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REVIEW ARTICLE

Modulation of the Cannabinoid System: A New Perspective for the Treatment of the Alzheimer's Disease

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Abstract: The pathogenesis of Alzheimer's disease (AD) is somewhat complex and has yet to be fully understood. As the effectiveness of the therapy currently available for AD has proved to be limited, the need for new drugs has become increasingly urgent. The modulation of the endogenous cannabinoid system (ECBS) is one of the potential therapeutic approaches that is attracting a growing amount of interest. The ECBS consists of endogenous compounds and receptors. The receptors CB1 and CB2 have already been well characterized: CB1 receptors, which are abundant in the brain, particularly in the hippocampus, basal ganglia and cerebellum, regulate memory function and cognition. It has been suggested that the activation of CB1 receptors reduces intracellular Ca concentrations, inhibits glutamate release and enhances neurotrophin expression and neurogenesis. CB2 receptors are expressed, though to a lesser extent, in the central nervous system, particularly in the microglia and immune system cells involved in the release of cytokines. CB2 receptors have been shown to be upregulated in neuritic plaque-associated microglia in the hippocampus and entorhinal cortex of patients, which suggests that these receptors play a role in the inflammatory pathology of AD. The role of the ECBS in AD is supported by cellular and animal models. By contrast, few clinical studies designed to investigate therapies aimed at reducing behaviour disturbances, especially night-time agitation, eating behaviour and aggressiveness, have yielded positive results. In this review, we will describe how the manipulation of the ECBS offers a potential approach to the treatment of AD.

Keywords: Alzheimer's disease, endocannabinoid system, dementia, CB1 receptors, CB2 receptors, new therapeutic approach.

1. ALZHEIMER'S DISEASE

Dementia is a leading cause of ill health. Alzheimer's disease (AD) is the most common form of dementia, accounting for 50-60% of all cases. The prevalence of AD increases exponentially with age, affecting between 24% and 33% of all people over 80 years of age [1]. The number of cases of dementia is expected to rise dramatically over the next 50 years owing to the increase in life expectancy and will consequently present a growing medical challenge.

Clinically, AD is a neurodegenerative disorder characterized by an impairment in cognitive abilities, a dysfunction in activities of daily living and behavioural disturbances. Although the pathogenesis of this disease is still unclear, it has been postulated that Abeta (Aβ) aggregation and deposition, associated with neuritic plaque formation, and tau hyperphosphorylation, associated with the development of neurofibrillary tangles, play important roles in the development of this disease. Other mechanisms known to be involved in the pathogenesis of AD are synapsis dysfunction, inflammation oxidative stress and mitochondrial metabolism alterations [2].

The pathophysiological process underlying AD is believed to start many years before the disease is diagnosed. Therefore, in order to provide an overview of the biomarkers and epidemiological and neuropsychological evidence collected on AD over the last 20 years, the National Institute on Aging and the Alzheimer's Association developed recommendations to determine the factors that best predict the risk of progression from "normal" cognition to mild cognitive impairment and AD dementia [3]. This "preclinical" phase of AD may benefit from new therapeutic interventions, particularly disease-modifying drugs. The ability of the drugs currently available to modify the clinical symptoms of AD has, however, been limited and relatively short-lasting.

The main therapeutic approach adopted in recent years has been to increase the availability of acetylcholine by inhibiting acetylcholinesterase. The cholinergic hypothesis in AD is based on the degeneration of cholinergic neurons in the basal forebrain, which causes a dysfunction in cholinergic neurotransmission, particularly in the hippocampus and

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neocortex [4]. Donepezil and galantamine are selective acetylcholinesterase inhibitors, whereas rivastigmine inhibits both acetylcholinesterase and butyrylcholinesterase.

Another current therapeutic strategy in AD consists in modulating glutamate neurotransmission and consequently reducing excitotoxicity [5]. Memantine, a non-competitive N-methyl-D-aspartate (NMDA) receptor, is the only such drug used in AD.

Translating the pathogenetic findings of AD into potential therapeutic strategies based on disease-modifying drugs has been the target of researchers in recent years. Inhibiting Aβ production and aggregation or increasing Aβ clearance from the brain as a means of acting on tau hyperphosphorylation have been the main strategies adopted to date.

Epidemiological and observational studies have also laid the bases for testing various substances in randomized controlled clinical trials, though with often disappointing results.

1.1. The Endogenous Cannabinoid System

A novel approach in AD consists in exploiting the endogenous cannabinoid system (ECBS) insofar as this system has been shown to modulate the main pathological process that occurs during the neurodegeneration process (Fig. **1**) [6].

The ECBS consists of lipid compounds, specific receptors and several enzymes responsible for their synthesis and degradation (fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MAGL) and diacylglycerol lipase (DAGL) [7].

The best-known endocannabinoids (CB) are arachidonoylethanolamine (AEA), also referred to as anandamide, and 2-arachidonoylglycerol (2-AG), both of which are eicosanoids derived from the hydrolysis of membrane phospholipids. They are synthesized in the post-synaptic terminal and work as retrograde messengers on presynaptic CB receptors [6-8]. Following the activation of CB receptors, endo-

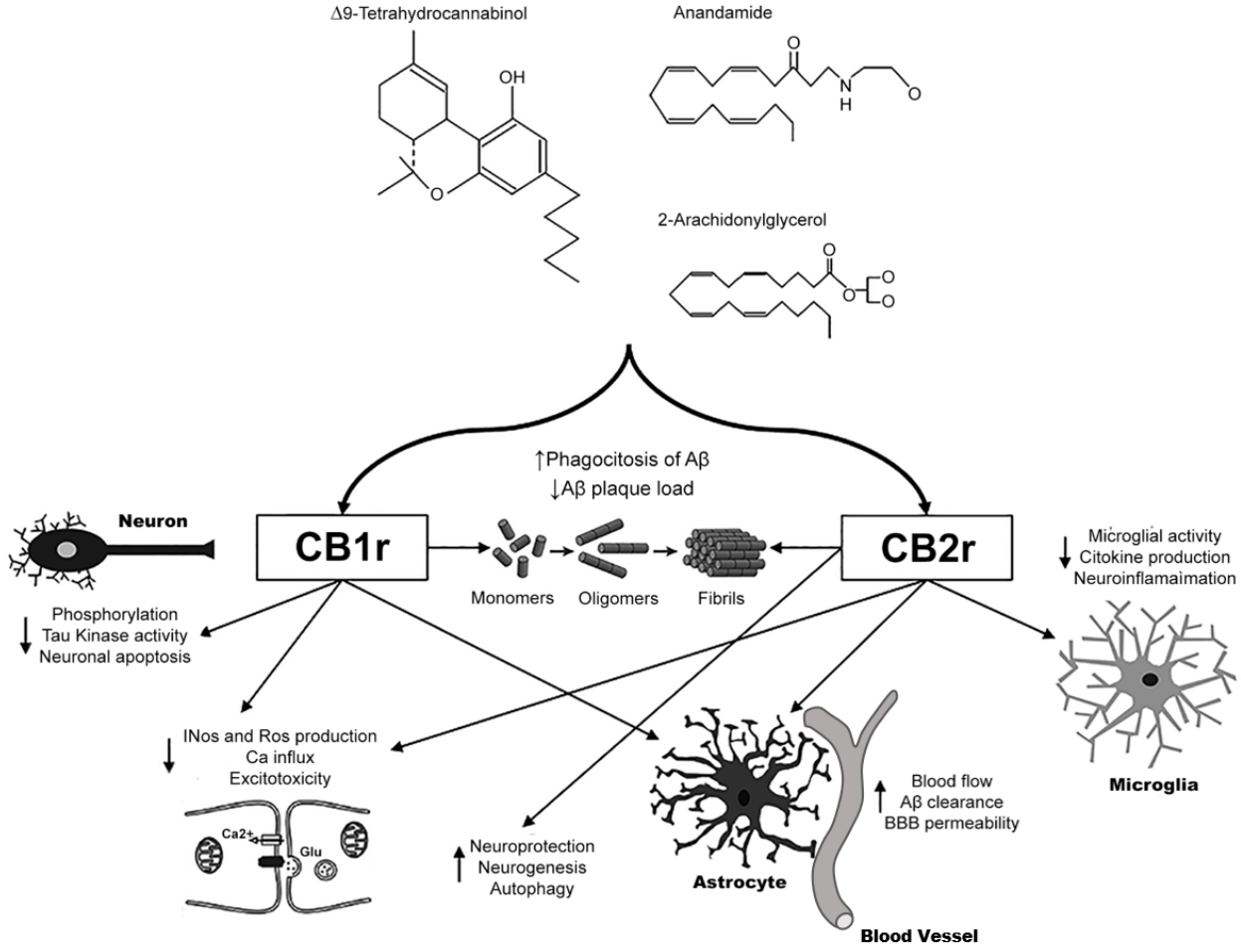

Fig. (1). Summary of the beneficial effects of the cannabinoid compounds in Alzheimer's disease pathology. Cannabinoid treatment can modulate multiple disease processes including Aβ and tau processing, neuroinflammation, microglial activation, mitochondrial dysfunction and excitotoxicity. CB2 receptor agonists reduce the release of pro-inflammatory molecules, facilitate Aβ clearance by promoting microglia phagocytic phenotype, reduce Aβ neurotoxicity. Moreover, CB2-mediated activity reduces oxidative stress damage produced by reactive oxidative species (ROS) and tau hyperphosphorylation. CB1 receptor agonists regulate iNOS protein expression and NO production, inhibit hyperphosphorylation of tau protein through the inhibition of tau kinase, reduce Abeta plaque load throught the activation of PPAR gamma receptor and the stimulating the expression of LPRP1. 337x257mm (96 x 96 DPI).

cannabinoids are removed by means of enzymatic hydrolysis from the synaptic junction; anandamide is hydrolysed by FAAH in post-synaptic neurons, while 2-AG is hydrolysed prevalently by MAGL in pre-synaptic neurons [9].

Other endocannabinoids have been identified more recently (virodhamine and 2-arachidonylglyserylether (2-AGE), among others), though their action is still unclear [10].

The cannabinoid receptors CB1 and CB2 are G protein-coupled receptors. CB1 receptors, which are the most abundant cannabinoid receptors, are expressed mainly in neurons in the central nervous system, as well as in glial cells, and thus regulate important brain functions, including cognition and memory, emotion, motor control, feeding and pain perception [11]. The activation of these receptors is believed to inhibit adenylate cyclase activity and calcium influx into the axon terminal [12]. CB1 is also expressed in peripheral tissue, *i.e*. in heart, uterus, testis, liver and small intestine, as well as in immune cells and adipose tissue [13].

CB2 receptors are expressed to a lesser extent in the central nervous system but are located above all in microglia and in immune system cells that act on the release of cytokines [14], their expression being significantly increased following stressful conditions [15]. Recent experimental studies have detected CB2 receptor activity in neural progenitor cell proliferation, axon guidance and synaptic transmission [16-17]. CB2 receptors can act through the initiation of the phospholipase C (PLC) and inositol 1 4 5-triphosphate (IP3) signalling pathway, which results in increased levels of intracellular calcium [18]. Like CB1 receptors, CB2 receptors can inhibit adenylyl cyclase, thereby reducing intracellular cAMP levels [7]. Activation of CB2 receptors is coupled with other cellular pathways, including PKA, ERK1/2 and P38. Besides being found in cells of the immune and hematopoietic system, CB2 receptors have also been found in peripheral organs such as muscle, liver, intestine and testis [19].

2. ENDOCANNABINOID SYSTEM IN AD BRAIN

The analysis of human post-mortem samples has revealed some ECBS alterations in AD brain.

Data on the changes induced by the ECBS on CB1 receptors are somewhat contrasting. A reduction in the number of CB1 receptors, particularly in the frontal cortex, has been described in some studies [20, 21], whereas no changes in the expression, distribution or availability of CB1 receptors has been reported in the hippocampus of AD patients [22-25] or during normal aging [26]. Lastly, changes in cognitive status or in biochemical variations are not reported to correlate with CB1 receptor levels, with the exception of an improvement in hypophagia, which supports the potential of CB1 receptor antagonists in the treatment of obesity [27] and, more specifically, in the management of eating disorders associated with AD [21].

By contrast, most studies consistently report an increase in CB2 receptor numbers expressed on microglia surrounding senile plaques [20-22]. Interestingly, this increased expression correlates with Aβ42 levels and plaque deposition, though not with cognitive status [21], thereby suggesting that such pathogenic events induce CB2 receptor expression and that the activation of these receptors stimulates amyloid removal by human macrophages [28].

It is also noteworthy that CB receptors in AD brain are nitrosylated [20], which may lead to impaired connections between receptors and their effectors.

Moreover, it has been demonstrated that changes in CB receptor expression might be time-dependent. In this regard, CB1 and CB2 have different patterns of expression: the level of activity displayed by the hippocampal and frontal CB1 receptors is greater in the early stages of AD, but is reported to decrease as the disease progresses [29]. By contrast, CB2 receptors are expressed to a greater extent during the advanced stages of AD, when neuroinflammation is more evident and microglia and astrocytes are activated [30].

3. A ROLE OF CBS IN AD PATHOGENESIS

The role of the ECBS as a therapeutic strategy in AD is based on the potential of cannabinoids to target several processes involved in the pathogenesis of AD, such as Abeta and tau metabolism, inflammation, mitochondrial dysfunction and excitotoxicity.

Clinical data have revealed an improvement in behavioural symptoms in AD patients following treatment with cannabinoids, whereas no correlation between cannabinoid and cognitive status has been reported by the few clinical studies that have investigated this aspect.

3.1. Neuroprotection Against A Beta and Tau

Several *in vitro* and *in vivo* studies have demonstrated that certain cannabinoid compounds confer neuroprotective effects against Abeta. There are several underlying mechanisms of action, some of which act in parallel while others interact with one another. Although most of these mechanisms indirectly mitigate the harmful effects of Abeta, direct effects of the ECBS on Abeta processing have also been reported [31, 32]. These direct effects, which consist in the removal of Abeta by macrophages and the facilitation of Abeta transport through the choroid plexus after CB2 receptor stimulation, are corroborated by mouse models that have highlighted a reduction in Abeta levels and plaque burden following chronic treatment with CB2 or Cb/CB2 receptor agonists. Endocannabinoid treatment or MAGL inhibition appears to facilitate Abeta transit across the blood-brain barrier (BBB) *in vitro* and *in vivo* as a result of increased expression of the low density lipoprotein receptor-related protein 1 (LRP1), which is known to influence Abeta removal from the brain [33]. Moreover, a significant reduction in Abeta plaques, likely due to the activation of neprilysin, which is an Abeta degradation enzyme, has been demonstrated in APP transgenic mice treated with Delta 9-THC [34].

The role of CB1 appears less clear. It has been suggested that the ECBS activates, through CB1 receptors, the peroxisome proliferator-activated gamma receptor (PPAR-γ), which in turn stimulates the expression of lipoprotein receptor protein 1 (LRP1) and increases Abeta clearance across the blood-brain barrier [35].

A role of cannabinoids has also been described in tau hyperphosphorylation, with the effect being mediated by the reduction in the phosphorylated active form of glycogen synthase kinase 3beta (GSK3), one of the tau kinases. This effect of tau hyperphosphorylation is selectively mediated by CB1 receptors [36-38]. Moreover, a specific role of CB2 receptors in the modulation of tau is also suggested by the potentiation of autophagy and improvement in the redox state [39].

3.2. Mimic the Current Medication

To date, the only approved therapy for AD, which consists of drugs such as donepezil, galantamine and rivastigmine, is based on the cholinergic hypothesis, according to which acetylcholinesterase (AchE) is inhibited and acetylcholine levels are increased in the synaptic slit [4]. In addition to these drugs, another substance called memantine has also been approved. As a non-competitive antagonist of the N-methyl D-aspartate (NMDA) receptors, memantine reduces excitotoxicity by conferring neuronal protection [5]. Interestingly, certain cannabinoid compounds act on the same targets as current medications. For instance, 9-THC competitively inhibits AchE by binding to its peripheral anionic site, thus increasing Ach levels [40]. The synthetic cannabinoid HU-211 (inactive enantiomer of HU-210) acts as a stereoselective inhibitor of NMDA receptors, and thus protects cells from NMDA-induced neurotoxicity [41-43]. HU-211 binds directly to NMDA but not to cannabinoid receptors because this compound shares the chemical structure though not the pharmacological properties with other cannabinoids.

Neuroprotection can thus be conferred by cannabinoids through a range of mechanisms, including inhibition of presynaptic glutamate release [44], blockage of voltage-dependent calcium channels [45, 46] and inhibition of calcium release from ryanodine-sensitive stores [47], most of which involve CB1 receptors.

3.3. Effects on Neuroinflammation

As neuroinflammation is present in every stage of AD and contributes to the evolution of this pathology, activated microglial cells and macrophages are found surrounding amyloid plaques. There is a growing body of evidence suggesting that that the ECBS can modulate neuroinflammation and neurotoxicity by reducing the release of proinflammatory cytokines [48] and preventing microglial activation by CB2 receptor agonists [49]. It has been demonstrated that CB2 receptors are upregulated in brain regions in which senile plaques are abundant and that their activity leads to the transformation of microglial cells from the M1 to M2 phenotype [14, 15], thereby favouring phagocytosis and cell repair mechanisms [22, 50]. Other known mechanisms are the inhibition of glutamate release [51] and reduction in Ca NMDA mediated [52, 53] by CB1/CB2 agonists and the prevention of NO signalling and PKA by CB1 receptors [54].

3.4. Activity on Mitochondria

Numerous studies have shown that mitochondrial function is altered in the early stages of AD, with a reduced energy production as well as increased demand and consequent excessive oxidative stress being observed [2]. It has been suggested that the ECBS may prevent ROS production and lipid peroxidation [55] and reduce NO levels by inhibiting the expression of iNOS, an enzyme that is responsible for NO synthesis [51, 56, 57].

CB2 receptors, in particular, play an important role by reducing oxidative stress and promoting responses against such damage, as is demonstrated by the ability of JWH-133 (selective CB2 receptor agonist) to reduce hydroxylated adducts, derived from lipid peroxidation, and enhance superoxide dismutase (SOD1 and SOD2) levels in the vicinity of plaques in APP/PS1 mice [31]. Another possible mechanism of action of the ECBS is its ability to regulate the neuronal energy metabolism by reducing the respiratory chain complex I activity and oxygen consumption, probably through cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) signalling [51]. It has also been demonstrated that partial CB1 agonists, such as AEA, 9-THC and HU-210, may slow down the oxidative metabolism, thereby regulating the activity of mitochondria [56].

4. CLINICAL EVIDENCE

Few clinical studies have tested cannabinoids on AD patients, and the majority of those that do exist were conducted on populations in the late stages of the disease and were based on a short-lasting treatment [58-60]. A first attempt to determine whether cannabinoids are clinically effective in the treatment of dementia was made by the Cochrane Dementia and Cognitive Improvement Group (CDCIG) in 2009 [61]. Only one study met the selection criteria, which only allowed the inclusion of double blind and single (rater)-blind randomized placebo-controlled trials [59]. The participants (15 severe, hospitalised, AD patients) were randomly assigned to two groups to receive either dronabinol (2.5 mg twice daily) or placebo for six weeks before treatment was crossed over for a further six weeks. The authors suggested that dronabinol is a promising agent for the treatment of anorexia and other behavioural disturbances such as agitation and aberrant nocturnal motor activity. However, the authors of the review also concluded that more randomized double-blind placebo-controlled trials are needed to determine whether cannabinoids are clinically effective as a means of treating dementia.

Some years later, another systematic review [62] sought to extend the evidence on the indications, efficacy, safety and pharmacokinetics of medical cannabinoids in older subjects. PubMed, EMBASE, CINAHL and Cochrane Library were used as a source of research and only controlled studies or data reporting only including subjects over 65 years of age, who did not need to be affected by AD, were selected. Only two studies [58, 59] have shown that THC might be useful in the treatment of anorexia and behavioural symptoms in dementia. The adverse events linked to cannabinoid treatment are sedation-like symptoms. The authors concluded that, despite the fascinating therapeutic potential, adequately powered trials are needed to assess the efficacy and safety of cannabinoids in older subjects [62].

In a more recent retrospective systematic review on 40 demented inpatients with behavioural and appetite disturbances, dronabinol intake, at a mean dose of 7.03 mg/day, was found to lead to a significant decrease in all domains of the Pittsburgh Agitation Scale (PAS) and an improvement in Clinical Global Impression (CGI) scores, sleep duration and meal consumption, as well as being well tolerated [63].

In a recent open label study on 11 AD patients with behavioural and psychological symptoms, medical cannabis oil (1.65% THC) administered up to 7.5 mg twice daily over 4 weeks proved to be both safe and well tolerated. A significant reduction in the CGI severity score, in caregiver distress and in the NeuroPsychiatric Inventory (NPI) domains, particularly in delusions, agitation/aggression, irritability, apathy and sleep disturbances, were observed, as were good safety and tolerability profiles [64].

In another study, a phase 2, randomized, multi-center, double-blind, parallel group trial was designed to explore the efficacy and safety of THC in tablet form (dose of 4.5 mg daily) to treat behavioural disturbances and pain in patients with mild to severe dementia. Behavioural disturbances were defined as a score of ≥10 on the NPI, which also served as the primary outcome measure. THC showed no benefit over placebo, though it was well tolerated. The authors suggested that the target dose might have been too low to detect any psychomimetic effects and the treatment period too short (3 weeks) [65].

Table 1. Overview on CB clinical studies.

References	Numbers of Partecipants	Type of eCB	Type of Study	Duration of Treatment	Clinical Evidences
Volicer L, 1997 [55]	15 severe AD patient hospitalised	dronabinol (2.5 mg twice daily) or placebo	placebo controlled study (double blind and single-rater blind randomized	six week and crossover of treatment for a further six week.	Improvement on anorexia, agitation and aberrant nocturnal motor activity
Walther S, 2006 [54]	6 demented patients (5AD, 1 VaD)	Dronabinol (2,5 mg daily)	Open label pilot study	2 weeks	Reduction of night-time agitation
Koppel J, 2009 [56]	19 late-onset AD subjects and 12 controls	No drugs	A case-control and cohort study		No significant differences in AEA and 2AG plasma concentration between AD and CTR
Woodward M.R, 2014 [59]	40 inpatients with severe dementia	Dronabinol	Retrospective study	1 week	Significant decreases in all domains of PAS, significant improvements in CGI scores and in sleep duration
Shelef, A, 2016 [60]	11 AD patients	THC oil up to 7.5 mg twice daily	an open label, add-on, pilot study	4 weeks	Improvement on delusions, agitation/aggression, irritability, apathy, and sleep disturbances. Reduction in CGI severity score and in caregiver distress
Van den Elsen G.A.H, 2015 [61]	patients with mild to severe dementia (24 with TCH and 26 with placebo)	THC in tablet form (dose of 1.5 mg 3 times daily)	a phase 2, randomized, double-blind, placebo-controlled study	3 weeks	No benefit in NPS
clinicaltrial.gov NCT02351882	40 AD patients	nabilone at 2 mg daily dose versus placebo	randomized placebo controlled study	14 weeks: participants take nabilone for 6 weeks, placebo for 6 weeks (order randomized) with 1 week between treatments	Estimated Study Completion Date: march 2018
clinicaltrial.gov NCT02792257	160 AD patients	2.5 mg per dose (5mg daily) during Week 1, then 5 mg per dose (10mg daily) for Weeks 2 and 3 versus placebo	randomized placebo controlled study	3 weeks	Estimated Study Completion Date: august 2020

Abbreviations: AD: Alzheimer's disease; THC: tetrahydrocannabinol; VaD: Vascular Dementia; CTR: controls; AEA: arachidonoylethanolamide; 2AG: 2-arachidonoylglycerol, PAS: Pittsburgh Agitation Scale; GCI: Clinical Global Impression; NPS: neuropsychiatric symptoms.

Two studies are currently being conducted (clinicaltrial.gov) to evaluate the safety and efficacy of cannabinoids (nabilone in one and dronabinol in the other) in the treatment of AD, particularly of agitation, pain, weight, sleep and memory. The first study is a 14-week crossover pilot study designed to compare a 2 mg daily dose of nabilone for 6 weeks with placebo in 40 patients with AD. The second is a 3-week pilot study designed to compare a 5 mg daily dose of dronabinol with placebo in 160 in-patients with AD. See Table **1** for a better overview.

CONCLUSION

Manipulation of the cannabinoid system may offer a novel pharmacological approach to the treatment of AD. Manipulation should be aimed prevalently at inhibiting neuroinflammation through CB2r activation so as to prevent ROS formation and cytokine release from microglia, on the one hand, and at reducing neurotoxicity by CB1 receptor activation so as to inhibit glutamate release and enhancing neurotrophin expression and neurogenesis, on the other.

It has also been demonstrated that the ECBS reduces tau phosphorylation and Delta9-THC inhibits AchE, which result in enhanced cholinergic transmission and reduced amyloidogenesis. However, since the potential value of the ECBS on cognitive and behavioural performances remains unclear, randomized controlled trials are warranted to evaluate whether the ECBS may modify disease development or progression.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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All the authors made a significant contribution to this study. Talarico Giuseppina wrote the article. Trebbastoni Alessandro contributed to the drafting of the clinical evidence paragraph and prepared the figure. Bruno Giuseppe and de Lena Carlo were involved in revising the draft and reviewing the article from a linguistic point of view.

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