Fall 2019

Therapeutic potential of cannabinoids in the management of Alzheimer's Disease and other neurodegenerative conditions

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Biomedical Sciences

Creative Component

Fall 2019

Abstract

Alzheimer's disease (AD) is the most pervasive form of neurodegenerative dementia affecting the global population today. Efforts to develop pharmacological intervention to halt or reverse the progression of AD have proven unsuccessful, resulting in insufficient treatment options for patients with AD which possess limited therapeutic benefits such as symptom management and slowing the inevitable progression of the disease. The lack of effective medications to treat AD has led to an urgent effort to fill this therapeutic gap, prompting researchers to further investigate the proposed anti-inflammatory and neuroprotective properties of cannabinoids in neurological diseases. This paper will review the current literature related to cannabinoids as potential therapeutic agents in the context of neurodegenerative disease.

Introduction

Neurodegenerative disease is defined by the slow and chronic progression of neuronal loss in specific areas of the nervous system. These types of disorders can affect either motor control and movement or cognitive function and dementia. The neurodegeneration associated with these types of diseases occurs over an extended period of time, often a decade or more. Individuals with a neurodegenerative condition typically do not consciously experience symptoms or clinical manifestations of the disease until irreparable cell damage has already occurred (Iuvone et al., 2009).

As a result of today's aging population, an estimated 115 million people are expected to be affected by neurodegenerative disease impacting cognitive function and dementia by the year 2050 (Vauzour, 2014). The most common form of neurodegenerative dementia in aging individuals is Alzheimer's disease (AD) (Benito et al., 2008). At present there is no known cure for the disease, and the limited treatment options available to AD patients merely slow the progression of the disease and aid in managing its symptoms (Jayant et al., 2016). The prevalence of this condition in combination with the lack of effective pharmacological intervention makes AD one of the most significant global health challenges today. This review will discuss the symptoms and hallmark characteristics of AD, the factors that contribute to its pathology, and the current research available related to cannabinoids as possible compounds of interest in the development of novel therapeutics for patients with AD.

Overview of AD

AD is highly prevalent in modern society, with an estimated 35 million people suffering from the disease worldwide (Sorrentino et al., 2014). The disease is especially common among senior individuals, as aging is the largest risk factor for AD (Sarlus and Heneka, 2017). The average age of onset for this debilitating neurodegenerative condition occurs in adults aged 60-

65 and older (Ahmed et al., 2015). Over time, its progression results in decreased neuronal function, cognitive impairment, behavioral deficits, and dementia (Ramirez et al., 2017).

Although the precise cause of AD is not well established, several links to understanding its complex pathology have been identified. It is widely accepted that there are two pathological hallmarks of the disease which are consistently present in brain tissue of individuals afflicted with AD. The first is extracellular senile plaques, which are composed of an accumulation of misfolded β-amyloid (Aβ) proteins (Sarlus and Heneka, 2017). The second is the presence of neurofibrillary tangles, or tau tangles, which contain an accumulation of hyperphosphorylated tau protein (Morales et al., 2014).

Protein and peptide deposits, microglial activation, neurotoxins, oxidative stress, neuronal loss, and progressive cognitive deficiencies have all been closely linked with AD (Watt et al., 2017). Many of these factors contribute to a strong, persistent immune response resulting in heightened inflammation in the brain (Morales et al., 2014). This neuroinflammation can become chronic, a trait shared by many neurodegenerative diseases. This chronic neuroinflammation has been widely acknowledged as a major contributor to the neurodegeneration associated with AD (Iuvone et al., 2009, Morales et al., 2014). Understanding the specific mechanisms underlying the pathology of AD is a crucial step in identifying effective targets to alter the progression of the disease as either preventative or therapeutic measures.

Overview of Cannabinoids

Types of Cannabinoids

There is increased therapeutic interest in cannabinoids in recent years due to their demonstrated neuroprotective and anti-inflammatory properties in various studies. Cannabinoids are separated into three distinct categories based on source. The first are exogenous or plant-based cannabinoids (also known as phytocannabinoids), then endogenous

cannabinoids (or endocannabinoids), and finally manufactured or synthetic cannabinoids (Iuvone et al., 2009, du Plessis et al., 2015).

The first group are naturally occurring compounds derived from the *Cannabis sativa* plant, more commonly referred to as marijuana (Lu et al., 2017). Recreational use of *C. sativa* has been popular for nearly five thousand years due to its psychoactive and calming properties. Byproducts of the plant were first introduced to Western medicine in the nineteenth century and were harnessed to treat symptoms ranging from anxiety to fever and pain (Iuvone et al., 2009; Lu et al., 2017).

In the 1930s and 1940s, cannabinol (CBN) and cannabidiol (CBD) were isolated and identified as the two major non-psychotropic agents naturally present in *C. sativa* (Iuvone et al., 2009, Walter et al., 2003). It was not until the 1960s that the main psychoactive component of *C. sativa* was first isolated and identified as Δ^9-tetrahydrocannabinol (THC). This discovery was critical in uncovering the endocannabinoid system (Hillard, 2015). Although the use of medicinal cannabis remains controversial, its clinical applications have become increasingly acknowledged and accepted over time. Phytocannabinoids are currently being clinically prescribed in several states in the U.S. and in countries such as Canada, Australia, and Spain to mitigate symptoms such as nausea and pain associated with chemotherapy and cancer, inhibit spastic movements of patients with multiple sclerosis, and increase the appetite of patients suffering from AIDS, among other applications (Lu et al., 2017).

The endocannabinoids are those produced by the endocannabinoid system. This system modulates nearly every region of the brain and influences many functions of the central nervous system (CNS) (Hillard, 2015). This system has been linked to neurodegenerative disorders and has been shown to play a complex role in CNS signaling. The system is composed of endogenous cannabinoids, degradative and synthetic enzymes that assist in the processing of cannabinoids, and cannabinoid receptors (Basavarajappa et al., 2017). Endogenous cannabinoids are comprised of lipids that act on cannabinoid receptors and therefore are

capable of mimicking some of the physiological actions of THC found in exogenous cannabinoids (du Plessis et al., 2015).

Synthetic cannabinoids are a family of heterogeneous compounds designed and manufactured to stimulate the endocannabinoid system through functional resemblance to THC (Antoniou and Juurlink, 2014). For a brief period of time in the early 2000s, synthetic cannabinoids such as Spice and K2 gained unfavorable notoriety for providing individuals seeking to abuse marijuana with a legal opportunity to achieve similar effects. Since that time, scientific studies of various synthetic cannabinoids have demonstrated pharmacological effects similar to THC, although vastly more potent, both *in vitro* and *in vivo* (Castaneto et al., 2014).

Cannabinoid Receptor Subtypes

All three classifications of cannabinoids achieve their physiological effects on the CNS through stimulation of cannabinoid receptors. Two subtypes of cannabinoid receptors are well characterized to date: cannabinoid receptor type 1 (CB1R) and type 2 (CB2R) (Basavarajappa et al., 2017). Both are transmembrane spanning G-protein coupled receptors (GPCRs) (du Plessis et al., 2015). These receptors are coupled to G_i proteins, which have an overall inhibitory physiological effect (Iuvone et al., 2009).

CB1Rs are considered the predominant cannabinoid receptor of the brain due to the high level of expression in the CNS, although they are present in the peripheral nervous system as well (Alger, 2014). CB1Rs are located in various regions of the brain including the hippocampus, basal ganglia, and cerebellum and are thought to play a regulatory role in memory and cognition (Alger, 2014, Talarico et al., 2019). In addition to their neuronal presence, CB1Rs are also present in astrocytes and microglia (Ramírez et al., 2005). Activation of these receptors is believed to decrease intracellular Ca2+ concentrations, inhibit glutamate release (Talarico et al., 2019), enhance GABAergic signaling (Alsasua del Valle, 2006), and increase neurotrophin expression and neurogenesis (Talarico et al., 2019). Endogenous, exogenous, and synthetic cannabinoids can all act on CB1Rs. Activation of these receptors by

cannabinoids has demonstrated neuroprotective effects in various studies (Benito et al., 2008). However, CB1Rs are also responsible for mediating the psychoactive effects of cannabinoids, thereby complicating and greatly limiting the pharmacological possibilities of CB1R as a potential drug target (Maldonado et al., 2011).

CB2Rs, while substantially less abundant in the CNS, appear to play an interesting role in neurodegenerative disease (Talarico et al., 2019). They are primarily located in the periphery, specifically in cells and tissues of the immune system (Benito et al., 2008). However, they also have limited presence in the brain and have been found in microglial cells (Talarico et al., 2019). Interestingly, recent studies found that CB2Rs were significantly upregulated in microglia surrounding senile plaques in the hippocampus of post-mortem AD brains (Talarico et al., 2019), suggesting that these receptors may be involved in the inflammatory pathway of AD (Aso et al., 2016). In contrast to CB1Rs, CB2R activation is not associated with psychoactive effects, making this receptor subtype a more desirable therapeutic candidate than CB1Rs (Buckley et al., 2000). In addition, CB2Rs have demonstrated an ability to be manipulated under neuroinflammatory conditions whereas CB1Rs have not, further promoting increased interest in CB2Rs as a potential therapeutic target for the treatment of neurodegenerative disease (Benito et al., 2008).

Current Literature on AD Pathology and Cannabinoids

Effect of Cannabinoids on Immune Function and Microglial Activation

In recent years, cannabinoids have been shown to decrease Aβ-induced microglial activation and modulate microglial activity, thereby preventing neuroinflammation (Schmöle et al., 2018). Microglia are resident immune cells of the brain and function to detect assaults on the CNS and defend against attacks by initiating an immune response. They also serve a protective role in the brain by promoting phagocytosis, assisting in tissue repair, and maintaining cerebral homeostasis. Microglial detection of CNS damage prompts glial cells to undergo various

phenotypical and secretory changes resulting in what is referred to as an “activated state” (Sarlus and Heneka, 2017). Two different types of microglial activation can occur.

One phenotype, M1 activation, involves an extensive inflammatory response including the release of inducible nitric oxide synthase (iNOS) as well as the release of pro-inflammatory substances such as cytokines, chemokines, and interleukins. Microglia in an M1 state of activation demonstrate increased motility and enhanced phagocytic activity. Under specific conditions, M1 activated microglia can clear protein aggregates and other unwanted materials from the brain. M1 microglia can eventually reach a state of unchecked activation, resulting in continuous neuroinflammation. This chronic M1 activation generates an unrelenting release of neurotoxic factors (pro-inflammatory substances, nitric oxide (NO), hydrogen peroxide, and glutamate), further exacerbating local cell damage and contributing to neuronal death and degeneration (Walter et al., 2003; Ramírez et al., 2017). This state of reactive gliosis is widely accepted to be a key factor in AD pathology (Sarlus and Heneka, 2017).

In contrast, microglia activated in the M2 phenotype serve a restorative anti-inflammatory role. M2 microglia function to reestablish a homeostatic environment in the brain and release anti-inflammatory cytokines and interleukins, thereby alleviating neuroinflammation. Actions of M2 microglia promote the expression of repair genes and stimulate neuronal growth and survival. Microglia possess the ability to change from the M2 to the M1 state throughout the progression of various neurological diseases, thereby changing the landscape of inflammation in the brain (Cherry et al., 2014; Ramírez et al., 2017).

Once pathological concentrations of misfolded Aβ protein are reached in the early-stage AD brain, microglia detect the conditions as harmful and enter an M1 activated state. These M1 microglial cells then initiate a fierce immune response involving the activation of complement, cytokine release, neurotoxic secretions, and phagocytosis in an effort to remove damaging Aβ deposits from the brain (Ramírez et al., 2005; Martín-Moreno et al., 2011; Akiyama et al., 2000). Significant chronic neuroinflammation develops as a result of M1 activation, leading to the

characteristic hyperphosphorylation of the tau protein and subsequent accumulation of neurofibrillary tangles associated with AD (Serrano-Pozo et al., 2011). The inflammation also triggers M1 microglia to migrate toward dying neurons, resulting in increased cell damage in the brain (Walter et al., 2003).

Aβ-induced M1 activation is therefore widely considered to be a significant contributor to the vicious cycle of immune activity that results in chronic neuroinflammation and ultimately neurodegeneration (Kreisl, 2017). As a result, there has been heightened therapeutic interest in pharmacologically inhibiting M1 microglial activation in an effort to reduce the intensity of the immune response and prevent the subsequent chronic neuroinflammation which results in neurodegenerative conditions (Martín-Moreno et al., 2011).

Although CB2Rs do appear to play a role in the neuroinflammatory process, the underlying mechanisms responsible for these actions are poorly understood at present, as both the genetic deletion and pharmacological activation of CB2Rs have been shown to reduce neuroinflammation (Schmöle et al., 2018). A CB2R agonist (JWH-133) has been shown to significantly decrease neurological impairment, mitigate the intensity of microglia and macrophage activity, and decrease the gene expression of markers for microglial and macrophage activation in an *in vivo* mouse model of infarction, thereby contributing to a reduced immune response (Zarruk et al., 2012). When exposed to a mouse model of AD, CBD also decreased cytokine expression resulting in a reduced neuroinflammatory response *in vivo*. In addition, CBD, which can act on both CB1Rs and CB2Rs, successfully blocked microglial activation *in vitro*, preventing the migration of microglia toward dying cells and eliminating their potential to further exacerbate destruction of these neurons (Martín-Moreno et al., 2011).

Martín-Moreno also demonstrated that CB2R agonists have the ability to decrease free radical NO production and reduce harmful levels of oxidative stress affecting Aβ-exposed microglia in culture (Martín-Moreno et al., 2011). Another CB2R-selective agonist (JWH-015) effectively decreased immune activity by limiting microglial production of TNF-α and NO,

thereby reducing levels of pro-inflammatory substances released in the brain (Ehrhart et al., 2005). CBD also reduced factors related to oxidative stress and apoptosis in an *in vivo* model of newborn mice, further supporting the neuroprotective capabilities of cannabinoids (Castillo et al., 2009).

A study utilizing an AD model in mice found that microglial cells harvested from CB2R negative mice were less responsive to pro-inflammatory stimuli than microglia harvested from CB2R positive mice. The transgenic mice lacking CB2R also had lower percentages of microglia and macrophages, reduced expression of pro-inflammatory chemokines and cytokines in the brain, and reduced concentrations of Aβ peptides, again suggesting CB2R likely plays a role in neuroinflammation (Schmöle et al., 2015). THC has also been shown to successfully reduce immune activity by inhibiting macrophage functions *in vivo* in wild type mice but not in CB2R knockout mice, indicating that phagocytic action may be mediated by CB2Rs (Buckley et al., 2000).

Treatment with a CB2R-selective modulator (1-phenylisatin) also significantly reduced neuroinflammation and neuronal damage and reduced Aβ deposits *in vivo* in two different mouse models of AD (Jayanat et al., 2016). CB2R modulators have also been shown to play a role in the migration of activated microglia. One endocannabinoid, 2-arachidonylglycerol (2-AG) triggered microglial motility and has been found to be mediated by CB2Rs. CBN and CBD both prevented 2-AG-induced microglial migration by acting as CB2R antagonists, suggesting the cannabinoid signaling system may be involved in the regulation of microglial migration (Walter et al., 2003).

Effect of Cannabinoids on Tau Hyperphosphorylation and Aβ Deposition

Cannabinoids have also demonstrated the ability to modulate tau hyperphosphorylation and Aβ accumulation and deposition. CB1R agonists have been shown to protect against tau hyperphosphorylation both *in vivo* and in cultured neurons, thereby preventing the formation of

neurofibrillary tangles. CB1R agonists also mitigated cellular changes and behavioral consequences in Aβ-induced mice (Iuvone et al., 2003).

A study analyzing post-mortem AD brain tissue indicated that CB2R expression levels were nearly 40% higher in individuals with AD compared to an age-matched control. Many of these CB2Rs were found surrounding senile plaques in subjects with AD. CB2R levels were also positively correlated with both Aβ protein levels and senile plaque score in post-mortem AD coritcal brain tissues (Solas et al., 2013), providing strong evidence that CB2Rs may play a functional role in Aβ accumulation.

Schmöle et al. showed that CB2R deletion in mice expressing genetic variants of amyloid precursor protein (APP), an Aβ precursor, resulted in neuroprotection by decreasing neuronal loss, lowering senile plaque levels, and correlating with increased Aβ degrading enzyme expression (Schmöle et al., 2018). Activation of CB2R by selective agonist JWH-015 also resulted in neuroprotective effects *in vitro* by reducing the expression of a tumor necrosis factor receptor (CD40) which is directly correlated with Aβ peptide levels. In addition, JWH-015 enhanced microglial phagocytosis of Aβ by suppressing inhibition efforts mediated by CD40 *in vitro* (Ehrhart et al., 2005).

A study by Scuderi et al. indicated that CBD may also be capable of decreasing APP, thereby hindering Aβ formation and diminishing its potential to activate microglia (Scuderi et al., 2013). Iuvone et al. established that CBD-treated cells provided neuroprotection *in vitro* by significantly increasing cell survival following Aβ exposure and provided protection against oxidative stress and apoptotic effects associated with Aβ toxicity (Iuvone et al., 2004). Vallée et al. also demonstrated that CBD inhibits Aβ-induced tau hyperphosphorylation and inhibits another Aβ precursor, thereby decreasing Aβ levels overall. This study also supports the mitigating effects of CBD on oxidative stress, reactive oxygen species production, and pro-inflammatory signaling (Vallée et al., 2017).

Additionally, current literature indicates that cannabinoids may play a role in memory function and cognitive deficits. A study by Albayram et al. showed that mice lacking CB1Rs had increased neuroinflammation in the hippocampus, suggesting that CB1R activity on GABAergic neurons in the hippocampus may protect against age-dependent dementia-like cognitive decline by reducing neuroinflammation (Albayram et al., 2011). Similarly, the genetic deletion of CB1Rs also led to accelerated memory impairment in transgenic CB1 knockout mice, reinforcing the notion that CB1Rs may play a critical role in the progression of AD-related pathology (Aso et al., 2016).

The role of CB2Rs in memory and cognitive functioning again appears to be mechanistically complex. CB2R activation has been shown to improve cognitive impairment in animal models of AD (Aso et al., 2016). Similarly, Jayant et al. demonstrated that a selective CB2R agonist (1-phenylisatin) was able to successfully mitigate learning-memory impairment *in vivo* in an AD model in mice (Jayant et al., 2016). However, Schmöle et al. reported improvements in cognitive and learning deficits following CB2R deletion in mouse models expressing APP (Schmöle et al., 2018). This data reinforces that CB2Rs do appear to play a role in memory and cognition, although their precise mechanism of action still requires further investigation.

Administration of a synthetic cannabinoid, WIN55,212-2, by Ramírez et al. prevented cognitive impairment and loss of neuronal markers in rats (Ramírez et al., 2005). CBD has also demonstrated the ability to both prevent and reverse the progression of cognitive impairment in rodent models of AD (Watt et al., 2017). A new study shows that cells pre-treated with CBD yielded neuroprotective effects against Aβ-mediated hippocampal long-term potentiation (LTP), which is a common cellular mechanistic standard used in the study of memory (Hughes et al., 2019). Another study by Fagherazzi et al. demonstrated that a single high dose injection of CBD was able to rescue memory in rats with iron-induced memory impairment. Chronic CBD administration also demonstrated improved recognition memory in iron-treated rats, suggesting

the potential use of CBD in the treatment of cognitive deficiencies associated with neurodegenerative disease (Fagherazzi et al., 2011).

Conclusion

Current research suggests that cannabinoids may be a promising therapeutic candidate for prevention and protection against neurodegenerative diseases such as AD in the future. Cannabinoids have demonstrated wide-ranging beneficial neuroprotective and therapeutic effects related to Aβ levels, tau hyperphosphorylation, immune activity, neuroinflammation, neuronal loss, and cognition. Recent studies indicate that cannabinoids may be a source of continued therapeutic focus for conditions related to inflammatory conditions and neurodegenerative disease.

Future studies are necessary in order to better understand the mechanistic actions and therapeutic potential of cannabinoids in treating conditions related to neurodegenerative disease. Although research related to cannabinoids in cell culture and in animal models of AD is available, clinical studies of cannabinoids as therapeutic targets in humans are scarce. These studies are necessary to better understand the effects of various cannabinoids in humans with AD pathology. Research related to long-term safety and efficacy of medicinal cannabinoids in humans moving forward is also an area of great need, particularly related to newer cannabinoids such as CBD and synthetic cannabinoids, which have very little long-term data available at present. Although significant research remains to be done, cannabinoids have proven to promising therapeutic potential in the treatment of AD and other inflammatory and neurodegenerative conditions.

LITERATURE CITED

1. Ahmed, A., Van der Marck, M., Van den Elsen, G., and Olde Rikkert, M. (2015). Cannabinoids in late-onset Alzheimer's disease. Clinical Pharmacology and Therapeutics, 97(6), 597-606.

2. Akiyama, H., Barger, S., Barnum, S., Bradt, B., Bauer, J., Cole, G. M., Cooper, N. R., Eikelenboom, P., Emmerling, M., Fiebich, B. L., Finch, C. E., Frautschy, S., Griffin, W. S. T., Hampel, H., Hull, M., Landreth, G., Lih-Fen, L., Mrak, R., Mackenzie, I. R., Rydel, R., Shen, Y., Streit, W., Strohmeyer, R., Tooyoma, I., Van Muiswinkel, F. L., Veerhuis, R., Walker, D., Webster, S., Wegrzyniak, B., Wenk, G., Wyss-Coray, T. (2000). Inflammation and Alzheimer's disease. Neurobiology of aging, 21(3), 383–421.

3. Albayram, O., Alferink, J., Pitsch, J., Piyanova, A., Neitzert, K., Poppensieker, K., Mauer, D., Michel, K., Legler, A., Becker, A., Monory, K., Lutz, B., Zimmer, A., and Bilkei-Gorzo, A. (2011). Role of CB1 cannabinoid receptors on GABAergic neurons in brain aging. Proceedings Of The National Academy Of Sciences Of The United States Of Ame, 108(27), 11256-11261.

4. Alger, B. (2014). Seizing an Opportunity for the Endocannabinoid System. Epilepsy Currents, 14(5), 272-276.

5. Alsasua del Valle, A. (2006). Implication of Cannabinoids in Neurological Diseases. Cellular and Molecular Neurobiology, 26(4-6), 577-589.

6. Antoniou, T., and Juurlink, D. (2014). Synthetic cannabinoids. CMAJ: Canadian Medical Association Journal, 186:3, 210.

7. Aso, E., Andrés-Benito, P., and Ferrer, I. (2018). Genetic deletion of CB1 cannabinoid receptors exacerbates the Alzheimer-like symptoms in a transgenic animal model. Biochemical Pharmacology, 157, 210-216.

8. Aso, E., and Ferrer, I. (2016). CB2 Cannabinoid Receptor As Potential Target against Alzheimer's Disease. Frontiers In Neuroscience, 10: 243.

9. Basavarajappa, B., Shivakumar, M., Joshi, V., and Subbanna, S. (2017). Endocannabinoid system in neurodegenerative disorders. Journal of Neurochemistry, 142(5), 624-648.

10. Benito, C., Tolón, R., Pazos, M., Núñez, E., Castillo, A., and Romero, J. (2008). Cannabinoid CB2 receptors in human brain inflammation. British Journal Of Pharmacology, 153(2), 277-285.

11. Buckley, N. E., McCoy, K. L., Mezey, E., Bonner, T., Zimmer, A., Felder, C. C., Glass, M., and Zimmer, A. (2000). Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoid CB(2) receptor. European Journal of Pharmacology, 396, 141-149.

12. Castaneto, M., Gorelick, D., Desrosiers, N., Hartman, R., Pirard, S., and Huestis, M. (2014). Synthetic cannabinoids: Epidemiology, pharmacodynamics, and clinical implications. Drug and Alcohol Dependence, 144, 12-41.

13. Castillo, A., Tolón, M. R., Fernández-Ruiz, J., Romero, J., and Martinez-Orgado, J. (2010). The neuroprotective effect of cannabidiol in an in vitro model of newborn hypoxic-ischemic brain damage in mice is mediated by CB2 and adenosine receptors. Neurobiology of Disease, 37(2), 434-440.

14. Cherry, J. D., Olschowka, J. A., and O'Banion, M.K. (2014). Neuroinflammation and M2 microglia: the good, the bad, and the inflamed. Journal of Neuroinflammation, 11(1), 98.

15. Dani, M., Wood, M., Mizoguchi, R., Fan, Z., Walker, Z., Morgan, R., Hinz, R., Biju, M., Kuruvilla, T., Brooks, D. J., and Edison, P. (2018). Microglial activation correlates in vivo with both tau and amyloid in Alzheimer's disease. Brain : A Journal of Neurology, 141(9), 2740-2754.

16. du Plessis, S. S., Agarwal, A., and Syriac, A. (2015). Marijuana, phytocannabinoids, the endocannabinoid system, and male fertility. Journal of assisted reproduction and genetics. 32(11), 1575–1588.

17. Ehrhart, J., Obregon, D., Mori, T., Hou, H., Sun, N., Bai, Y., Klein, T., Fernandez, F., Tan, J., and Shytle, R. D. (2005). Stimulation of cannabinoid receptor 2 (CB2) suppresses microglial activation. Journal of Neuroinflammation, 2:29.

18. Fagherazzi, E., Garcia, V., Maurmann, N., Bervanger, T., Halmenschlager, L., Busato, S., Hallak, J., Zuardi, A., Crippa, J., and Schröder, N. (2011). Memory-rescuing effects of cannabidiol in an animal model of cognitive impairment relevant to neurodegenerative disorders. Psychopharmacology, 219(4), 1133-40.

19. Hillard, C. (2015). The Endocannabinoid Signaling System in the CNS: A Primer. Endocannabinoids, 125, 1-47.

20. Hughes, B., and Herron, C. E. (2019). Cannabidiol Reverses Deficits in Hippocampal LTP in a Model of Alzheimer's Disease. Neurochemical Research, 44(3), 703-713

21. Iuvone, T., Esposito, G., Esposito, R., Santamaria, R., Di Rosa, M., and Izzo, A. (2004). Neuroprotective effect of cannabidiol, a non-psychoactive component from Cannabis sativa, on beta-amyloid-induced toxicity in PC12 cells. Journal of Neurochemistry, 89(1),

134-141.

22. Iuvone, T., Esposito, G., Filippis, D., Scuderi, C., and Steardo, L. (2009). Cannabidiol: A promising drug for neurodegenerative disorders? CNS Neuroscience and Therapeutics, 15(1), 65-75.

23. Jayant, S., Sharma, B. M., Bansal, R., and Sharma, B. (2016). Pharmacological Benefits of Selective Modulation of Cannabinoid Receptor Type 2 (CB2) in Experimental Alzheimer's Disease. Pharmacology Biochemistry and Behavior, 140: 39-50.

24. Kreisl, W. (2017). Discerning the relationship between microglial activation and Alzheimer's disease. Brain, 140(7), 1825-1828.

25. Lu, Y., and Anderson, H. (2017). Cannabinoid signaling in health and disease. Canadian Journal of Physiology and Pharmacology, 95(4), 311-327.

26. Maldonado, R., Berrendero, F., Ozaita, A., and Robledo, P. (2011). Neurochemical basis of cannabis addiction. Neuroscience, 181, 1-17.

27. Martín-Moreno, A., Reigada, D., Ramírez, B., Mechoulam, R., Innamorato, N., Cuadrado, A., and De Ceballos, M. (2011). Cannabidiol and other cannabinoids reduce microglial activation in vitro and in vivo: Relevance to Alzheimer's disease. Molecular Pharmacology, 79(6), 964-73.

28. Morales, I., Guzman-Martinez, L., Cerda-Troncoso, C., Farias, G., and Maccioni, R. (2014). Neuroinflammation in the pathogenesis of Alzheimer's disease. A rational framework for the search of novel therapeutic approaches. Frontiers In Cellular Neuroscience, 8.

29. Ramírez, A., De Hoz, R., Salobrar-Garcia, E., Salazar, J., Rojas, B., Ajoy, D., López-Cuenca, I., Rojas, P., Alberto, T., Ramírez, J. (2017). The Role of Microglia in Retinal Neurodegeneration: Alzheimer's Disease, Parkinson, and Glaucoma. Frontiers in Aging Neuroscience, 9, 214.

30. Ramírez, B., Blázquez, C., Gómez del Pulgar, T., Guzmán, M., and de Ceballos, M. (2005). Prevention of Alzheimer's Disease Pathology by Cannabinoids: Neuroprotection Mediated by Blockade of Microglial Activation. Journal of Neuroscience, 25(8), 1904-1913.

31. Sarlus, H., and Heneka, M. (2017). Microglia in Alzheimer's disease. The Journal of Clinical Investigation, 127(9), 3240-3249.

32. Schmöle, A-C., Lundt, R., Toporowski, G., Hansen, J., Beins, E., Halle, A., and Zimmer, A. (2018). Cannabinoid Receptor 2-Deficiency Ameliorates Disease Symptoms in a

Mouse Model with Alzheimer's Disease-Like Pathology. Journal of Alzheimer's Disease, 64(2): 379-392.

33. Schmöle, A-C. Lundt, R., Ternes, S., Albayram, Ö., Ulas, T., Schultze, J. L., Bano, D., Nicotera, P., Alferink, J., and Zimmer, A. (2015). Cannabinoid receptor 2 deficiency results in reduced neuroinflammation in an Alzheimer's disease mouse model. Neurobiology of Aging, 36(2), 710-719.

34. Scuderi, C., Steardo, L., and Esposito, G. (2014). Cannabidiol promotes amyloid precursor protein ubiquitination and reduction of beta amyloid expression in SHSY5Y^{APP+} cells through PPARγ involvement. Phytotherapy Research, 28(7), 1007-1013.

35. Serrano-Pozo, A., Mielke, M., Gómez-Isla, T., Betensky, R., Growdon, J., Frosch, M., and Hyman, B. (2011). Reactive Glia not only Associates with Plaques but also Parallels Tangles in Alzheimer's Disease. The American Journal of Pathology, 179(3), 1373-1384.

36. Solas, M., Francis, P., Franco, R., and Ramirez, M. (2013). CB2 receptor and amyloid pathology in frontal cortex of Alzheimer's disease patients. Neurobiology of Aging, 34(3), 805-808.

37. Sorrentino, Pierpaolo, Iuliano, Antonietta, Polverino, Arianna, Jacini, Francesca, and Sorrentino, Giuseppe. (2014). The dark sides of amyloid in Alzheimer's disease pathogenesis. FEBS Letters, 588(5), 641-652.

38. Talarico, G., Trebbastoni, A., Bruno, G., and De Lena, C. (2019). Modulation of the Cannabinoid System: A New Perspective for the Treatment of the Alzheimer's Disease. Current Neuropharmacology, 17(2), 176-183.

39. Vallée, A., Lecarpentier, Y., Guillevin, R., and Vallée, J. (2017). Effects of cannabidiol interactions with Wnt/β-catenin pathway and PPARγ on oxidative stress and neuroinflammation in Alzheimer's disease. Acta Biochimica Et Biophysica Sinica, 49(10), 853-866.

40. Vauzour, D. (2014). Effect of flavonoids on learning, memory and neurocognitive performance: Relevance and potential implications for Alzheimer's disease pathophysiology. Journal of the Science of Food and Agriculture, 94(6), 1042-1056.

41. Walter,L., Franklin, A., Witting, A., Wade, C., Xie, Y., Kunos, G., Mackie, K., and Stella, N. (2003). Nonpsychotropic cannabinoid receptors regulate microglial cell migration. Journal Of Neuroscience, 23(4), 1398-1405.

42. Watt, G. and Karl, T. (2017). *In vivo* Evidence for Therapeutic Properties of Cannabidiol (CBD) for Alzheimer's Disease. Frontiers in Pharmacology, 8, 20.

43. Zarruk, J., Fernández-López, D., García-Yébenes, I., García-Gutiérrez, M., Vivancos, J., Nombela, F., Torres, M., Burguete, M., Manzanares, J., Lizasoain, I., and Moro, M. (2012). Cannabinoid type 2 receptor activation downregulates stroke-induced classic and alternative brain macrophage/microglial activation concomitant to neuroprotection. Stroke, 43(1), 211-219.