-2 increased PPAR-

809 γ pathway by increasing its transcription activity
810 and provided neuroprotection [140]. Furthermore, the
811 effects of cannabinoids were studied in transgenic
812 murine models of AD. Prolonged oral treatment of CB₂
813 receptor agonist (JWH-133) was able to improve cog-
814 nitive impairments and decrease microglial activation
815 in Tg2576 mice, while Win55,212-2 was ineffec-
816 tive [141]. Moreover, both cannabinoids significantly
817 reduced the expression of CB₂ receptor, TNF- α and
818 COX-2 suggesting a critical role of CB₂ in inflam-
819 matory processes in AD [141]. Recently, it has been
820 shown that long-term treatment with cannabidiol was
821 able to prevent the development of social recognition
822 deficits in the A β PP/PS1 mouse model of AD [170].
823 The authors further revealed that these effects were not
824 associated with decreased A β plaque load or oxida-
825 tive changes while they noticed subtle effects induced
826 by cannabidiol on neuroinflammation and cholesterol
827 levels [170]. Moreover, a different study conducted
828 on the same model showed that a combined treat-
829 ment with cannabidiol and Δ^9 -THC reduced learning
830 impairment, decreased soluble A β_{42} peptide levels and
831 caused a change in plaques composition [171].

832 However, there are few reports that do not support
833 beneficial effects of cannabinoids in AD treatment.
834 Chen and colleagues found that chronic administration
835 of the cannabinoid agonist HU-210 to A β PP23/PS45
836 double transgenic mice did not improve water maze
837 performance or a contextual fear conditioning task
838 [172]. HU-210 neither altered A β PP processing and
839 neuritic plaque formation nor enhanced hippocam-
840 pal neurogenesis in A β PP23/PS45 transgenic mice. It
841 has been reported that CB₁ blockade by rimonabant
842 improved A β -induced memory impairments in mice
843 tested in a passive avoidance paradigm. The authors
844 suggested that such memory improvement might be
845 due to the increased acetylcholine release in the brain
846 [173].

847 *Additional effects of cannabinoids*

848 Apart from aforementioned mechanisms, few
849 cannabinoids exert their therapeutic effects in sim-
850 ilar way of currently US-FDA approved drugs for
851 AD treatment. Most of the drugs currently used in
852 AD treatment (donepezil, rivastigmine, and galan-
853 tamine) are inhibitors of AChE. AChE is involved in
854 degradation of neurotransmitter acetylcholine (ACh),
855 which is reduced in AD [174]. Active component of
856 marijuana, Δ^9 -THC, has been demonstrated to com-
857 petitively inhibit AChE and to thus increase ACh
858 levels [175]. Moreover, Δ^9 -THC prevented AChE-

859 induced aggregation of A β which can reduce plaques
860 formation [175]. In addition to Δ^9 -THC, other CB
861 agonists also showed to have AChE and butyryl-
862 cholinesterase inhibition properties [176]. Alternative
863 strategies based on multiple targets such as CB recep-
864 tors and cholinesterase with single compound is
865 gaining acceptance for treatment of AD.

866 Besides AChE inhibitors, current AD treatment
867 includes memantine, a NMDA receptor antagonist,
868 which reduces excitotoxicity by inhibiting Ca⁺² influx.
869 In similar way, HU-211 (synthetic cannabinoid devoid
870 of CB₁ and CB₂ agonist activity) protects neurons
871 from excitotoxicity by antagonizing NMDA receptors
872 [177–179].

873 Moreover, recently it was demonstrated that eCBs
874 can modulate A β -induced alterations in Notch sig-
875 naling. Notch signaling plays a pivotal role in
876 neurodevelopment, and it is also involved in control of
877 neurogenesis, neuritic growth, synaptic plasticity, and
878 long term memory [180, 181]. In advance neurodegen-
879 eration, Notch signaling is reduced [180]. Long term
880 spatial deficits were observed in Notch mutant mice
881 [182]. It has been shown that A β negatively regulates
882 Notch-1 signaling by increasing expression of Numb,
883 the endogenous negative regulator of Notch-1 cleav-
884 age [183]. Interestingly, AEA, through CB₁ receptors,
885 was able to reverse this effect by increasing expres-
886 sion of Notch-1 signaling components like nicastrine,
887 Notch intracellular domain, Hes1 and Hes5 (see Fig. 1).
888 Moreover, AEA and 2-AG were also able to inhibit
889 A β -induced expression of Numb [183].

890 Furthermore, cannabinoids could provide beneficial
891 effects by modulating cerebral blood flow functions.
892 AD is characterized by a decreased regional cerebral
893 blood flow that could result in decrease brain sup-
894 ply of oxygen, glucose, and nutrients. Cannabinoids
895 can improve blood flow to the brain as CB₁ receptor
896 activation can elicit vasodilatation [184]. Moreover,
897 as discussed earlier, cannabinoids can increase A β
898 clearance at blood brain barrier [112]. CB₂ receptor
899 activation has been shown to improve blood-brain bar-
900 rier integrity by decreasing adhesion of leukocytes to
901 endothelial cells under inflammatory conditions [185],
902 which may reduce further exaggeration of inflamma-
903 tion.

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

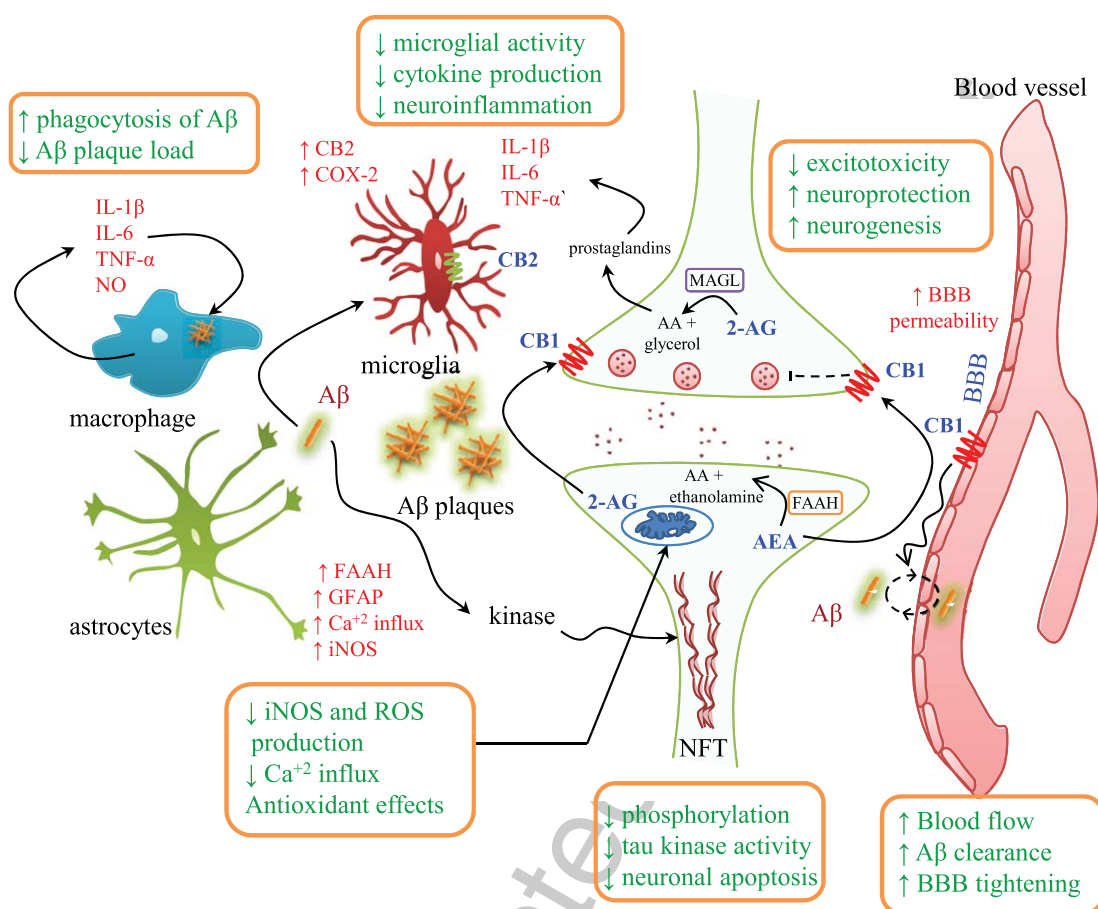


Fig. 3. Schematic diagram showing the beneficial effects of cannabinoid treatment in AD. Cannabinoid treatment can modulate multiple disease processes, which could reduce A β and phosphorylated tau deposition, neuroinflammation, oxidative damage, microglial activation, and excitotoxicity. Moreover, it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

911 and anxiety-related disorders (for more details, see
912 [190]). Furthermore, CB1 agonists may worsen AD by
913 inhibiting acetylcholine release in the brain [7]. CB2
914 agonist and inhibitors of endocannabinoid deactivating
915 enzymes seems to be devoid of such side effects.
916 Therefore, much attention has been focused on this
917 kind of compounds as potentially useful for the AD
918 treatment.

919 CONCLUSIONS

920 The advances in AD research in the last decade
921 have revealed that this disease is multifaceted in nature
922 and is linked to different multiple mechanisms in the
923 brain. A novel, more effective therapeutic approach
924 for AD treatment should target multiple mechanism
925 of disease progression. A large body of evidence sug-
926 gested the involvement of the eCB system in the
927 neurodegenerative process associated with AD. A β

928 deposition in the brain is linked to significant changes
929 in the expression pattern of CB2 receptors and FAAH
930 enzyme. CB2 receptors and FAAH are selectively and
931 abundantly overexpressed in microglia and astrocytes,
932 respectively, in vicinity of A β neuritic plaques.
933 AEA and its precursor NarPE levels are decreased in frontal
934 cortex. In contrast, 2-AG degrading enzymes MAGL
935 and ABHD6 activity is reduced in plaques and sur-
936 rounding area. Over all AEA signaling is lowered and
937 2-AG signaling is increased in the vicinity of plaques.
938 CB1 receptors expression in AD is still controversial
939 and brain region specific. Although results of different
940 groups are sometimes conflicting, a decline in the eCB
941 system activity in AD is probable.

942 This review proposes cannabinoids as potential
943 therapeutics, which can target simultaneously neuro-
944 degeneration, neuroinflammation, oxidative dam-
945 age, cognitive impairments, and clearance of A β from
946 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 [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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