

Drożak, Paulina, Skrobas, Urszula, Drożak, Martyna. Cannabidiol in the treatment and prevention of Alzheimer's disease – a comprehensive overview of in vitro and in vivo studies. *Journal of Education, Health and Sport*. 2022;12(9):834-845. eISSN 2391-8306. DOI <http://dx.doi.org/10.12775/JEHS.2022.12.09.097>
<https://apcz.umk.pl/JEHS/article/view/39922>
<https://zenodo.org/record/7083451>

The journal has had 40 points in Ministry of Education and Science of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of December 21, 2021. No. 32343. Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Physical Culture Sciences (Field of Medical sciences and health sciences); Health Sciences (Field of Medical Sciences and Health Sciences). Punkty Ministerialne z 2019 - aktualny rok 40 punktów. Załącznik do komunikatu Ministra Edukacji i Nauki z dnia 21 grudnia 2021 r. Lp. 32343. Posiada Unikatowy Identyfikator Czasopisma: 201159. Przynależność dyscypliny naukowej: Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu).

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The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 01.09.2022. Revised: 02.09.2022. Accepted: 15.09.2022.

Cannabidiol in the treatment and prevention of Alzheimer's disease – a comprehensive overview of in vitro and in vivo studies

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Abstract

Introduction and purpose. Dementia is a major public health problem. Alzheimer's disease (AD) accounts for 60% of dementia cases. However, AD is currently considered as an incurable disorder and the only few drugs available for its treatment are mostly symptomatic. In the quest for novel drugs for this devastating disease, cannabidiol (CBD) has been recently gaining attention due to its multiple properties. The aim of this review article was to summarize findings on the effect of CBD on AD with a focus on molecular mechanisms of CBD's action and therapeutic effects which it exerts. The review was performed based on available literature on the PubMed platform by entering key words: Alzheimer's disease, cannabidiol.

Description of the state of knowledge. In vitro studies, carried out on cell models of AD, showed anti-inflammatory and antioxidative properties of CBD through a suppression of pro-inflammatory genes and causing a reduction of production of, among others, nitric oxide. Moreover, CBD was proven to decrease amyloid β ($A\beta$) production, reduce formation and aggregation of tau fibrils, protect against microglial and $A\beta$ -induced neurotoxicity and prevented the $A\beta$ -induced deficit in long-term potentiation in hippocampus. In vivo studies, conducted mostly on mice models of AD, indicated that CBD improves memory and spatial learning, reverses deficit in social and object recognition, reduces anxiety-like behaviors and improves glucose metabolism. One study including humans demonstrated that CBD enhances cerebral blood flow in hippocampus. Currently there are two ongoing clinical trials on the use of CBD in AD-associated agitation.

Conclusions. Both in vitro and in vivo studies indicate that CBD appears promising in the treatment and prevention of Alzheimer's disease.

Key words: Alzheimer's disease; cannabidiol

Introduction

Dementia is a major public health problem with a rapidly growing number of people affected. According to the Global Burden of Disease Study 2019, a number of people living with dementia is expected to increase from 57.4 million cases globally in 2019 to 152.8 million cases in 2050 [1]. The most common types of dementia are Alzheimer's disease (AD) and vascular dementia (VD) accounting for 60% and 30% of dementia cases, respectively [2]. The coexistence of AD and VD is termed mixed dementia (MD) [3]. Neuropathological studies indicate that the prevalence of MD is between 20-22% [4,5].

Currently, there are multiple hypotheses concerning the pathogenesis of AD, i.e. abnormal accumulation of amyloid β ($A\beta$) in the extracellular spaces of neurons, formation of neurofibrillary tangles of tau protein inside neurons, alterations in the cholinergic system, oxidative stress and inflammation [6]. Moreover, observations of the vascular alterations which appear early in the AD lead to a formation of the two-hit vascular hypothesis which was first stated by Zlokovic. It says that a damage in the brain microvasculature (hit one) induces neuronal dysfunction which is mediated by the leakage of neurotoxic molecules through blood-brain barrier (BBB) and decreased brain capillary blood flow which leads to multiple ischemic foci. A dysfunction of BBB also causes impaired clearance of $A\beta$. These changes promote the accumulation of $A\beta$ in the brain (hit two). Whereas, according to this hypothesis, tau pathology develops secondary to the vascular and $A\beta$ abnormalities [7].

AD is currently considered as an incurable disorder. However, several symptomatic drugs for this disease exist. These include: 3 acetylcholinesterase inhibitors (AChEIs): donepezil, galantamine and rivastigmine, 1 noncompetitive N-methyl-d-aspartate (NMDA) receptor antagonist: memantine, and a very newly Food and Drug Administration (FDA)-approved drug called aducanumab which is a monoclonal antibody targeting aggregates of $A\beta$ [8,9]. Furthermore, in a quest for novel, effective drugs for this devastating disease, currently 143 agents are undergoing a thorough examination in a total of 172 clinical trials (as of January 25, 2022) [10]. Among them, there are currently two clinical trials (under identifiers NCT04075435 and NCT04436081) investigating cannabidiol (CBD) for the treatment of AD [11,12]. So far, CBD has proven promising for the treatment and prevention of AD in multiple studies, both in vitro and in vivo [13,14].

The aim of this review article was to summarize findings on the effect of cannabidiol on Alzheimer's disease with a focus on molecular mechanisms of CBD's action and therapeutic effects which it exerts.

The review was performed based on available literature on the PubMed platform by entering key words: Alzheimer's disease, cannabidiol.

Cannabidiol (CBD)

The cannabis plant (*Cannabis sativa* L.) is an ancient medicinal plant which extract contains over 100 various cannabinoids [15]. Cannabidiol (CBD) is one of the most common phytocannabinoids found in this plant and constitutes up to 40 percent of its extract [16]. Contrary to the Δ 9-tetrahydrocannabinol (Δ 9-THC), another abundant phytocannabinoid which is psychoactive, CBD is non-psychoactive [17]. Contrary to most cannabinoids, CBD does not interact with cannabinoid receptors in the endocannabinoid system. Instead, it is highly pleiotropic and exerts its action through a wide variety of other receptors, such as: adenosine receptors, glycine receptors (GlyRs), non-endocannabinoid G protein-coupled receptors (GPCRs), serotonin (5-HT) receptors, opioid receptors (ORs), peroxisome proliferator-activated receptors γ (PPAR γ), nicotinic acetylcholine receptors (nAChRs), and Transient Potential Vanilloid Receptor Type 1 (TRPV1), as well as multiple other targets (i.e. enzymes and ion channels) [18]. Due to its remarkable diversity of mechanisms of action, CBD has also multiple beneficial properties which have been proven in studies. These include antioxidative, anti-inflammatory, analgesic, antitumor, anticonvulsant, anxiolytic, antidepressant, antipsychotic and many other properties [19 - 23]. In June of 2018 cannabidiol has been approved by FDA for the treatment of two epileptic syndromes: Lennox-Gastaut syndrome and Dravet syndrome for individuals older than 2 years of age. However, there is also interest in the off-label use of CBD [24]. Apart from AD, cannabidiol also shows promise in the treatment of other neurological disorders, such as: chronic pain, trigeminal neuralgia, essential tremors, amyotrophic lateral sclerosis, Parkinson's disease, and Huntington's disease, as well as in psychiatric disorders, such as anxiety, depression and psychosis [25 - 28].

In vitro studies on CBD in Alzheimer's disease

The neuroprotective, anti-apoptotic and anti-oxidative effects of CBD against A β -induced neurotoxicity and oxidative stress were shown in a study conducted by Iuvone et al. The authors demonstrated that a treatment of PC12 neuronal cells with CBD prior to an exposure to A β significantly increased cell survival together with decreasing lipid peroxidation, reactive oxygen species (ROS) production and caspase 3 levels (a crucial enzyme in apoptosis) [29]. Moreover, two studies by Esposito et al., performed on the same PC12 cell line, indicated that CBD inhibits the hyperphosphorylation of tau protein through Wnt/beta-catenin pathway rescue and suppresses expression of the nitric oxide synthase protein and nitric oxide production through an inhibition of phosphorylated p38 MAP kinase and an activation of NF-kappaB [30,31]. Thus, CBD suppresses expression of pro-inflammatory genes. Furthermore, in a study conducted by Martín-Moreno et al. CBD promoted microglial cell migration and decreased an ATP-induced intracellular calcium augmentation in cultured N13 microglial cells and in rat primary microglia. This finding is promising, since microglial activation occurs in AD [32]. A study by Janefjord et al. confirmed findings from the above-mentioned researches. In this study CBD was shown to protect the SH-SY5Y cells (neuroblastoma cell line which is relevant for AD studies) from microglial and A β neurotoxicity [33]. What is

interesting, a study by Scuderi et al. which was performed on SHSY5Y(APP+) neurons, demonstrated CBD to induce the ubiquitination of amyloid precursor protein (APP) which lead to its reduction and, in consequence, a decrease in production of A β . The authors stated that this action was performed through the involvement of PPAR γ . Furthermore, a reduction of apoptotic rate and an increase in the cell survival was observed [34]. Cannabidiol was also proven to modulate the expression of AD-related genes in the mesenchymal stem cells. A study carried out by Libro et al. indicated that CBD downregulated genes encoding proteins involved in A β generation and tau phosphorylation [35]. Another impressive study conducted by Hughes et al. demonstrated that a pre-treatment of mice hippocampal slices with CBD prevented the A β -induced deficit in long-term potentiation (LTP). However, a treatment of cells with CBD after an application of soluble A β did not show such effect. This indicates that cannabidiol protects synaptic plasticity [36]. The newest study by Alali et al. indicated that CBD has a capacity to inhibit tau aggregation. With the use of Thioflavin T assay, circular dichroism and atomic force microscopy authors demonstrated that CBD suppresses formation of the tau fibrils [37].

In vivo studies on CBD in Alzheimer's disease

Recent years have particularly expanded our understanding of CBD's effect on AD through *multiple in vivo* studies conducted mostly on mice models of this disease. A study conducted by Esposito et al. on mice inoculated with human A β confirmed anti-inflammatory properties of CBD which manifest themselves through a decrease in expression of inducible nitric oxide synthase (iNOS) and IL-1 β and thus a limitation of related NO and IL-1 β release [38]. Furthermore, a recent study carried out by Hao et al. demonstrated that CBD enhanced immune response and autophagy in hippocampus of A β PP \times PS1 transgenic mice [39]. A study by Watt et al., also carried out on A β PP \times PS1 transgenic male mice, confirmed the finding previously obtained in *in vitro* studies: CBD caused reduction in insoluble A β 40 levels in mice's brains [40]. A very recent study by de Paula Faria et al. unraveled another important property of CBD which is an ability to improve glucose metabolism. This is especially crucial, since glucose hypometabolism is an early sign of AD and persists through all stages of this disorder [41]. Studies by Martín-Moreno et al., Watt et al. and Coles et al. revealed that CBD improves spatial memory and learning [40, 42-44]. CBD was proven to reverse and also prevent cognitive deficits in social recognition in two researches by Cheng et al. [45,46]. Whereas an improvement in novel object recognition was observed in studies by Cheng et al. and Coles et al. [45,43]. An overall ameliorated memory and cognitive function after CBD administration was observed in studies by de Paula Faria et al. and Khodadadi et al. [41,47]. The latter study also concluded that the CBD-mediated improvement of cognitive abilities occurs through an enhancement of expression of IL-33 and triggering receptor expressed on myeloid cells 2 (TREM2) [47]. In a study by Wang et al. CBD was demonstrated to improve lifespan and overall health span in *C. elegans* AD model [48]. The only study which did not note any changes after CBD administration was a study carried out by Watt et al. in which no behavior, sociability and social recognition changes were noticed after CBD treatment [49]. Different results were obtained in a study by Kreilaus et al. which was also conducted on TAU58/2 transgenic mice, however, CBD dosage was twice higher than in a study by Watt et al. (100 mg/kg/day for 3 weeks compared to 50 mg/kg/day for 3 weeks) and also female mice were incorporated in the study, instead of male mice [44,49]. Kreilaus et al. found that CBD caused a reduction in anxiety-like behaviors and decreased contextual fear-associated freezing [49]. [Table 1.]

Table 1. A summary of in vivo studies concerning the use of CBD in AD.

Author (year)	Model	CBD dosage	Results
Esposito et al. (2007) [38]	C57BL/6J mice inoculated with human A β (1–42) into hippocampus	2.5 or 10 mg/kg/day intraperitoneally for 7 days	CBD prevented neuroinflammatory response caused by A β through a decrease of iNOS and IL-1 β expression and thus a decrease of the related release of NO and IL-1 β .
Martín-Moreno et al. (2011) [42]	C57/Bl6 mice with A β administered intraventricularly	20 mg/kg/day intraperitoneally daily during the first week, then 3 days/week for next 2 weeks	CBD improved spatial memory.
Cheng et al. (2014) [45]	A β PP \times PS1 transgenic mice	20 mg/kg/day intraperitoneally for 3 weeks	CBD reversed cognitive deficits in social recognition and novel object recognition.
Cheng et al. (2014) [46]	A β PP \times PS1 transgenic mice	From 2.5 months of age mice obtained 20 mg/kg/day orally for 8 months	CBD treatment prevented development of deficits in social recognition.
Watt et al. (2020) [49]	TAU58/2 transgenic male mice	50 mg/kg/day intraperitoneally for 3 weeks	CBD treatment did not affect changes in behavior, sociability and social recognition.
Watt et al. (2020) [40]	A β PP \times PS1 transgenic male mice	50 mg/kg/day intraperitoneally for 3 weeks	CBD reversed deficits in social recognition and spatial learning. Moderate reductions in insoluble A β 40 levels were observed in specific brain regions.
Coles et al. (2020) [43]	A β PP \times PS1 transgenic female mice	5 mg/kg/day intraperitoneally for 3 weeks	CBD reversed deficits in object recognition and spatial learning. However, CBD did not affect impairments in

			sensorimotor gating.
Khodadadi et al. (2021) [47]	5xFAD mice	10 mg/kg/day intraperitoneally every other day for 8 doses over 2 weeks	CBD ameliorated cognitive function through an enhancement of IL-33 and triggering receptor expressed on myeloid cells 2 (TREM2) expression.
Wang et al. (2021) [48]	Transgenic CL2355 strain of <i>Caenorhabditis elegans</i> (<i>C. elegans</i>)	<i>C. elegans</i> eggs were placed on nematode growth medium plates and seeded with <i>E. coli</i> OP50 at a 5 μ M CBD concentration.	CBD extended lifespan and improved health span of <i>C. elegans</i> .
Hao et al. (2021) [39]	A β PP \times PS1 transgenic mice	5 mg/kg/day intraperitoneally for 30 days	CBD enhanced immune response and autophagy in hippocampus.
de Paula Faria et al. (2022) [41]	Wistar male rats with intracerebroventricularly injected streptozocin (to mimic hypometabolism associated with AD)	20 mg/kg/day intraperitoneally for 1 week	CBD improved memory and glucose metabolism.
Kreilaus et al. (2022) [44]	TAU58/2 transgenic female mice	100 mg/kg/day for 3 weeks; route of administration not specified	CBD reversed an impairment in spatial reference memory, reduced anxiety-like behaviors and decreased contextual fear-associated freezing.

Human studies on CBD in Alzheimer's disease

According to our best knowledge, to date no study concerning the use of CBD alone in AD in humans was published. Currently, there are 2 ongoing clinical trials, under identifiers of NCT04075435 and NCT04436081, which investigate CBD effect on behavioral symptoms and agitation accompanying AD, respectively [11,12]. Moreover, in a study by Broers et al. CBD with THC were proven effective and well-tolerated in improving rigidity, behavioral problems, direct contact, and daily care in severely demented patients. What is interesting, in this study patients obtained orally a THC/CBD-based oil in higher doses than in other studies: 7.6 mg THC/13.2 mg CBD a day after 2 weeks, 8.8 mg THC/17.6 mg CBD a day after 1 month, and 9.0 mg THC/18.0 mg CBD a day after 2 months [50]. A very interesting study by Bloomfield et al. demonstrated that an acute CBD oral administration at a dose of 600 mg increased cerebral blood flow (CBF) in the hippocampus detected by arterial spin labelling.

This indicates that CBD could be beneficial in conditions with impaired memory processing, such as AD [51].

Conclusions

Both in vitro and in vivo studies indicate that cannabidiol appears beneficial in the treatment and prevention of Alzheimer's disease through its multiple properties. The next research step is to evaluate safety, efficacy and dosage of CBD in human studies.

Abbreviations

AD – Alzheimer's disease

VD – vascular dementia

MD – mixed dementia

A β – amyloid β

BBB – blood-brain barrier

AChEI - acetylcholinesterase inhibitor

NMDA - N-methyl-d-aspartate

FDA – Food and Drug Administration

CBD – cannabidiol

5-HT – 5-hydroxytryptamine (serotonin)

OR – opioid receptor

PPAR γ - peroxisome proliferator-activated receptor γ

nAChR - nicotinic acetylcholine receptor

GlyR – glycine receptor

GPCRs - non-endocannabinoid G protein-coupled receptors

TRPV1 - Transient Potential Vanilloid Receptor Type 1

ROS – reactive oxygen species

FAAH - fatty acid amide hydrolase

2-AG - 2-arachidonoyl-glycerol

LTP – long-term potentiation

iNOS - inducible nitric oxide synthase

TREM2 - triggering receptor expressed on myeloid cells 2

CBF – cerebral blood flow

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