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EXPERT OPINION

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The therapeutic potential of the endocannabinoid system for Alzheimer's disease

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Introduction: Dementia currently affects over 35 million people worldwide. The most common form of dementia is Alzheimer's disease (AD). Currently, treatments for AD do not stop or reverse the progression of the disease and they are accompanied by side effects.

Areas covered: The main features of AD pathology, treatment options currently available, the endocannabinoid system and its functionality in general and its role in AD pathology in detail will be outlined. A particular focus will be on the therapeutic potential of the phytocannabinoid cannabidiol.

Expert opinion: Based on the complex pathology of AD, a preventative, multimodal drug approach targeting a combination of pathological AD symptoms appears ideal. Importantly, cannabinoids show anti-inflammatory, neuroprotective and antioxidant properties and have immunosuppressive effects. Thus, the cannabinoid system should be a prime target for AD therapy. The cannabinoid receptor 2 appears to be a promising candidate but its role in AD has to be investigated cautiously. Furthermore, the phytocannabinoid cannabidiol is of particular interest as it lacks the psychoactive and cognition-impairing properties of other cannabinoids. In conclusion, future research should focus on the evaluation of the effects of manipulations to the endocannabinoid system in established animal models for AD, combined with early-phase studies in humans.

Keywords: Alzheimer's disease, cannabidiol, cannabinoid receptor 2, cannabinoids, FAAH inhibition, inflammation, neurodegeneration, oxidative stress

Expert Opin. Ther. Targets [Early Online]

1. Introduction

As the world's population ages and life expectancy increases, many individuals are faced with an increased risk of developing dementia. In 2011, over 260,000 Australians and around 35 million people worldwide were affected by dementia. The most common form of dementia is Alzheimer's disease (AD), which is predicted to affect 1 in 85 people globally in 2050. AD is classified into three progressive clinical stages: mild, moderate and severe [1]. The mild stage encompasses short-term memory loss, subtle deficits in learning and communication and spatial disorientation. Memory decline in the moderate stage (e.g., pronounced decline of recent memory, impaired writing and reading skills) begins to affect everyday tasks and emotional control and the severe stage is characterized by a global disruption of cognitive abilities, severely impaired speech, inability to recognize familiar people and loss of control over functioning of the body. Eventually, individuals are in a weakened physical state where they are prone to other illnesses (e.g., infections). Most pharmacological interventions for AD are only effective in the early stages of the disease, do not reverse the progression of AD and are accompanied by side effects. Importantly, there is no curative treatment available for AD. Therefore, it is necessary to explore new therapeutic avenues. The cannabinoid system appears

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Article highlights.

- The complexity of Alzheimer's disease (AD) pathology and the limitations of current treatment options require new targets for research into AD therapeutics.
- The cannabinoid system appears to be a prime candidate based on its immunosuppressive, anti-inflammatory, neuroprotective and antioxidant characteristics.
- Past research and the non-psychoactive nature suggest cannabinoid receptor 2 (CB_2) manipulations and cannabidiol (CBD) as relevant targets for novel strategies for research into treatment of AD.
- Long-term, preventative strategies should be investigated using well-established multi-factorial preclinical animal models before double-blind placebo-controlled human clinical trials are carried out to assess the therapeutic utility of the most promising compounds for AD.

This box summarizes key points contained in the article.

to be a promising therapeutic target as it has the ability to modulate a range of aspects of AD pathology.

2. Pathology of Alzheimer's disease

AD can be classified as sporadic or familial. Most AD cases are sporadic (late onset) accounting for the majority of AD cases (~ 95%) whereas familial AD is the hereditary form (early onset, autosomal dominant) causing the remaining AD cases [2]. The factors responsible for sporadic AD remain to be fully elucidated. However, a great deal of information has been gained in the analysis of genetic risk factors. Apolipoprotein E (APOE) genotype is by far the most robust predictor of AD risk with the $\varepsilon 4$ allele affording increase risk and the $\varepsilon 2$ allele granting protection as compared with the most common $\varepsilon 3$ allele. Recent genome-wide association studies (GWAS) have confirmed the importance of APOE in AD risk and also identified several additional genetic risk factors, many of which are, like APOE, related to lipid homeostasis [3].

Familial AD is caused by mutations in the amyloid precursor protein gene (*APP*) or in genes encoding presenilins, a family of enzymes responsible for the processing of APP. Presenilin 1 and 2 (*PSEN1*, *PSEN2*) are responsible for the activity of γ-secretase, one of the enzymes responsible for the cleavage of APP into β-amyloid peptides (Aβ). Mutations in *PSEN1* or *PSEN2* cause the most common and aggressive forms of familial AD. In the amyloidogenic pathway, a two-step cleavage of APP by β-secretase and then γ-secretase results (predominantly) in the production of $A\beta_{40}$ and $A\beta_{42}$. Mutations in *APP* or APP-processing enzymes lead to overproduction of $A\beta_{42}$, resulting in Aβ depositions into amyloid plaques and rapid progression of AD [4].

2.1 Neuropathological hallmarks of AD

AD is a neurodegenerative cognitive disorder with an inflammatory component. The first hallmark of AD is the presence of extracellular Aβ deposits in AD brains due to accumulation of non-soluble fragments of APP. Aβ deposits form neuritic plaques in predominantly limbic regions. Amyloid plaques are thought to trigger local inflammatory responses, in which astrocytes and in particular microglia play a crucial role [5]. Importantly, an increased level of Aβ in the brain is correlated with AD-typical cognitive decline [6]. Aβ-induced neurodegeneration also causes long-term disruptions to various neurotransmitter systems: i) elevated levels of glutamate have been detected in the cerebrospinal fluid (CSF) of AD patients [7] and ii) a loss of cholinergic neurons in brain areas relevant for memory processing (i.e., amygdala, hippocampus and frontal cortex) and the accompanying decrease in acetylcholine (ACh) are major neurochemical deficits in AD pathology [8]. Importantly, ACh plays a role in cortical development and activity and the modulation of cognition, learning and memory.

The second hallmark of AD is the hyperphosphorylation of the cytoskeletal microtubule-associated protein tau [9], Tau phosphorylation promotes its aggregation leading to the formation of intracellular neurofibrillary tangles (NFT), thereby impairing intra-neuronal communication. The accumulation of tau and associated NFTs correlates with neurodegeneration and induces cognitive deficits [10]. The amyloid cascade hypothesis suggests that Aβ deposits may be responsible for the build-up of NFTs. Finally, neuritic plaques and NFTs provoke loss of functional synapses and subsequent degeneration of nerve cell bodies in the hippocampus and cortex resulting in a further decline of cognitive abilities and memory.

2.2 Neuroinflammation and neurotoxicity in AD

As aforementioned, AD is also characterized by a neuroinflammatory response involving the activation of astrocytes and microglia. The amyloid cascade-neuroinflammation hypothesis is based on the observation that activated microglia cluster at sites of Aβ depositions (especially at advanced senile neuritic plaques) due to i) the presence of Aβ itself or ii) concurrent neurodegenerative processes [11,12]. This extension to the amyloid cascade hypothesis suggests that microglia, following activation by Aβ, differentiate into phagocytic cells, which then ingest Aβ and secret pro-inflammatory cytokines (e.g., IL-1β, IFN-γ and TNF-α) and chemokines, thereby causing pronounced neuroinflammation and local tissue damage in the proximity of pathological structures [13]. Ironically, this enhances the production of Aβ even further [14]. These brain-damaging effects are partially caused by a pronounced release of glutamate and consequential excito-neurotoxicity [15]. Aβ may also direct blood-born cells (e.g., activated T cells) to amyloid plaques directly, further potentiating local inflammatory cascades and neurotoxicity [5].

Importantly, production of pro-inflammatory cytokines and chemokines and the activation of the complement cascade have been observed in AD patients [16] and post-mortem analysis of

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inflammatory markers is correlated with synaptic loss in AD brain tissue [11]. The greatest atrophy and highest concentration of senile plaques was found in brain regions that show a cascade of immunological events early in the progression of the disease [17]. Furthermore, the brain regions showing high levels of inflammation coincide with regions thought to be responsible for the memory impairments observed in the early stages of AD [18]

Interestingly, human studies suggest that there is also a pronounced defect in innate immunity in AD, which impairs Aβ phagocytosis [19]. Under normal conditions, the presence of Aβ activates microglia, which secrete pro-inflammatory enzymes for the removal of Aβ. In AD, the clusters of activated microglia seem to be incapable of completely removing Aβ, which is thought to be caused by an impaired phagocytic or clearance ability. This in turn allows Aβ plaques to develop along with the build-up of inflammatory cytokines that contribute to the pronounced inflammation and neurotoxicity seen in AD [12]. Thus, while the products of microgliosis may be locally toxic, stimulation of microglial phagocytosis of Aβ may be a reasonable goal of preventative immune therapy intervention in AD to inhibit plaque formation [16]. Interestingly, antibody treatment of *APP* transgenic mice reduced plaque load by inducing microglial phagocytosis [20] and active immunization with Aβ stimulated microglial phagocytosis and slowed cognitive decline [21,22]. Instead of all or nothing anti- or pro-inflammatory immune therapies, a balanced immune-modulation might be required for AD therapy (i.e., immune activation of microglia can clear plaques whereas chronic neuroinflammation can cause neuronal death/dysfunction) [23].

2.3 Oxidative stress in AD

Oxidative stress can be induced by Aβ, activated microglia and altered mitochondrial functioning [24]. Microglia are a source of reactive oxygen species (ROS). Extracellular ROS are highly neurotoxic thereby inducing oxidative damage, while intracellular ROS are crucial for pro-inflammatory functioning [25]. Studies have found prominent ROS-mediated injuries especially in regions with high senile plaque and NFT load and increased lipid peroxidation in AD brains [26,27]. Furthermore, upregulation of inducible nitric oxide synthase (iNOS) has been found in senile plaques of AD brains [28] and in microglia after Aβ administration *in vivo* [29]. Interestingly, myelin breakdown can be a consequence of oxidative stress and is an early and largely unrecognized feature of AD [30]. It causes decreased neurotransmission and may contribute to the onset of AD.

In the following sections, this review outlines treatment options currently available for AD patients, characterizes the endocannabinoid system and its functionality (with a focus on AD-relevant pathways) and describes the role of the endocannabinoid system in AD pathology. A particular focus is on the therapeutic potential of the non-psychoactive phytocannabinoid cannabidiol.

3. Current therapeutic strategies for Alzheimer's disease

Given the looming burden of AD, pharmacological regimens that could delay or even prevent the onset of AD would offer tremendous public health benefits. The slow progression of AD pathology and the characteristics of clinical stages suggest that a variety of treatment strategies over the course of the disorder would be ideal [31]. Interventions with neuroprotective agents should aim to slow or attenuate the progress of the early stages of AD to delay or avoid the later, symptomatic stages of the disease (primary intervention). Effective interventions in the mild to moderate forms of AD should attempt to slow or avert the progression from irritating mild symptoms to disabling dementia syndrome (secondary prevention). Finally, in the severe stages of AD, it is important to i) boost cognitive abilities and ii) prevent any further progression of AD symptoms and death of the patient. Furthermore, based on the complex pathology of AD, a preventative, multimodal drug approach that is able to target a combination of pathological AD symptoms would be ideal. The main treatment strategies currently available do not reverse or stop the progression of the disease and only relieve certain cognitive symptoms [11,32,33]. In the following sections, the current treatment options are discussed.

3.1 Acetylcholinesterase (AChE) inhibitors

AChE inhibitors (e.g., donepezil, rivastigmine, galantamine) are used to increase the cholinergic tone in AD patients. AChE inhibitor treatment has been shown to improve the cognitive performance as well as activities of daily living but only in patients with mild to moderate forms of AD [34] and only short-term (9 – 12 months to 5 years). Donepezil and galantamine have also been documented to inhibit NO-induced cytotoxicity (via production of radicals and mitochondrial dysfunction) and counteract neuronal cell death [35], which may have contributed to their initial treatment success. Potential side effects include diarrhea, nausea, vomiting, insomnia, fatigue and dizziness. Estrogen replacement therapies have been considered as well as they preserve cholinergic activity (and influence Aβ metabolism and oxidative stress [1]). However, the effects seen in observational and treatment studies are limited and require further investigations.

3.2 NMDA receptor antagonism

Evidence suggests both hypoactivity (i.e., cognitive deficits) and hyperactivity (i.e., neurotoxicity and neuronal cell death) of the glutamatergic system in AD patients [36]. The non-competitive NMDA receptor antagonist memantine is the only approved anti-glutamatergic compound to date and is used to reduce glutamate excitotoxic neurodegeneration. Importantly, memantine has no effect on cognitive function but reduces the decline in quality of daily living in moderate to severe stages of AD [37]. Another study suggests its

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effectiveness in the early stages of AD (mild to moderate) with limited efficacy after a few years [38]. However, a meta-analysis could not confirm efficacy of memantine treatment for mild AD [39]. Some of the reported side effects include hallucinations, dizziness and tiredness.

3.3 Anti-inflammatory drugs

NSAIDs have been employed to target neuroinflammation events in AD patients. These drugs inhibit COX enzymes, with older NSAIDs (e.g., ibuprofen and aspirin) non-selectively targeting COX-1 and COX-2 isoforms where as newer agents (e.g., celecoxib and rofecoxib) are COX-2-specific. Interestingly, the COX-2 isoform is upregulated at sites of local inflammation and correlates with Aβ levels in AD brains [31]. Suppression of COX-2 may also modulate consequences of excessive glutamatergic stimulation and reduce the production of superoxide and other free radicals by COX enzymes [31]. Ibuprofen has been shown to decrease plaque density, total Aβ burden and markers of glia cell activation in transgenic mouse models of AD [40]. Epidemiological studies and meta-analysis suggest that NSAIDs may offer some protection against AD, however, only to a point several years prior to the appearance of diagnosable dementia (primary intervention) [31]. Importantly, NSAIDs can cause serious side effects when used at high doses or over prolonged periods (e.g., renal and gastro-intestinal complications) [31].

3.4 Antioxidant treatment

To counteract the neurodegenerative effects of oxidants produced by microglia in AD, several antioxidative molecules are currently under study (e.g., vitamin E, vitamin C and estrogen). Antioxidants might have the capacity to prevent AD and to have continuous beneficial effects after the clinical onset of the disease [1] but prospective, randomized, controlled clinical trials are needed to validate antioxidants further. Importantly, no cognitive benefits of such treatments have been reported so far and vitamin E was found to increase the risk of morbidity and mortality [41].

3.5 Anti-Aβ therapies

As Aβ is the main constituent of neuritic plaques and has the capability to trigger a cascade of events leading to the death of neurons and the loss of functional synapses, a beneficial effect of the reduction in Aβ burden has been postulated. As mentioned earlier, Schenk and co-workers reported that active immunization with Aβ stimulated microglial phagocytosis in AD mice [22] and slowed cognitive decline in AD patients [21]. However, human trials have encountered secondary effects (i.e., encephalitis) that have halted the development of anti-Aβ treatments [42].

3.6 Therapeutics targeting vascular function

As discussed above, risk factors for late-onset AD are related to lipid metabolism and as such also to vascular disease. A large number of studies have therefore focused on interventions such as lipid-lowering drugs (principally stains), antihypertensive and anti-inflammatory drugs and several vitamins (principally B-group) as a means of preventing AD. A description of all of these avenues is beyond the scope of this review but this area has been covered in a recent publication [43].

4. The endocannabinoid system

Cannabinoids are a class of diverse chemical compounds that activate cannabinoid receptors (CB). Endocannabinoids (eCBs) are produced naturally in the body, phytocannabinoids [e.g., $\triangle^9$-tetrahydrocannabinol (THC) and cannabidiol (CBD)] are produced by the *Cannabis sativa* plant and cannabimimetics (e.g., CP55,940 and WIN55,212-2) are produced synthetically. The eCBs are endogenous lipid signaling molecules, which are synthesized on demand post-synaptically either stimulated by membrane depolarization in neurons or by immune cell activation [44]. The eCBs N-arachidonoylethanolamine (anandamide) and arachidonoylglycerol (2-AG) bind to the G-protein coupled cannabinoid receptors 1 and 2 (CB_1 and CB_2). Anandamide also activates the transient receptor potential vanilloid type 1 (TRPV1) channel. CB_1 is expressed in the brain in largely pre-synaptic neurons (i.e., highest levels in cerebral cortex, hippocampus, basal ganglia and cerebellum) and in peripheral neural tissue and organs. CB_2 is abundant in immune cells (e.g., macrophages and T cells) and is also highly expressed by activated microglia in the CNS. Importantly, most studies report that CB_2 is devoid of the psychotropic effects linked to CB_1 stimulation [45] but further research will have to clarify its behavioural properties in more detail. The intracellular enzymes monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH) metabolize 2-AG and anandamide respectively [46]. The actions of FAAH on 2-AG metabolism are under debate. The biosynthesis of 2-AG utilises diacylglycerol lipase (DAGL: α and β) whereas the enzymes responsible for the biosynthesis of anandamide require complete characterization but a N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) appears to be involved [46]. More recently, further eCBs have been discovered including noladin-ether and virodhamine [47].

5. Functions of the endocannabinoid system

Cannabinoids appear to be involved in the control of the immune response, have neuroprotective abilities and modulate inflammation through CB_1 and CB_2 [32]. The stimulation of immune cells with eCBs has immunosuppressive effects [16]: for example, WIN55,212-2 decreased IL-1β-induced production of TNF-α and chemokines via the stimulation of both CB_1 and CB_2 [48]. Importantly, selective CB_1 stimulation modulates the production of pro-inflammatory cytokines, which are associated with neurodegenerative processes

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(e.g., IL-1, IL-6, and TNF-α,), and increases the production of anti-inflammatory molecules (e.g., IL-10), trophic factors and neurotrophins that could prevent neuronal death in an inflammatory milieu (for review see [49]). However, CB_1 agonism also has pro-inflammatory effects as THC promoted hypertrophy, macrophage infiltration and increased expression of TNF-α in adipose tissue of rats [50]. The conflicting nature of the consequences of CB_1 stimulation on inflammatory processes suggests careful investigations into the benefits of CB_1-selective cannabinoids for AD therapy. Importantly, several reports indicate that CB_1 expression patterns might be dissociated from AD pathology or even unchanged in AD brains and that CB_1-independent mechanisms accompany the neuroprotective effects of cannabinoids [47].

A number of studies suggest anti-inflammatory properties for CB_2. Already high levels of CB_2 expression on microglia increase further under neuroinflammatory conditions [32]. 2-AG-induced migration of microglia occurs through CB_2 [51] and CB_2 stimulation by the synthetic CB_2 agonist JWH-015 attenuated microglial phagocytosis and the production of TNF-α [52]. The neuroprotective and anti-inflammatory abilities of CB_2 were confirmed *in vitro*, as CB_2-deficient T cells in the CNS exhibited reduced apoptosis, increased proliferation and enhanced production of inflammatory cytokines [53]. Furthermore, THC-induced inhibition of T cell activation was absent in CB_2-deficient mice [54]. These findings suggest CB_2 as a feedback inhibitor of immune responsiveness in the CNS [47]. Importantly, the specific actions of CB_2 on microglia are still somewhat unclear: pro- as well as anti-inflammatory effects have been reported (further details in [16,31]). Thus, CB_2 might have the capacity to play a role in a balanced immune-modulation as suggested earlier. Comprehensive studies on the characteristics of the role of CB_2' in inflammation are required.

Further evidence for neuroprotective characteristics of the eCB system comes from studies showing that 2-AG reduced brain edema and hippocampal cell death and was elevated in traumatized hemispheres in a mouse model of closed head injury [55]. Blockade of eCB transport (by AM404) and degradation (i.e., FAAH inhibition by AM374) also inhibited excitotoxic brain damage and associated memory impairments *in vitro* and *in vivo*. Stimulation of CB_1 (by R-methanandamide: AM356) produced similar effects although other receptors might be involved in these effects of FAAH inhibition as well [56].

Cannabinoids are neuromodulators that inhibit presynaptic neurotransmitter release. The non-psychotropic synthetic cannabinoid HU-211 (i.e., a cannabinoid derivative, which does not act at cannabinoid receptors but instead has NMDA receptor antagonistic effects) attenuated glutamate action by specifically blocking NMDA receptors [57]. Furthermore, THC inhibits AChE by binding its peripheral anionic site, which is the critical region involved in amyloidgenesis [58]. This suggests therapeutic implications for cannabinoids in AD beyond their anti-inflammatory and neuroprotective effects.

6. Endocannabinoid regulation of memory function

The role of cannabinoids in learning and memory appears to be complex. *In vitro* experiments report that eCBs promote changes in neural activities related to memory with a positive effect on long-term potentiation and depression [59]. A potentially beneficial role of eCBs in cognition has also been suggested by studies investigating the cognitive effects of manipulating the synthesis and metabolism of eCBs: FAAH inhibitors (i.e., URB597) and FAAH knockout in mice enhance working memory and the acquisition of passive avoidance learning [60,61].

However, our own work found that pharmacological FAAH inhibition (or THC treatment) impaired the cognitive flexibility of rats, which is a CB_1-mediated phenomenon [62]. Furthermore, exogenous cannabinoids such as the phytocannabinoid THC and the synthetic cannabinoid CP55,940 caused impairments in spatial learning and memory in rats [63]. CB_1 expression in the hippocampus was vital to the memory impairing effects of THC as intra-hippocampal injection of the CB_1 antagonist SR141716 reversed these working memory deficits [63]. Furthermore, THC's ability to decrease working memory performance correlated with reduced ACh release in the hippocampus [64,65].

Indeed, the abundance of CB_1 expression in the hippocampus and the parahippocampal and enthorinal cortices suggests the involvement of this receptor in the mediation of cannabinoid effects on learning and memory [47]. Interestingly, both enhanced and impaired cognitive performance has been described for CB_1 knockout mice [66,67], whereas CB_2 knockout mice displayed impairments in both short and long-term memory consolidation [68].

The current research does not suggest that targeting CB_1 in isolation would be helpful in treating AD-related cognitive impairments. Even more so as CB_1 activation affects ACh levels negatively, which affect long-term potentiation, a process critical for learning and memory [69,70]. Thus, comprehensive analyses of the effects of the different types of cannabinoids on CB_2 are needed to evaluate the potential of the eCB system for rescuing AD-relevant cognitive deficits.

7. The endocannabinoid system in Alzheimer's disease

There is a growing body of evidence that the eCB system is implicated in the regulation of events occurring during the course of AD progression, particularly in the regulation of Aβ clearance, inflammation, oxidative stress and ACh homeostasis (for overview see **Table 1** and [11,16,32,33,47,71-73]). The eCB system is activated in the pathology of AD, which is suggested to be an anti-inflammatory response of the CNS to protect neurons from degeneration. Farooqui and co-workers described a tissue selective upregulation of the 2-AG-biosynthesizing DAGL-α in the hippocampus of AD

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patients [74] suggesting upregulated 2-AG levels. This finding was confirmed in animal models of Aβ-induced brain damage [75,76]. Inhibition of endocannabinoid cellular reuptake reversed neuronal damage and prevented amnesia (only after early but not late enhancement of eCB tone) [76]. Furthermore, impaired recruitment of MALG in post-mortem AD tissue has been described, suggesting that disease progression slows the termination of 2-AG signaling [77]. Another study found reduced cortical levels of anandamide in AD patients, which were correlated with cognitive impairments and levels of neurotoxic $A\beta_{42}$ (but not $A\beta_{40}$ or amyloid plaque load or tau hyperphosphorylation) [78].

Increased activity of FAAH has been demonstrated in regions of Aβ-enriched neuritic plaques in AD patients and enhanced levels of FAAH have been found in astrocytes surrounding neuritic plaques [79,80]. Upregulated FAAH activity may actually not only affect the eCB tone but also be detrimental for AD directly, as FAAH located in astrocytes can metabolize eCBs into arachidonic acid, resulting in the increased production of prostaglandins and related pro-inflammatory mediators in the vicinity of senile plaques [16,47]. In this context, the inhibition of FAAH activity could be beneficial in the prevention of inflammatory processes and neurotoxicity associated with Aβ. Furthermore, the recent development of dual FAAH–AChE inhibitors is in line with the concept of a multimodal drug therapy approach in AD [81].

CB_2 appears to mediate at least some of the functions of the eCB system in AD. Increased CB_2 expression has been demonstrated in regions of Aβ-enriched neuritic plaques [79,80] and neuritic-plaque-associated microglia [76]. Furthermore, CB_2 stimulation by synthetic cannabinoids (i.e., JWH-015 and JWH-133) enhanced Aβ phagocytosis *in vitro* [52], blocked Aβ-induced activation of microglia [82] and induced removal of Aβ by human macrophages [83]. Finally, Aβ provoked upregulation of CB_2 expression (and 2-AG levels) and selective CB_2 antagonism (by SR144528 treatment) blunted Aβ-induced reactive astrogliosis [75].

The link between CB_1 and AD is less well established. Studies found reduced expression of CB_1 in areas of activated microglia and in the hippocampus and frontal cortex of AD patients [82]. However, the hippocampal reductions were dissociated from AD pathology and rather attributed to generalized aging processes. Furthermore, Benito and co-workers did not detect altered CB_1 expression in neuritic plaque-associated astrocytes and microglia [79]. Nevertheless, hippocampal atrophy is one of the promising diagnoses for AD [84] and there is a link between the AD risk allele *APOE4* and hippocampal atrophy [85]. *In vitro*, anandamide and noladin ether inhibited Aβ-induced neurodegeneration by CB_1-mediated mechanisms [86]. CB_1 was also involved in the WIN55,212-2-induced downregulation of iNOS levels in Aβ-stimulated C6 cells and consequential inhibition of tau hyperphosphorylation in PC12 neuronal cells [the synthetic CB_1 agonist arachidonyl-2-chloroethylamide (ACEA) produced similar effects] [87]. Animal model research found that WIN55,212-2 was able to prevent Aβ-induced cognitive deficits and microglia activation through CB_1 and CB_2 [82] and THC to decrease AChE-induced Aβ aggregation with higher potency than classic drugs such as donepezil [58]. Furthermore, Aβ provoked downregulation of CB_1 and a reduction in anandamide levels [75] and selective CB_1 agonism blunted Aβ-induced reactive astroglial cells [87]. In this context it is interesting to note that CB1 antagonism prevented Aβ-induced amnesia in mice [88] confirming the complexity of the nature of involvement of CB_1 in AD.

The few human studies on the effects of cannabinoids on AD patients revealed that THC had a positive effect as an appetite stimulant and antiemetic, increased body weight and improved disturbed behaviours in AD patients [89], although patients also experienced adverse effects such as tiredness and euphoria. Another study found that low-dose THC was effective in improving several clinical parameters including nocturnal motor activity and agitation, without undesired side effects [90]. Finally, a case report suggested the possible usefulness of THC in a 72-year-old woman as THC treatment reduced agitation and aggressiveness. Remarkably, this effect was rapid and dramatic, rendering better results than those observed with other medications [91]. Importantly, no cognitive effects were observed and the role of CB_1 and CB_2 in mediating the described effects is unknown. Unfortunately, no investigations into the specific effects of FAAH inhibition or CB_2 modulations in AD patients have been carried out to date (for overview see **Table 1**).

8. The phytocannabinoid cannabidiol

The therapeutic potential of cannabidiol (CBD) has received increasing attention over the last few years [92-96]. CBD has little affinity for cannabinoid receptors and the absence of intrinsic effects of CBD on CB_1 is likely to be responsible for CBD's non-psychoactive traits [97]. Recent evidence suggests it may be an antagonist/inverse agonist at CB_1 and CB_2. CBD also activates a myriad of receptor proteins including the abnormal-cannabidiol sensitive receptor, TRPV1 and 5-hydroxytryptamine$_{1A}$ receptors but the pharmacological relevance of this requires further clarification [92]. CBD has also been shown to inhibit FAAH expression in a mouse model for sepsis [98].

CBD has neuroprotective, anti-inflammatory and antioxidant properties, which are proposed to be mostly CB_1- and CB_2-independent [99] (for reviews see [92-96]). *In vitro*, CBD exhibited antioxidant properties and was neuroprotective against glutamate neurotoxicity through mechanisms independent of cannabinoid receptors [100,101]. Furthermore, CBD protected against hippocampal and entorhinal cortical neurodegeneration in a rat model of binge ethanol-induced neurotoxicity [102]. *Ex vivo*, CBD reduced IFN-γ production, TNF-α release and suppressed lymphocyte proliferation and reactive oxygen burst in a murine collagen-induced arthritis model [103]. Similar anti-inflammatory and antioxidant

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Table 1. Overview on role of the endocannabinoid (eCB) system in Alzheimer's disease (AD) (for reviews see [11,16,32,33,47,71-73]).

Study subject	Finding	Ref.
eCBs in the pathology of AD patients and *in vivo* and i*n vitro* AD models		
AD patients	Tissue-selective upregulation of the 2-AG-biosynthesizing DAGL-α in the hippocampus	[74]
Rat ($A\beta_{42}$ treatment)	Elevated levels of 2-AG and CB_2 Decreased levels of anandamide and CB_1	[75]
Rat ($A\beta_{42}$ treatment)	Elevated levels of 2-AG in the hippocampus VDM-11 (eCB reuptake inhibitor) reversed hippocampal damage and loss of memory retention	[76]
AD patients	Expression of CB_1 and CB_2 in senile plaques Reduced number of CB_1 in areas of microglia activation and in AD brain tissue	[82]
AD patients	Impaired recruitment of MAGL in post-mortem AD brain tissue	[77]
AD patients	Reduced cortical levels of anandamide correlated with cognitive impairments and levels of neurotoxic $A\beta_{42}$ (but not $A\beta_{40}$ or amyloid plaque load or tau hyperphosphorylation)	[78]
AD patients	Selective and abundant expression of FAAH and CB_2 in neuritic plaque-associated astrocytes and microglia (CB_1 unchanged)	[79]
Teratocarcinoma cells	Anandamide inhibited Aβ-induced neurodegeneration by CB_1-mediated mechanisms	[86]
Microglia cells	Cannabinoids (HU-210, WIN55,212-2, JWH-133) block Aβ-induced microglia activation	[82]
Macrophage cell line	CB_2 stimulation induced removal of Aβ by human macrophages	[83]
Microglia cells	CB_2 stimulation by JWH-015/JWH-133 enhanced Aβ phagocytosis	[52]
C6 rat glioma cells and PC12 neuronal cells	WIN55,212-2 induced downregulation of iNOS levels in Aβ-stimulated C6 cells and consequential inhibition of tau hyperphosphorylation in PC12 neuronal cells (similar results with CB_1 agonist ACEA)	[87]
Rat ($A\beta_{42}$ treatment)	CB_1/CB_2 stimulation by WIN55,212-2 blocked Aβ-induced microglia activation, cognitive impairment and loss of neuronal markers	[82]
Mouse (Aβ fragments, $A\beta_{25-35}$ or $A\beta_{42}$)	CB_1 antagonism by SR141716A prevented Aβ-induced amnesia	[88]
Rat ($A\beta_{42}$ treatment)	CB_2 blockade blunted Aβ-induced reactive astrogliosis	[75]
CBD in *in vivo* and *in vitro* models		
PC12 neuronal cells ($A\beta_{42}$ treatment)	CBD increased cell survival of PC12 neuronal cells after Aβ challenge and decreased ROS production and lipid peroxidation	[111]
PC12 neuronal cells ($A\beta_{42}$ treatment)	CBD inhibited Aβ-induced tau hyperphosphorylation as well as expression and production of iNOS and IL-1β	[112,113]
N13 microglia cells and rat primary microglia	CBD promoted microglia migration	[114]
Mouse ($A\beta_{42}$ treatment)	CBD attenuated Aβ-evoked neuroinflammatory responses (i.e., reduced protein expression of glial fibrillary acidic protein, iNOS and IL-1β and the release of NO and IL-1β)	[100]
Mouse ($A\beta_{42}$ treatment)	CBD prevented cognitive impairments as well as cytokine gene expression (i.e., IL-6 but not TNF-α)	[114]
Rat ($A\beta_{42}$ treatment)	CBD suppressed reactive gliosis and subsequent neuronal damage	[115]
Cannabinoid treatment in AD patients		
AD patients	THC had effects as an appetite stimulant and antiemetic, increased body weight and improved disturbed behaviours (adverse effects: tiredness and euphoria)	[89]
AD patients	Low-dose THC was effective in improving several clinical parameters including nocturnal motor activity and agitation (no adverse effects)	[90]
AD patients (Case report)	THC treatment reduced agitation and aggressiveness	[91]

Particular attention has been given to the role of cannabidiol (CBD) (see reviews [92-95]).

CB1 and CB2: Cannabinoid receptors; 2-AG: Arachidonoylglycerol; ACEA: Arachidonyl-2–chloroethylamide; Aβ: Amyloid beta; DAGL: Diacylglycerol lipase; FAAH: Fatty acid amide hydrolase; MAGL: Monoacylglycerol lipase; ROS: Reactive oxygen species; THC: $\triangle^9$-tetrahydrocannabinol; iNOS: Inducible nitric oxide synthase.

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characteristics of CBD have been described for a mouse model of hepatic ischemia [104]. CBD also effectively inhibited activated microglia migration [51] and reduced levels of lipid peroxide *in vitro* [105]. Finally, CBD markedly counteracted reactive enteric gliosis in LPS-mice through reduction of neurotrophin S100B and TNF-α expression (similar findings *ex vivo* using cultured human-derived colonic biopsies) confirming its anti-inflammatory properties [106]. Thus, CBD's neuroprotective, antioxidant and anti-inflammatory characteristics make it a prime candidate for AD therapy. Furthermore, a human study showed that CBD significantly augmented some of the behavioural effects of the NMDA antagonist ketamine [107]. Therefore, preclinical research should determine CBDs ability to potentiate the effects of another NMDA antagonist, memantine, which is approved for AD therapy. Importantly, CBD, even at high doses, has been shown to be well tolerated in humans [108].

Relative to THC, CBD appears largely behaviourally inert although its potential anti-epileptic, anxiolytic and anti-psychotic actions have received attention [92]. Importantly, CBD is inactive in a number of cognitive domains under normal physiological conditions [109] and improves cognitive impairments induced by iron overload [110].

9. The role of cannabidiol in Alzheimer's disease

There is only limited data regarding the potential therapeutic effects of CBD for AD available (**Table 1** and [92-95]). CBD has been shown to increase cell survival of PC12 neuronal cells after exposure to Aβ and to decrease ROS production and lipid peroxidation [111]. Furthermore, CBD inhibited Aβ-induced tau hyperphosphorylation as well as expression and production of iNOS and IL-1β in those cells [112,113] thereby attenuating Aβ-evoked neuroinflammatory responses (i.e., levels of glial fibrillary acidic protein) [100]. Importantly, a recent study revealed that subchronic administration of CBD promoted microglia migration *in vitro* and prevented cognitive impairments as well as cytokine gene expression (i.e., IL-6 but not TNF-α) in Aβ-injected mice [114]. Finally, in a rat model for Aβ-induced neurotoxicity, CBD effects on reactive gliosis and subsequently on neuronal damage were blunted by blockade of PPAR-γ, which is involved in the aetiology of AD pathology. Moreover, CBD stimulated hippocampal neurogenesis due to its interaction at PPAR-γ [115]. These studies show great promise and indicate that additional research into the therapeutic potential of CBD for AD is warranted using, for example, established transgenic mouse models for AD.

10. Conclusions

The immunosuppressive, anti-inflammatory and neuroprotective actions of cannabinoids suggest their potential for the treatment of AD. The available data indicate that eCBs are likely to represent an endogenous adaptive response aimed at counteracting the neurochemical and inflammatory consequences of Aβ deposition, Aβ-induced tau hyperphosphorylation and the imbalances to neurotransmitter systems involving ACh and glutamate. Furthermore, cannabinoids might also exert other protective effects, including, but not limited to, antioxidant actions. Thus, therapeutic targeting of the eCB system may offer protection from pathological processes typical of AD. Importantly, manipulations to the endocannabinoid system appear to have the capacity to provide balanced immune-modulation, which would be beneficial for AD therapy.

11. Expert opinion

As outlined in the current therapeutic strategies for AD section, treatment options available to AD patients to date do not stop or reverse the progression of AD, and no novel treatments for AD have been approved since memantine in 2003. Other therapeutic approaches not discussed here include statins, folic acid, histamine H_2 receptor antagonism [1], metal chaperons [116] and drugs targeting vascular functions [43]. Furthermore, a number of side effects accompany current treatments [34]. Based on the complex pathology of AD, researchers should ideally adopt a multimodal drug approach that targets a number of pathological processes of AD simultaneously [11]. An emerging theory, supported by the lack of efficacy of current treatments, is that existing interventions may be too late to have any lasting beneficial effects, as the extent of the damage caused by AD pathology may already be too severe. Thus, it has been suggested that new interventions should adopt a more preventative approach. Unfortunately, definite tools for early AD diagnosis (and the chance of early preventive treatment) are still missing, although imaging techniques are improving [117] and a wealth of information is now available regarding AD genetic risk factors [3] and biomarkers [118]. Only post-mortem analysis of cerebral tissue allows for a conclusive diagnosis.

This review proposes a multimodal therapeutic approach that targets simultaneously neuroinflammation, neurodegeneration and oxidative damage in AD. Interestingly, one clinical study using a combination of NMDA receptor antagonist and ACh inhibitor (memantine and donepezil) found greater treatment efficacy in improving cognitive functions [119], although another study showed that a similar combination (memantine and rivastigmine) worsened aspects of daily living [120]. Thus, one therapeutic strategy, which should be investigated in more detail, is the combination of different approved drugs to determine the value of interactive effects on a number of pathological features of AD. For example, CBD might have the ability to strengthen the therapeutic value of memantine [107]. Furthermore, the use of dual FAAH–AChE inhibitors [81] could lead to a potentially more effective treatment of AD, as they would target both ACh and eCB signaling and could thereby improve neuronal transmission and counteract neuroinflammation.

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The potential of the endocannabinoid system for a multimodal therapeutic approach must be emphasized as well. Based on its anti-inflammatory and neuroprotective properties as well as its inducible nature and the fact that CB_2 agonists are devoid of psychoactive effects in most studies, CB_2 appears to be a compelling candidate as a novel AD therapeutic. Stimulation of CB_2 expression in microglia in the vicinity of neuritic plaques may be part of an anti-inflammatory response of the brain, in order to protect neurons from degeneration. The inflammation-suppressing characteristics of CB_2 agonists are thereby probably linked to enhanced proliferation and recruitment of immune cells, which are involved in the immune-mediated repair of damaged neuronal tissue. This would be in line with the CB_2-mediated immunosuppressive effects of cannabis. The ability of CB_2 agonists to decrease neurotoxicity and cytokine secretion seems to reinforce this point. Thus, compounds that selectively stimulate CB_2 may have therapeutic potential in controlling Aβ-related pathology.

The blockade of eCB-degrading enzymes (i.e., FAAH inhibition) might represent another therapeutic target for AD. Reducing FAAH activity could be beneficial in preventing or dampening local inflammatory processes associated with Aβ depositions and could potentiate neuroprotection [33]. However, the potential involvement of CB_1-mediated mechanisms when enhancing the eCB tone might cause unwanted side effects, which have to be considered carefully.

Finally, the non-psychotropic CBD, which interacts with the eCB system but has actions that are distinct, offers promise as a new candidate for anti-inflammatory, antioxidant and neuroprotective drug development. CBD's characteristics make it a highly relevant drug target for AD (for reviews of the therapeutic potential of CBD for a number of other diseases see [92-96]). Importantly, CBD also lacks the cognition-impairing properties of other cannabinoids although further research has to address the uncertainties regarding its beneficial effects on cognition. In a first step, preclinical animal models using established pharmacological as well as genetic models for AD should be characterized comprehensively for their behavioural and biochemical response to long-term CBD exposure prior and post onset of AD pathology. Eventually, double-blind placebo-controlled clinical trial should then be performed to assess the therapeutic utility of CBD for AD. The fact that CBD is well tolerated by humans and has been tested under clinical conditions in the past will enable the fast translation of preclinical research into human trials [92,93,95,108].

In conclusion, the emerging data suggest the eCB system as a potential target for immune and/or cognitive intervention in AD. A wealth of available compounds manipulating the eCB system at a variety of levels and their success in animal models suggest the potential for human drug development. However, more comprehensive research using well established animal models for AD thereby including transgenic and knockout models, is needed. In particular, CBD's therapeutic potential for AD pathology has to be characterized in much more detail using *in vivo* approaches. Furthermore, combining AD models with genetic models available for cannabinoid researchers (e.g., CB_1 and CB_2 knockout mice) will increase our understanding of the role of particular cannabinoid receptors for the beneficial effects of cannabinoid manipulation in AD [16,32]. Importantly, *in vivo* models for both Aβ pathology and tau hyperphosphorylation must be considered (and ideally combined) to reduce/avoid some of the shortfalls of AD-relevant preclinical research in the past.

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