Review

Emerging potential of cannabidiol in reversing proteinopathies

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ABSTRACT

The aberrant accumulation of disease-specific protein aggregates accompanying cognitive decline is a pathological hallmark of age-associated neurological disorders, also termed as proteinopathies, including Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, amyotrophic lateral sclerosis and multiple sclerosis. Along with oxidative stress and neuroinflammation, disruption in protein homeostasis (proteostasis), a network that constitutes protein surveillance system, plays a pivotal role in the pathobiology of these dementia disorders. Cannabidiol (CBD), a non-psychotropic phytocannabinoid of Cannabis sativa, is known for its pleiotropic neuropharmacological effects on the central nervous system, including the ability to abate oxidative stress, neuroinflammation, and protein misfolding. Over the past years, compelling evidence has documented disease-modifying role of CBD in various preclinical and clinical models of neurological disorders, suggesting the potential therapeutic implications of CBD in these disorders. Because of its putative role in the proteostasis network in particular, CBD could be a potent modulator for reversing not only age-associated neurodegeneration but also other protein misfolding disorders. However, the current understanding is insufficient to underpin this proposition. In this review, we discuss the potentiality of CBD as a pharmacological modulator of the proteostasis network, highlighting its neuroprotective and aggregates clearing roles in the neurodegenerative disorders. We anticipate that the current effort will advance our knowledge on the implication of CBD in proteostasis network, opening up a new therapeutic window for aging proteinopathies.

1. Introduction

Disruption in proteostasis network and protein misfolding are two major drivers in the pathobiology of age-associated neurodegenerative diseases (NDDs), including Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), Amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS). NDDs are generally characterized by the presence of protein aggregates either in the nucleus or cytoplasm (Menzies et al., 2015; Sarkar, 2013), and the region-specific neuronal death with a consequence of motor and cognitive deficits (Lumkwana et al., 2017; Skovronsky et al., 2006). A line of evidence supports the concept that NDDs are proteinopathies, where they share fundamental features of protein aggregate, for example, tau and amyloid-β in AD, α-synuclein in PD, huntingtin (Htt) in HD, etc. (Boland et al., 2018; Golde et al., 2013; Marsh, 2019). Proteostasis network constitutes the protein surveillance system that regulates all aspects of the cellular proteome, from protein synthesis to clearance of misfolded proteins (Soares et al., 2019). Evidence from the recent studies correlates the higher incidence of NDD with the progressive failure of the proteostasis network, which results in proteotoxic stress that reduces both repair and/or clearance of misfolded protein; and thus contributes to pathological aging (Labbadia and Morimoto, 2015; López-Otín et al., 2013; Martínez et al., 2017; Morimoto and Cuervo, 2014). The proteostasis network is impaired by oxidative stress (OS), which is a pathological condition arising from excess production of reactive oxygen species (ROS) due to starvation, exposure to antibiotics (Morano et al., 2012),
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inflammation (Ravishankar et al., 2015), disease-associated mutations, polymorphisms, energetic deficits and aging (Ferrington and Gregerson, 2012; Luo et al., 2017; Powers et al., 2009; Reichmann et al., 2018), where it plays vulnerable roles in disrupting proteostasis by causing oxidative damage and neuroinflammation, leading to cell death (Korovila et al., 2017; Powers et al., 2009).

Accumulating evidence suggests that endocannabinoid systems regulate the functionality of redox homeostasis in different cell types (Ambrozewicz et al., 2018; Lipina and Hundal, 2016), thus maintain an equilibrium state between the redox system and pro-oxidant state (Gomes et al., 2018; Llanos-Gonzalez et al., 2020). The endocannabinoid systems, consisting of cannabinoid receptors (CB1 and CB2), are either activated or antagonized by endocannabinoids and phytocannabinoids (Palozzi et al., 2018). Phytocannabinoids, such as cannabidiol (CBD), cannabivarin, delta-9-tetrahydrocannabinol (THC), cannabidiolin, and cannabigerol, have been widely studied for their involvement in endo-
cannabinoid systems (Linge et al., 2016). CBD is one of the fascinating non-psychoactive phytocannabinoids with well-known anti-oxidant and anti-inflammatory properties (Giacoppo and Mazzon, 2016; Huestis et al., 2019).

CBD has shown to provide neuroprotection (Campos et al., 2016) and thus become a therapeutic option in neurodegenerative disorders like AD, PD, HD, ALS, and MS, where treatment slows down disease progression (Iavone et al., 2009). Remarkably, disease-modifying mecha-
nisms of CBD are attributed to its antioxidant, anti-inflammatory, and neuroprotective potentials: the precise mechanisms, however, remain unclear, specifically in the regulation of proteostasis network (HAMP-SON et al., 2000). In this review, an attempt has been made to link CBD-mediated pharmacological effects with the proteostasis network, providing a more extensive area for future research on CBD pharma-
cotherapy in the management of neurodegenerative disorders.

2. Cannabidiol chemistry, bioavailability, and toxicity

The plant, C. sativa, serves as a primary source of CBD, where CBD is available up to 40 % in the organic extract (Fernandez-Ruiz et al., 2013). CBD from cannabis was first reported in the late 1930s and pu-
rified in 1940; however, structure and stereochemistry were first elucidated in the 1960s by Mechoulam et al. (Mechoulam et al., 1970).

The biosynthesis of CBD is usually triggered by the leading precursor cannabigerolic acid, which is derived from a phytocannabinoid pre-
cursor, olivetolic acid. Cannabigerolic acid is further converted into c

annabinichromenic acid, tetrahydrocannabinolic acid, and cannabidiolic acid, where cannabidiolic acid forms CBD (Bonini et al., 2018; Hanus et al., 2016). Comparatively, CBD is the major non-psychomimetic compound present in the plant, well-tolerated (Mechoulam et al., 2007), and has a broad spectrum of potential therapeutic properties, including anxiolytic (Campos et al., 2012), anticonvulsant (Linge et al., 2016), anti-inflammatory (Esposito et al., 2011; Mecha et al., 2013; Napimoga et al., 2009), neuroprotective (Campos et al., 2015, 2012; Patel et al., 2012; Perez et al., 2013; Schiavon et al., 2014), and immunomodulatory (Kozela et al., 2010). Until now, the molecular pharmacology of CBD remains unclear, and little is specified about the potential signaling pathways that are regulated through the CBD.

CBD-induced pharmacological actions are based on their interactions with two classic cannabinoid receptors, CB1 and CB2, along with other various types of receptors. Despite a low affinity for both CB1 and CB2 receptors, CBD at doses equivalent to or lower than 1 μM can still interact with these receptors and capable of antagonizing CB1/CB2 re-
cptor activity (Machado Bergamaschi et al., 2011). CBD and its enantiomer, (−)-CBD interact with TRPV1 receptor with an EC50 calculated between 3.2 and 3.5 μM in vitro (Bisogno et al., 2001) and at dose 10 mg/kg in the rat in vivo model of acute inflammation (Costa et al., 2004). Besides, using cell-based Ca2+ mobilization and electrophysiological assays, CBD was identified as a potent TRPV2 receptor agonist with EC50 value 3.7 μM in cultured rat DRG neurons (Qin et al., 2008). Research

studies have shown that CBD also linked with 5-HT1A receptor, and this interaction has been suggested for preventing cerebral infarction during ischemia in MCA occluded mice at dose 3 mg/kg, i.p. (Mishima et al., 2005) as well as for anxiolytic impact in rat model (dose: 30 nmol, i.p.) (Campos and Guimaraes, 2008). Using CHO cell line, Russo et al. provide evidence that 16 μM CBD acts as an agonist at the 5-HT1A serotonin receptor (Russo et al., 2005). Moreover, CBD at dose 100 μM allosteri-
cally modulates both μ and δ receptors with EC50 values 4.38 μM and 4.10 μM in rat cerebral cortex membrane homogenates (Kathmann et al., 2006).

Interestingly, in an in vitro evaluation, CBD has also been observed to remarkably antagonize the novel cannabinoid receptor GPR55 with an IC50 of 445 nM (Ryberg et al., 2007). Studies also suggest that CBD (1 μM) blocks voltage-gated Ca2+ channel (T-type) by 45 % in mouse sensory neuron (Ross et al., 2008) and directly increase the ac-
tivity of the inhibitory glycine receptors including, α1-glycine receptor with EC50 value 132.4 μmol/l and α1β-glycine receptor with EC50 value 144.3 μmol/l (Ahrens et al., 2009). CBD markedly stimulate the activity of FAAH (fatty acid amide hydrolase, AEA degrading enzyme) in a concentration-dependent manner both in vitro treatment of CD-1 nude mice (0.5 mg/mouse) and in vitro experiments of U87 cells (16 μmol/l, 24 h incubation) (Massi et al., 2008). CBD (20 μM) also significantly binds to and stimulates transcriptional activity of PPARY (O’Sullivan et al., 2009).

Studies have suggested that CBD (100 μM) exerts robust

neuroprotection in mice with hypo-ischemic brain damage through acting on adenosine A2 receptor (Castillo et al., 2010). In addition, CBD (1–30 μM) suppressed the functions of α2-Ach receptor recorded in rat hippocampal slices with an IC50 value of 12.7 μM (Mahgoub et al., 2013). In combination therapy, CBD alleviates some of the adverse ef-
tects of THC, such as cognitive impairment, psychosis, schizophrenia-like effects (D’Souza et al., 2004; Morgan and Curran, 2008; Morgan et al., 2010). The pharmacokinetics of CBD is quite complex, and various studies suggest several potential routes of administration. The oral bioavailability of CBD is ranged from 13 % to 19 % with a substantial first-pass impact, whereas the systemic bioavailability of inhaled CBD was 31 % (range 11–45 %) for a com-
munity of cannabis users. The specification of plasma was identical to THC. At chronically administered oral daily doses of CBD 10 mg/kg/day, the average plasma concentration of CBD was 5.9–11.2 mg/mL (Scuderi et al., 2009). When injected, CBD is absorbed rapidly and easily crosses the blood-brain barrier (BBB), owing to its lip-
ophlicity, which in turn provides sustained release of CBD (Gro-
tenhermen, 2003). CBD delivery is controlled by its high lipophilicity and an approximate volume of distribution ~32 l/kg with prompt dissemination in the fat tissue, brain, and other organs (Devinsky et al., 2014). CBD is also exceedingly protein-bound, and ~10 % is associated with red blood cells (Koo and Kang, 2017). It is predominantly metab-
olized by the liver, as in other cannabinoids, whereby cytochrome P450 (CYP) enzymes, primarily by CYP3A and CYP2C isozymes groups hy-
droxylating it to 7-OHCBD. This metabolite is then substantially more metabolized in the liver, and the subsequent metabolites are eliminated to feces and slightly less into the urine. The half-life of CBD is 18–32 hours in humans, with a clearance of 960–1560 ml/min after the single dosage given in prolonged cannabis users (Corrigan, 2008). Without worsening of psychotic symptoms, CBD has well endured in patients with dosages up to 600 mg (Weltly et al., 2014). No significant CNS impacts or consequences for vital signs or mood changes were identified in a minority of placebo-controlled studies conducted at dosages up to 1500 mg/day (p.o.) of 4.8 mg (i.v.) in both placebo and persistent administration (Bergamaschi et al., 2011). For adults, there is a possible hazard of immunosuppression because CBD has been identified to repress anti-inflammatory factors IL-10 and IL-8 as well as to induce apoptosis of lymphocytes (Weltly et al., 2014; Wu et al., 2008). In humans and other species, CBD exhibits very low toxicity: with an LD50 of 212 mg/kg after administered intravenously to rhesus monkeys (Rosenkrantz et al., 1981). The oral LD50 has not been reported yet; however, Rosenkrantz has demonstrated, an oral dose of CBD that was
20–50 times higher than intravenous dose was sufficiently high to cause severe toxicity (Rosenkrantz et al., 1981). Besides, a broad range of studies has failed to identify CBD-inducing mutagenic or teratogenic effects (Scuderi et al., 2009).

3. Molecular hallmarks of neurodegeneration

The aberrant accumulation of misfolded proteins or protein aggregates in the brain is the main hallmarks of neurodegeneration, where the NDDs are categorized based on the type of protein deposition or by known genetic mechanisms. These disorders, caused by misfolded proteins, are also known as proteinopathies, where the protein conformation is being critically altered (Golde and Miller, 2009; Uversky, 2009). For each disease, the clinical manifestation is initiated with the repeated production of a specific protein, which is misfolded, aggregated, and, hence, affects specific neurons (Dickson et al., 1971). In the basal state, misfolded proteins are either refolded correctly or degraded by the quality control system, like by chaperone proteins (Gandhi et al., 2019). During cellular aging, under proteotoxic stress or mutation, misfolded proteins escape this system and then aggregated into amorphous assemblies and oligomers, ranging to highly ordered amyloid fibrils plaques. Once formed, higher-order amyloid aggregates are highly resistant to degradation. Several factors play a critical role in favor of this transition, including post-translational modifications, environmental changes, chemical alterations, in addition to genetic mutations. These factors alter the hydrophobicity or net charge of the protein, which reduces conformational stability and also affect the protein quality control system (Vanni et al., 2020).

Furthermore, cellular insults, like calcium-induced protein misfolding, mitochondrial dysfunction, and inflammation, are also associated with protein aggregation, where mitochondrial dysfunction confers upregulation of ROS/RNS (reactive nitrogen species) in the cells, which leads to cell death (dos Santos, 2015). On the other hand, overstimulation of NMDA receptors also caused excessive intracellular calcium accumulation, which promotes ROS/RNS production that impedes quality control mechanisms. Misfolded proteins also activate microglia and astrocytes, causing the release of proinflammatory mediators and cytokines that activate several mechanisms, which result in ROS/RNS generation, mitochondrial dysfunction, and neuronal apoptosis (Gu et al., 2010). Moreover, excessive aggregation leads to develop a vicious cycle of cell toxicity, where they interact with the membrane systems and establish transmembrane pores, further causing an influx of calcium (Solomon et al., 2012). In this perspective, CBD could be a fascinating molecule for a particular interest in neurodegenerative disorders because of having anti-inflammatory, antioxidant, and neuroprotective potentiality. A wealth of literature highlights the therapeutic roles of CBD in a wide range of neurotoxicity and NDD models, which are discussed in subsequent sections.

4. CBD-mediated neuroprotection against oxidative stress (OS)

OS is a pathological condition resulting from an imbalance of pro- and anti-oxidant molecules (Melo et al., 2011). Because of high metabolic demand and huge turnover in brain cells, neurons are particularly highly prone to OS. Prolonged OS causes a depletion of cellular anti-oxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and non-enzymatic components like glutathione (GSH), leaving the cellular antioxidant defense system exhausted (Hannan et al., 2020). OS may also lead to inflammation, protein misfolding, mitochondrial dysfunction, impairment of the DNA repair system, glial activation, and ultimately cellular damages, which are critically implicated in the development of neurodegenerative disorders (Chen et al., 2012; Kim et al., 2015).

Several studies demonstrate the neuroprotective effects of CBD, owing to its antioxidant activity. A study by Pan et al. showed that CBD mitigated cisplatin-induced oxidative/nitrosative stress by downregulating the expression of superoxide-generating enzymes RENOX (NOX4) and NOX1 in mice (Salazar et al., 2009). In the Fenton reaction based system, CBD can transfer electrons under variable voltage potential as well as prevent dihydrodorhodamine oxidation similar to the synthetic antioxidant butylated hydroxytoluene (BHT). CBD also showed protection against tert-butyl hydroperoxide-induced neurotoxicity in a concentration-dependent manner (Hampson et al., 1998). Moreover, in the glutamate neurotoxicity model, CBD was shown higher neuroprotective efficacy than the popular antioxidants, α-tocopherol (vitamin E), and ascorbate (vitamin C) (Hampson et al., 1998). Iuvone et al. demonstrated that CBD (10 μM) attenuated apoptosis in PC12 cells by reducing intracellular calcium accumulation, lipid peroxidation, ROS generation, and downregulating caspase-3 level. CBD also demonstrated an antioxidant effect by inhibiting inducible nitric oxide synthase protein expression and nitric oxide production, followed by blocking p38 MAP kinase phosphorylation and the NF-κB activation (Esposito et al., 2006a; Iuvone et al., 2004). In the H2O2 induced OS model, CBD has shown to protect primary hippocampal neurons, oligodendrocyte progenitor cells, and cerebellar granule cells (Lupica et al., 2017; Mecha et al., 2012; Ricart and Fiszman, 2001). Juknat and his colleagues have paid an effort to identify underlying molecular mechanisms of CBD-mediated antioxidation in BV-2 microglial cells (Juknat et al., 2012). The study reveals that CBD can modulate redox homeostasis and ROS generation by regulating Nrf2/ARE-1 axis and (FpRe/ARE-E)-Nr2/ATF4 system, respectively. Similar results were observed in a study with keratinocyte (Jastrzab et al., 2019). However, a recent study identified CBD as a relatively weak inducer of Nrf2, although it strongly upregulates HMOX1 by inhibiting BACH1 (Casares et al., 2020). Nrf2 is a transcription regulator of various antioxidant factors, whereas HO-1, one of the targets of Nrf2, is an enzyme that provides antioxidant properties by the rate-limiting reaction in heme catabolism (Gozzelino et al., 2010).

5. CBD-mediated neuroprotection against neuroinflammation

The phenomenon of neuroinflammation includes a complex reaction of glial activation related to inflammatory mediators like chemokines or cytokines secretion and ROS/RNS generation (Milatovic et al., 2017). Accumulation of misfolded protein or protein aggregates is often triggered by ROS/RNS (Branca et al., 2019; Mecha et al., 2012), which in turn activates proinflammatory responses and thus sustains neuroinflammation (Solleiro-Villavicencio and Rivas-Arancibia, 2018). Notably, molecular pathways regulated by CBD, as described in OS, are also implicated in neuroinflammation. As a result, CBD can manage neuroinflammation not only by reducing OS but also by producing anti-inflammatory substances and regulating proinflammatory responses (De Ternay et al., 2019; Dos-Santos-Pereira et al., 2020; Esposito et al., 2011; Hartmann et al., 2019; Olah et al., 2014).

Several studies demonstrated that proinflammatory responses, including chemokines and cytokines, are mediated through NF-κB signaling, which promotes inflammatory cascade upon the microglial activation (Campos et al., 2016; Karunaweera et al., 2015). NF-κB signaling consequently plays an essential role in neuronal plasticity as well as in the cellular response to brain injury by upregulating cytokines in astrocytes and microglia, especially TNF-α, and IL-6 and many others reviewed elsewhere (Mattson and Camandola, 2001). The regulation of NF-κB signaling, however, is repressed by the activation of peroxisome proliferator-activated receptor γ (PPAR-γ) (Necela et al., 2006). A line of studies demonstrated that CBD reduced the amount of IL-1β, IFN-β, TNF-α, IFN-γ, IL-6, IL-17, NO, and COX-2 through the activation of PPAR-γ (Al-Ghezi et al., 2019a; Cabral and Griffin-Thomas, 2009; Giancoppo et al., 2015a; O’Sullivan et al., 2009; Peres et al., 2016; Rajan et al., 2016); while increasing the production of anti-inflammatory cytokines IL-4 and IL-10, and impeding iNOS expression (Rajan et al., 2016). In the lipopolysaccharide-stimulated animal model, CBD reduced the secretion of proinflammatory cytokines (IL-1β and TNF-α) and other
By inhibiting ROS/NF-κB pathway, CBD can lower glucose uptake in the microglial cell, which is essential for the activation of microglia (Dos-Santos-Pereira et al., 2020; Gloire et al., 2006), followed by downregulating NADPH oxidase and IκB kinase-2 (Dos-Santos-Pereira et al., 2020).

Additionally, CBD showed a suppressive role in the immune system,
as evidenced by improving innate and adaptive immune responses in a chronic inflammatory model (Lee et al., 2016). Ruiz-Valdepeñas et al. represented that CBD reduced leucocyte recruitment and TNF expression in the central nervous system (Ruiz-Valdepeñas et al., 2011). Furthermore, Juknat et al. found that CBD regulates Th17 proliferation and STAT1/STAT3 balance, which suppresses microglial cell activation (Juknat et al., 2012) and reduces inflammatory cytokine IL-6 and IL-17 secretion (Kozela et al., 2015). Immune regulatory effects of CBD are based on the strong upregulation of CD4+ and CD25 molecules LAG3 and CD69 (Kozela et al., 2015). Besides, activation of mitogen-activated protein kinases like p38/MAP-kinases may lead to the upregulation of proinflammatory mediators during inflammation. Interestingly, CBD can inhibit the p38 phosphorylation, which sequentially reduces the neurotoxic effects with uncontrolled immune reactions (Esposito et al., 2006b).

The positive effects of CBD are linked to the expression of brain-derived neurotrophic factor (BDNF) and proinflammatory cytokines to interact with intracellular pathways in neuronal survival (Campos et al., 2012; Fernandez-Ruiz et al., 2013). BDNF is a vital neurotrophin for neuronal development and survival, cognitive function, and synaptic plasticity (Travaglia and La Mendola, 2017). Barichello et al. found that low brain BDNF levels and augmented proinflammatory cytokines in rats exposed to an experimental model of meningitis were associated with poor cognitive performance. In this regard, CBD therapy minimized these effects (Barichello et al., 2012). Using rat hippocampus, a study based on an amphetamine-induced OS model showed that CBD increased the levels of BDNF as a model to investigate mania (Valvassori et al., 2011). On the other hand, the upregulation of BDNF expression by CBD was also correlated with anti-inflammatory activity, decreasing TNF-α and IL-6 levels in the prefrontal cortex and the hippocampus (Campos et al., 2015).

In combination therapy, CBD supplementation with THC suppresses miRNA-mediated neuroinflammation (Felli et al., 2015; Moreno-Martet et al., 2015). This conjugated therapy reduces TH1 and TH2 expression and neuroinflammation in murine experimental autoimmune encephalomyelitis (EAE) model system, which was mediated through CB1 and CB2 receptors. Again, CBD therapy combination with THC has been reported to reduce CD4+ T cell proliferation in the brain and pro-inflammatory cytokines IL-1β, IL-6, INF-γ, IL-17, TNF-α, and TBX21 and enhanced the production of anti-inflammatory molecules like STAT5b, Foxp3, TGF-β, IL-4, and IL-10. The miRNA microarray data revealed that THC + CBD upregulated miR-706-5p and miR-7116, whereas, suppressed miR-21a-5p, miR-31-5p, miR-122-5p, miR-146a-5p, miR-150-5p, miR-155-5p, and miR-27b-5p (Al-Ghezi et al., 2019b). The pathway analysis revealed that most of the downregulated miRNA’s targeted cell cycle-arrest and apoptosis molecules, such as CCNG1, CDKN2A, and BCL2L11, and anti-inflammatory molecules such as Foxp3 and SOCS1 (Al-Ghezi et al., 2019b).

Studies suggested that CBD has no or little effect on endocannabinoid receptors. However, depending on the concentration, CBD can act as both agonist or antagonist to the various receptors (Table 1), including ionotropic (TRP) as well as voltage-gated sodium channel, nuclear (PPAR) receptors, and also cannabinoid receptors (CB1 and CB2), albeit (De Petrocellis et al., 2017; Ghovanloo et al., 2018; Giacoppo et al., 2017).
6. CBD-mediated protection against calcium-induced protein misfolding

Calcium (Ca\(^{2+}\)) ions are the critical factor in intracellular signaling by regulating second messengers in the systems and used as a cofactor for some enzymes. Although Ca\(^{2+}\) is prominent in cell physiology, its imbalance severely disrupts protein conformation (Grzybowska, 2018). Growing evidence supports the concept that the accumulation of excessive Ca\(^{2+}\) in the cell induces OS, which promotes protein aggregation, leading to cell death. Oxidative reactive species, such as ROS/RNS, modify misfolded proteins highly oxidized and cross-linked, leaving them more prone to aggregates. These aggregated forms act as endogenous protease inhibitors (Chen et al., 2012). Consequently, reduced activity of the proteasomal system, the primary machinery for the removal of oxidized and misfolded protein, leads to further accumulation of protein aggregate (Ciechanover and Brundin, 2003; Dahlmann, 2007; Jung et al., 2009; Lee et al., 2010; Seifert et al., 2010). These protein aggregates can interact with the lipid bilayer of the cell membranes, causing membrane disruption or pore formation (Andreasen et al., 2015; Di Scala et al., 2016), which eventually disrupts ion homeostasis (Shrivastava et al., 2017; Soto, 2003). Studies also showed an interaction between protein aggregates with cellular receptors, including mGluR5, causing gain or loss of function in the signaling platform, resulting in the upregulation of NMDAR (N-methyl-D-aspartic acid receptor)-dependent Ca\(^{2+}\) response (Shrivastava et al., 2017).

NMDAR is one of the ionotropic glutamate receptors dealing with Ca\(^{2+}\) regulation, along with Na\(^{+}\) and K\(^{+}\) in the cytoplasm (Carvajal et al., 2016; Zhang et al., 2016). However, overstimulation of NMDAR exaggerates the massive influx of Ca\(^{2+}\), which leads to energy loss with depolarization of mitochondrial Ca\(^{2+}\) and neuronal apoptosis by the activation of caspase pathways (Leist et al., 1997). An excessive influx of Ca\(^{2+}\) gives rise to the production of ROS successively with the rising oxygen tension (Tenneti et al., 1998).

Several studies showed that CBD has an antioxidant activity (Conroe et al., 1982; Jones et al., 2010; Wallace et al., 2001), focusing its effect on NMDAR regulation (Fig. 2). Indeed, Azza B.El-Remessy et al. found that CBD decreased nitrate/nitrite, lipid peroxides, and nitrotyrosine expression, which subsequently protects neurons from NMDA induced injury (El-Remessy et al., 2003). Moreover, CBD was shown to inhibit glutamate release in the brain hypoxia model by acting on both CB2 and adenosine receptors but mainly on A\(_{1}\)R (Castillo et al., 2010). Linge et al. found a correlation between CBD-mediated glutamate signaling and serotonergic systems, where glutamate regulation is maintained by 5-HT\(_{1A}\) receptor-dependent mechanism (Linge et al., 2016). Strikingly, Gobira et al. found that activation of mTOR by CBD is associated with a subsequent reduction in glutamate release (Gobira et al., 2015). However, substantial evidence indicates that CBD behaves as an antagonist for chaperone protein e\(_{1}\)R, which is a viable target to treat neuroapathy pain by reducing the influence of glutamate NMDARs (Diaz et al., 2009; Kim et al., 2006; Rodriguez-Munoz et al., 2018; Romero et al., 2012). The e\(_{1}\)R antagonist also inhibits G protein-coupled receptors (GPCRs), which subsequently reduces the actions of NMDARs (Rodriguez-Munoz et al., 2015; Rodriguez-Munoz et al., 2015). They produce secondary messengers and control homeostasis of calcium by triggering PKA, which is responsible for activating the calcium channels (Du et al., 2019).

A variety of GPCRs such as CB1 and CB2, orphan GPCRs such as GPR6, GPR3, GPR18, GPR12, and GPR55, along with adenosine, serotonin, and opioid receptors are found to be modulated by CBD (Morales and Reggio, 2017). Along with GPCRs, voltage-gated calcium channels (VGCCs) increase calcium influx due to constant hyperpolarization and activation of NMDAR (Demuro et al., 2005), and these are implicated in aging and neurodegeneration (Fukunaga et al., 2019). The higher concentration of calcium ions affects Calcineurin (CaN) and CaMKII signaling pathways and results in memory deficits and long-term depression in AD (Egorova et al., 2015; Marambaud et al., 2009; Schampel and Kuerten, 2017). Evidence demonstrated by Ross et al. has shown that CBD acts as VGCC antagonist and can fully inhibit T-type voltage-gated calcium channels (VGCCs), expressed from CaV3 gene (Ross et al., 2008). Furthermore, CBD is also demonstrated to suppress L-type VGCC with IC\(_{50}\) of 0.1 \(\mu\)M, where the effect was not mediated in a voltage-dependent manner (Ali et al., 2015).

Additionally, CBD also balances intracellular Ca\(^{2+}\) level, as the study found that CBD acts as a transient receptor potential cation channel subfamily V1 (TRPV1) stimulant in HEK-TRPV1 cells, lacking any subtractive effects (Bisogno et al., 2001). TRPV1 can act both as ion channel and receptor, and more prolonged activation of TRPV1 reduced pain through desensitization. TRPV1 can be activated upon any pain stimuli (Muller et al., 2019). Some recent studies have indicated that TRPV1’s channel unlocks upon activation, allowing ions to pass through the membrane from one side to another. Calcium passes over the pore systemically into the cell and activates various calcium-dependent pathways that finally lead to desensitization of the channel resulting in a reduction of inflammation pain (Costa et al., 2004; Muller et al., 2019; Whalley et al., 2018). Similarly, CBD exerts anti-hyperalgesic effects that may result from underlying peripheral and spinal activation via TRPV1 desensitization (De Petrocellis et al., 2011). In vivo study shows that CBD derived TRPV1 agonistic activity can act as anti-inflammatory agents (Costa et al., 2004; Tsuji and Aono, 2012).

7. CBD regulates proteostasis

Proteostasis is the protein homeostasis network that regulates all aspects of the cellular proteome, from protein synthesis to degradation. As a part of this network, several signaling pathways, which are usually activated in response to misfolded protein and protein aggregation, are also known as quality control systems (Soares et al., 2019). Once a protein is misfolded, chaperone control systems assist protein folding and disaggregation; however, if escaped, clearance systems are activated, leading aggregates into proteolytic degradation (Labbadia and Morimoto, 2015). The clearance system consists of two main types of machinery, including the ubiquitin-proteasome system (UPS) and autophagy, where UPS functions in the cytoplasm and nucleus, while autophagy only in the cytoplasm (Hipp et al., 2014). The degradation is directed by unfolded protein response (UPR) that follows either UPS or autophagy, which can be in the form of macroautophagy (including mitophagy), microautophagy, and chaperone-mediated autophagy (CMA) (Blašiak et al., 2019).

During the unfolded protein response, misfolded peptides are recruited by GRP78; eventually, IRE1\(_a\), PERK, and ATF6 dissociate from the luminal domains of UPR\(_{\alpha}\) sensors, which promotes parallel downstream signalings to reduce protein load by activating protein degradation and transport pathway. Lim et al. identified that CBD can alter endoplasmic reticulum (ER) morphology and initiate signaling cascades of PERK, ATF6, and IRE1, and thus elicits an endoplasmic reticulum (ER) stress response, which is not mediated by cannabinoid receptor (Lim et al., 2011). In oligodendrocyte progenitor cells, CBD (1 \(\mu\)M) decreased phosphorylation of eIF2\(a\), enhanced Bcl-2 expression, and thus protected against OS, and similarly, those effects were not mediated through CB1, CB2, TRPV1 or PPAR-\(\gamma\) receptors (Mecha et al., 2012). Moreover, a study on cadmium (Cd)-treated differentiated neuronal cells showed that CBD (1 \(\mu\)M) increased GRP78 upregulation and thus prevented Cd-mediated ROS generation. Accordingly, CBD ameliorated Cd-induced neuronal injury, as well as prevented the cellular distribution of the cytochrome C, while down-regulated BAX (Branca et al., 2015b). In this way, CBD regulates redox balance and collectively provides an anti-inflammatory effect by reducing OS (Wang et al., 2017). For a detailed understanding, readers are referred to a comprehensive review (Atalay et al., 2020). Besides, based on our discussion, we illustrate, highlighting CBD mechanism of action in OS and inflammation-mediated through PPAR-\(\gamma\) receptor (Fig. 1).
CBD enhanced phosphorylation of PERK-chop and thus upregulated DR5 (Kim et al., 2019), where DR5/TRAIL-R2 signaling regulated UPR mediated cell death (Yamaguchi and Wang, 2004). More recent studies showed that CBD regulated noxa ROS signaling pathway, resulting in the upregulation of IRE1α, PERK, Bip, GRP94, and CHOP in a dose and time-dependent manner (Jeong et al., 2019b). Moreover, due to the upregulation of CHOP, CBD can regulate Smac, which inhibits XIAP, and thus plays a role against mitochondrial damage (Jeong et al., 2019a).

The autophagy is considered as the non-selective system, where aggregates are degraded by the lysosome, while UPS is target-specific protein for lysosomal degradation using ubiquitin like cargo-recognition molecules and chaperons (Nixon, 2013; Tanaka and Matsuda, 2014; Wong and Cuervo, 2010). In this aspect, CBD is also reported to induce autophagy, appeared in several studies. The report represented by Shrivastava et al. showed that CBD could regulate autophagy by inhibiting Akt and mTOR signaling pathway by downregulating cyclin D1 and reducing the phosphorylation of mTOR and 4EBP1 (Fig. 3) (Shrivastava et al., 2011). Similarly, CBD was also shown to induce autophagy in vivo and prevented alcohol-mediated autophagy inhibition while downregulating JNK MAPK pathway and OS (Yang et al., 2014). Supporting this finding, Giacoppo and colleagues observed that CBD regulates in PI3K/Akt/mTOR pathway Encephalomyelitis (EAE) MS model and also promotes neuroprotection by inhibiting JNK and p38 MAP kinases (Giacoppo et al., 2017). Hossein Zadeh et al. showed that repeated treatment of 0.100 ng CBD as an intra-cerebroventricular injection in epileptic rats induce several autophagy markers such as conjugation of Atg5/12, Atg7, Atg12, and LC3II/LC3I expression, especially in hippocampal cells, confirming protective effect in epilepsy followed by autophagy pathway (Hosseinzadeh et al., 2016). A study using Glioma stem-like cells suggested that induction of autophagy by CBD was triggered by activating transient receptor potential vanilloid-2 (TRPV2) (Nabissi et al., 2015; Salazar et al., 2009), and thus increased response to radiosensitivity (Scott et al., 2014).

Although the precise mechanisms of CBD remain to be further investigated, it is unlikely that activation of autophagy is mediated through the CB1 receptor (Koay et al., 2014), localized in lysosomal compartments (Rozenfeld and Devi, 2008). However, a very recent study showed that CBD could potentially inhibit BACH1 (Casares et al., 2020), which acts as a repressor of p62 expression, a component that is
involved in selective autophagy (Ichimura et al., 2013).

8. Cannabidiol as a therapeutic option for aging-related proteinopathies

8.1. Huntington’s disease

Huntington’s disease (HD) is a lethal and progressive neurodegenerative disorder, which is featured by motor impairment, cognitive deficits, and behavioral shortages that mostly occur due to mutation of the huntingtin gene encoding Htt protein. The mutation caused the inclusion of CAG repeat in the exon of the huntingtin gene, resulting in an expansion of polyQ region near the N-terminus of the Htt protein, which causes aggregation of Htt protein (McCollan and Tabrizi, 2016). The major pathogenic mechanisms of Htt aggregates include neuronal dysfunction and death, followed by transcriptional dysregulation, altered proteostasis, and mitochondrial dysfunction (Kumar et al., 2020). Furthermore, these aggregates enhance OS, dopamine toxicity, metabolic imbalance, excitotoxicity, apoptosis, and autophagy (Gil and Rego, 2008). Accumulating evidence suggested that neuronal death by oxidative and inflammatory stress can be reduced by activating anti-inflammatory PPAR-γ signaling (Sánchez-Lopez et al., 2012), and thus, CBD can be a therapeutic option to ameliorate HD pathogenicity.

In a preclinical study, based on 3-nitropropionic acid (3-NP)-acid-lesioned rat model of HD, CBD injections at dose 5 mg/kg/day for a total period of 5 days reverse the striatal neurodegeneration induced by 3-NP. The 3-NP is a mitochondrial complex II inhibitor that provokes striatal damage through the activation of calpain (a Ca2+-dependent protein) and oxidative injury (Sagredo et al., 2007). Interestingly, the neuroprotective effects of CBD are not blocked by the selective antagonists of A2A, CB1, and TRPV1 receptors, suggesting that intrinsic antioxidant potentiality of CBD may be efficacious for slowing down the progression of HD striatal degeneration (Sagredo et al., 2007). Subjecting to malonate-induced rat model of HD, where striatal damage is produced mainly by glial activation and apoptosis, the CBD administration was not significant to reverse the condition (Sagredo et al., 2009). However, in combination with THC at 1:1 ratio, which is similar to Sativex, CBD reduces inflammatory markers (IGF-1 and iNOS), decreases the number of degenerating cells, enhances the number of surviving cells and decreases edema and glial reactivity, when injected combined at dose 4.63 mg/kg (Valdeolivas et al., 2012). The beneficial effect of this combination was exerted by both CB1 and CB2 agonistic effects (Valdeolivas et al., 2012). In a subsequent study of 3-NP induced model, treatment with a combination of THC and CBD (4.63 mg/kg; i.p.; for 5 days) at equimolar rate attenuates GABA and Nissl-stained neurons deficiency, up-regulates CB1, SOD1 expression, and downregulate IGF-1, calpain and iNOS expression in Sprague-Dawley rats (Sagredo et al., 2011). Interestingly, these results are also found similar when CBD effect was potentiated by enhancing ratio in the combination (1:2 of THC and CBD). Valdeolivas et al. found that Sativex® treatment at dose 4.5 mg/kg/day intraperitoneally for 8 weeks (1:1 ratio of pure CBD and THC) mitigates the elevated claspung behavior and reduces basal ganglia metabolism in R6/2 transgenic HD mice model (Valdeolivas et al., 2017). Moreover, this treatment also changed the prognostic markers of HD animals, including mitochondrial dysfunction, energy failure, and excitotoxicity.

However, in a double-blind, randomized crossover study, CBD alone administered orally (10 mg/kg/day for 6 weeks) in 15 HD patients did not improve chorea severity in HD patients or even did not cause any untoward consequence (Consroe et al., 1991). Similarly, a double-blind, placebo-controlled experiment with 25 HD patients conducted by Moreno and colleagues also claimed that CBD/THC combination therapy (2.7 mg CBD/2.5 mg THC) at a dose of 12 oral sprays/day for 12 weeks did not interfere with disease progression nor did it show any adverse effects or further clinical worsening (Moreno et al., 2016). As these findings in clinical settings are not conclusive, further clinical trials using CBD alone or in combination with THC need to be performed to estimate their efficacy following the use of a higher dose and more extended periods. Moreover, the role of CBD in Htt clearance has not been reported yet, thus needs to be further investigated.

8.2. Alzheimer’s disease

Alzheimer’s disease (AD) is a chronic neurodegenerative disorder, which is characterized by the intra-neuronal neurofibrillary tangles (tau-NFTs), extracellular senile plaques (aggregation of Aβ protein), neuronal atrophy, and progressive cognitive decline (Huang and Jiang, 2009). In AD, aggregated Aβ peptide (β-amyloid) is recognized as a typical hallmark (Hickman et al., 2008; Streit, 2004), which triggers the imbalance of different phosphatases and protein kinases that maintains several cellular signals. Moreover, Aβ aggregation promotes tau hyperphosphorylation, which eventually aggregates to neurofibrillary tangles, another hallmark of AD (Medeiros et al., 2011). Thereby, inhibition of tau hyperphosphorylation and Aβ aggregation has been recognized as a promising approach to target AD pathogenesis.

Iuvone et al. addressed that CBD was effective in reducing Aβ-mediated neurotoxicity, where they found that treatment of CBD in PC12 cells at a dose of 0.1 μM–100 μM decreased expressions of caspase-3, ROS and intracellular Ca2+ level. Furthermore, CBD reduced lipid peroxidation, which eventually protects cells from apoptosis (Iuvone et al., 2004). In addition, Esposito et al. found that CBD at a dose of 1μM to 100 μM inhibits iNOS expression and p38 MAPK phosphorylation, and thus reduces intracellular NO level and NF-kB activation in the same cell line (Esposito et al., 2006b). It was also found that intra-peritoneal administration of CBD treatment (2.5, 10 mg/kg for 7days) in C57BL/6 J mice promotes neurons against Aβ induced OS and reactive gliosis by downregulating the expressions of iNOS and IL-1β (Esposito et al., 2007). In astrocyte culture, CBD treatment at a dose of 0.001μM to 1μM was seen to ameliorate Aβ induced inflammation by lowering pro-inflammatory cytokines in astrocytes, which was mediated by activating PPAR-γ receptor (Esposito et al., 2011). Moreover, when CBD treated (5 mg/kg for 30 days) in Sprague-Dawley rat (2 days old), a similar phenomenon was also observed in addition to hippocampal neurogenesis and reducing reactive gliosis (Esposito et al., 2011).

Consistently, Scuderi et al. assessed the PPARγ mediated CBD effects on Aβ pathology in human neuroblastoma SHSY5Y APP′′ cells, which usually produce high levels of Aβ. They found that CBD (at dose 0.001μM to 0.1μM) activates PPARγ that induces APP protein ubiquitination and thus reduces Aβ production. Furthermore, CBD inhibits apoptosis of SHSY5Y (APP′′) neurons and eventually reduce the long-term apoptotic effects (Scuderi et al., 2014). Schubert et al. showed that CBD (100 nM) induces the degradation and removal of preformed Aβ aggregates, reduces inflammation, and inhibits the death of MC65 cells (Schubert et al., 2019). Benjamin et al. found that CBD (10 μM) protects PC12 cells from OS; however, CBD does not protect PC12 cells against H2O2 influenced cell death and found ineffective against preformed Aβ plaques, which suggests that the effects of CBD depend on Aβ formation mechanism (Harvey et al., 2012).

Moreno et al. demonstrated that intraperitoneal CBD injection in C57/B16 AD mice (3 months old) model at a dose of 20 mg/kg for 21 days prevents the Aβ induced cognitive impairment, and downregulates IL-6 expression, but causes no change in the expression of increased TNF-α (Martin-Moreno et al., 2011). Recently, Watt et al. showed that a higher dose (50 mg/kg, i.p. for 3 weeks) of CBD treatment reduces hippocampal insoluble Aβ40, and reverses spatial learning and social recognition of APPsw/PS1ΔE9 double transgenic AD male mice model with an age of 12-months (Watt et al., 2020). Nevertheless, CBD provides insignificant effects against PPARγ markers in the cortex, neuroinflammation, or neurodegeneration in this AD model (Watt et al., 2020). Cheng et al. showed that chronic CBD treatment (20 mg/kg, 3 weeks, i.p. injections) reduces cognitive deficits and improves novel object recognition and social recognition on APPsw/PS1ΔE9 AD mouse
model (Cheng et al., 2014a). Cheng et al. also demonstrated that oral CBD treatment reduces OS and prevents social recognition deficit on AβPP × PS1 mice at a dose of 20 mg/kg daily for 8 months (Cheng et al., 2014b). Nevertheless, CBD shows no effect on anxiety and associated learning and provides no effect on Aβ load management (Cheng et al., 2014b).

In Sativex®-like combination (CBD + THC), intraperitoneal injection of combined dose (0.75 mg/kg for five weeks) upregulated Wnt16 and thioredoxin-2 expressions in APPxPS1 transgenic AD mice. Furthermore, the combination improved memory impairments and reduced Aβ42 peptide-induced neurotoxicity and microgliosis. They thereby altered plaque composition and Aβ processing (Asa et al., 2015), although individual compounds failed to exert these effects. Moreover, when only CBD was used, learning performance was observed to reduce (Asa et al., 2015). In a similar experimental model and dose range, Aso et al. also found that a combination of CBD and THC reduces the AD-like phenotype and restored memory shortages. The combination changed the clearance of Aβ, CBD agonists could lower Aβ plaque, whereas CBD acted as an agonist of CB2 receptor (Harris et al., 2014).

CBD also demonstrated to inhibit tau hyperphosphorylation in various AD models. In a preclinical study, CBD treatment reduces Aβ production in gingiva derived mesenchymal stem cells (GMSCs). CBD also upregulated PI3K/Akt expressions while decreasing the expression of certain genes related to tau phosphorylation, including kinases MAPKs expression, and also reduced β- and γ-secretase secretions at a dose of 5 μM (Diomede et al., 2017). Evidence also shows that CBD inhibits GSK-3β expression, as well as inhibits hyperphosphorylation of tau by upregulating Wnt/catenin signaling, and reduces the death of PC12 neuronal cells at a dose of 0.1–10 μM (Esposito et al., 2006a). Moreover, CBD also upregulates Aβ degradation genes ACE1, IDE, and ECE1, and heat shock proteins (HSPs), like the HSP70 and the HSP90 (Diomede et al., 2017). The upregulated HSPs may inhibit tau and Aβ misfolding and accumulation (Patterson et al., 2011) by boosting proteostasis. CBD combination with THC was also effective against AD tauopathy. In this regard, Casarejos et al. reported that combined treatment reduced reduced abnormal and aggressive behavior in (PKL/−/−/ TauU/LW) mice (6 months old). Furthermore, CBD (4.63 mg/kg, i.p., for one month) reduced OS, which, in turn, downregulated astrogliosis and microgliosis, decreased iNOS and neuroinflammation. Along with reducing neuritic plaques, tau phosphorylation, and enhancement in dopamine metabolism and autophagy induction have also been observed (Casarejos et al., 2013).

In a perspective observational study, Broers et al. showed that an oral cannabis extract with THC/CBD (7.6 mg THC/13.2 mg CBD daily after 2 weeks, 8.8 mg THC/17.6 mg CBD after 1 month, and 9.0 mg THC/18.0 mg CBD after 2 months) was well-tolerated and improved rigidity, daily care, and behavioral problems of female patients (average 79.5 years old) with severe dementia and these improvements persisted after two months (Broers et al., 2019). Some patients developed pain during swallowing, and mouths ulcers, which were mitigated when given with CBD/THC-based oil (Broers et al., 2019).

All the above findings emphasize the relevance of CBD as a promising pharmacological compound compatible with ameliorating Aβ-intoxicated neuroinflammatory and neurodegenerative responses. However, further research is required for the detailed understanding of CBD actions on protein clearance (Aβ and tau degradation) and phosphorylation pathway modulation along with the alteration of glial reactivity or Aβ load. Moreover, clinical trials with allocating a sufficient number of patients using chronic-low doses of CBD alone or in combination and subsequent patient follow-up are also warranted.

8.3. Parkinson’s disease

Parkinson’s disease (PD) is a progressive neurodegenerative disorder that is characterized by a lack of dopamine, deposition of α-synuclein, and the gradual loss of dopaminergic neurons. The pathogenesis of PD is also associated with neuroinflammation and oxidative damage to the neurons (Sveinbjørnsdottir, 2016), which leads to the loss of gross movement, dysregulation of the sleep-wake cycle, cognitive deficit and dementia, psychosis, slow thinking capacity, anxiety disorders, depression, and mood disorders (Moon and Paek, 2015; Seppi et al., 2011; Thomas and Beal, 2010).

The promising neuroprotective effects of CBD, at a dose of 1 μM, has been observed by in vitro studies on PC12 neuronal cells related to PD (Santos et al., 2015). Santos et al. reported that CBD exerts neuroprotection against MPP+ (PD disease-causing neurotoxin)-induced neurotoxicity in PC12 cells, by upregulating neuritogenesis following the activation TrkA receptor (Santos et al., 2015). However, in murine mesencephalic cultures, CBD treatment at a dose of 10 μM failed to promote neurite outgrowth, although it significantly mitigates the degenerative effects of MPP+ (Moldzio et al., 2012). Taken together, both studies only the antidegenerative effect of CBD in the PD model, but the underlying mechanism is still unclear; thereby, further analysis is required. Recently, Pollastro et al. showed that CBD treatment at a dose of 10 μM regulates autophagy-related pathways, including ERK and AKT/mTOR signaling, followed by the activation of CB2 and TRPV1 receptors, and reverses MPP+ induced neurotoxicity in SH-SY5Y cells (Gugliandolo et al., 2020). Using SD rats of PD model (≥8 weeks), Lastres-Becker et al. showed that the antioxidative properties CBD are capable of reverting 6-hydroxydopamine induced dopaminergic injury when the compound was applied for 2 weeks (3 mg/kg) (Lastres-Becker et al., 2005). Furthermore, García-Arencibia reported that CBD (3 mg/kg, i.p. daily for 2 weeks) reverses 6-hydroxydopamine-tempted dopamine reduction, increases Cu, Zn-superoxide dismutase mRNA expression, and subsequently reduces dopaminergic neurons degeneration in the nigrostriatal of PD male SD rats (age: ≥8 weeks) (Garcia-Arencibia et al., 2007). Peres et al., showed that CBD (0.5/5 mg/kg, i.p. with 1 mg/kg reserpine for 7 days) could reduce reserpine induced motor and cognitive damages, improved memory deficit, and oral movements in male Wistar rats (3 months old), although they found no alterations in animals anxiety (Peres et al., 2016).

Zuardi et al. for the first time reported that CBD (150 mg/day, p.o., for 4 weeks) decreased the total scores of PD rating scale and did not get worse the motor function of six patients (mean age 58.8 ± 14.9 years) and no side effects or cognitive decline were observed. (Zuardi et al., 2009). In a double-blind trial, Chagas et al. showed that CBD (300 mg/day, p.o. for 6 weeks) improves PD patients (age >45 years) life quality in terms of emotional well-being, cognition, mobility, and communication. Moreover, no psychiatric comorbidities were found in patients (Chagas et al., 2014b). CBD at a dose of less than 300 mg/day showed no significant benefits in this study (Chagas et al., 2014b). Chagas et al. also showed that CBD (75 mg/day and CBD 300 mg/day for 6 weeks) is sufficient to reduce REM sleep behavior disorder of PD patients with age between 59–71 years (Chagas et al., 2014a). In a randomized, double-blind crossover study, Carroll et al. found that the combined (THC + CBD, 2.5 mg + 1.5 mg, p.o. for 4 weeks) therapy does not affect dyskinesia in PD (Carroll et al., 2004).

However, these clinical studies were conducted with limited patients. Also, no significant modulation of the BDNF level was found, or even whether CBD can modulate dopaminergic functions has not been observed during the treatment period. Thus, to conclude the comprehensive pharmacological benefits of CBD in PD patients, further randomized, double-blind clinical trials with a systematic assessment of a large number of patients are required.
8.4. Multiple sclerosis

Multiple sclerosis (MS) is an inflammatory demyelinating disease, which destroys the spinal cord and brain nerve cells of the CNS (Trapp and Nave, 2008) and considered to affect the young and middle-aged individuals (Frohman et al., 2006). It impedes signal transmission with the following symptoms; trouble with sensation or coordination, blindness in one eye, double vision, muscle weakness, and bladder dysfunction (Compston and Coles, 2008; Murray et al., 2012). The downregulation of the PI3K/AKT/mTOR pathway is known to be associated with the pathogenesis of MS (Mammana et al., 2018). However, several studies suggested that MS disease has complicated pathophysiology, and immunotherapy was not so responsive in the progressive stages of MS (Coles et al., 2006; Confavreux and Vukusic, 2006; Martin et al., 2007).

Evidence suggests that the use of cannabis can reduce MS symptoms (Consroe et al., 1997). The cannabinoid-based compounds, CBD, was used to alleviate pain and spasticity in MS (Nielsen et al., 2018; Pertwee, 2012). Mecha et al. found that CBD improves motor deficits, reduces microglial activity, leukocyte homing, and inflammation in TMEV-IDD susceptible female SJL/J mice model (4 weeks old), when administered CBD at a dose of 5 mg/kg for ten days. Besides, CBD reduced blood leukocyte migration by downregulating VCAM-1, CCL2 and CCL5, TNFα/IL-1β expression, and microglial activation (Mecha et al., 2013).

The experimental autoimmune encephalomyelitis (EAE) model system is widely used to understand the molecular and cellular mechanisms of MS and actions of associated therapeutics (McCarthy et al., 2012). In a murine induced EAE C57BL/6 mice (age: 6–8 weeks, female) model system of MS, Elliott et al. found that CBD treatment (20 mg/kg for 16 days) reduced T cell penetration, IFNγ, and IL-17 and increased MDSCs (myeloid-derived suppressor cells) with attenuation of EAE (Elliott et al., 2018). In contrast, when CBD given orally at a dose of 215 mg/kg (from day 6–18) in female Lewis rat (age 9–14 weeks) model of MS, a reduction of pain and spasticity were observed along with the downregulation of TNF-α, and the stimulation of BDNF gene expression (Zhou et al., 2019). Giacoppo et al. found that CBD at a dose of 10 mg/kg i.p. for 14 days improves EAE illness and inhibits phosphorylation of ERK p42/44 in 12 weeks old male C57BL/6 mouse model of MS (Giacoppo et al., 2015c). CBD also activates Fas pathway, triggers caspase-3 cleavage, modulates mitochondrial permeability, and axis activation of p53-p21 (Giacoppo et al., 2015c). Giacoppo et al. also found that CBD treatment at the same dose improves MS characteristics symptoms of male C57BL/6 MS mice (age 12 weeks) model. Moreover, the phosphorylation of PI3K, Akt, and mTOR was significantly increased together with BDNF expression and reduced proinflammatory cytokine IFN-γ and IL-1β after CBD treatment (Giacoppo et al., 2017). Recently, Gallily et al. found that CBD attenuates EAE symptoms in female SJL/J mice (6–7 weeks old) model at a dose of 5 mg/kg, 5 days/week for 60 days (Gallily and Yekhtin, 2019). Kozela et al. found that CBD (5 mg/kg/day, i.p. for 30 days) improves EAE symptoms, reduced microglial activation, inflammation, axonal impairment, and T-cell filtration in the spinal cord of female MS C57BL/6 mice (8 weeks old) model (Kozela et al., 2011). Recently, Al-Ghezi et al. reported that the combined treatment (CBD + THC) reduces neuroinflammation and subsequently ameliorates MS at a dose of 10 mg/kg/day each, i.p. for 18 days in female C57BL/6 mice (6–8-weeks old) model (Al-Ghezi et al., 2019b). The improvement of MS is associated with altering brain-infiltrating cells miRNA profiles (Al-Ghezi et al., 2019b).

Brady et al. showed that the combined clinical therapy (CBD + THC 2.5 mg of each per spray for 8 weeks) reduces pain, spasticity, excessive urination frequency, and increases the quality of sleep of MS patients (age: 18–65 years, female) (Brady et al., 2004). The therapy also showed some side effects, including dry mouth, mild drowsiness, altered time perception and confusion, short hallucinations (3 patients), and mouth soreness (2 patients) (Brady et al., 2004). Recently, Meuth et al. reported that the combined THC: CBD (for 12 weeks, patients age: >50 years); oromucosal spray is able to relieve pain and MS spasticity in randomized clinical trials (Meuth et al., 2020). Vermersch et al. reported that THC + CBD combined treatment (6 oromucosal spray/day for 3 months) improves patients (age ≥ 18, male and female) pain, sleep quality, spasms, bladder dysfunction, and fatigue of some patients also shows adverse drug effects (Vermersch and Trojano, 2016). Though this study was conducted with many patients (433 patients), a more extended treatment period and follow-up are required to provide a finite conclusion about this combined therapy.

CBD treatment reduces neuroinflammation in the MS model system but, whether it is due to the activation of PI3K/Akt/mTOR signaling or others is not mentioned in previous studies. Moreover, the upregulation of PI3K/Akt/mTOR signaling could reduce proinflammatory cytokines that need extensive studies to confirm. Besides, the anti-inflammatory effects of CBD through PPARγ receptor activation requires further studies.

8.5. Prion disease

Prion disease occurs due to the abnormal assembly of the protease-resistant prion protein (PrPres, misfolded isoform of PrPsen) aggregates on the surface of many cells forming clump in the brain, causing brain damage, which split the typical tissue structure. As vacuole forms in the neurons, some holes are formed in the tissue like spongy architecture (Cotran et al., 1999; Dirikoc et al., 2007). The PrPres aggregate causes extensive ER stress, which subsequently disrupts Ca2+ homeostasis. The maintenance of Ca2+ homeostasis is an essential event for continuing neuronal signaling (Verkhratsky and Toescu, 2003). Ca2+ is released in the cytoplasm when cells are exposed to the misfolded prion proteins (Verkhratsky and Toescu, 2003). Mecha et al. found that CBD (1 μM) attenuated ER stress, oxidative stress, and induced an anti-apoptotic pathway in oligodendrocyte progenitor cells (Mecha et al., 2012). In a study, it was found that CBD inhibited PrPres assembly at a dose of 5 μM (in vitro) and 60 mg/kg (in vivo) for 4 weeks in sheep scrapie-infected cells and C57BL/6 mice model, respectively (Dirikoc et al., 2007). Peripheral CBD injection restricted PrPres cerebral accumulation and increased the lifespan of the infected mice after intraarterial infection (Dirikoc et al., 2007).

Additionally, it was found that neuron triggers microglial cell migration in response to PrPres exposure (Marella and Chabry, 2004). CBD suppressed PrPres neurotoxic effects and impaired the concentration-dependent migration of microglial cells induced by PrPres (Dirikoc et al., 2007). Thus, during the prion infection, CBD may tend to prevent neurodegeneration through the inhibition of multiple molecular and cellular influencers associated with prion disease. As a corollary, CBD could be a favorable drug showing anti-prion properties, which could be used in prion disease, but it requires a high concentration of CBD to achieve its survival efficacy (Dirikoc et al., 2007).

The exact mechanism of CBD on PrPres, as well as the role of CBD in the destabilization of preexisting PrPres aggregates remain unclear. Additionally, as previous evidence suggests that low membrane cholesterol reduces PrPres generation (Tarabooulos et al., 1995), a pharmacological invention that lowers the neuronal membrane cholesterol level would inhibit PrPres formation. Whether CBD could have the cholesterol-lowering effect in the neuronal membrane, however, needs to be investigated.

8.6. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a progressive and lethal neurodegenerative disease, which affects both lower and upper motor neurons, resulting in spasticity, weakness, and, lastly, death due to respiratory collapse (Hardiman et al., 2011; Miller et al., 2009). Although the pathobiology of ALS much remains unknown, like other NDDs, the pathogenic mechanisms, including OS, inflammation, excitotoxicity, mitochondrial dysfunction, and protein misfolding, are also
Table 2: Pre-clinical and clinical evidence of CBD in the treatment of aging proteinopathies.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Drug combination</th>
<th>Experimental model</th>
<th>Dose regimen</th>
<th>Cellular effects</th>
<th>Molecular pharmacology</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huntington disease</td>
<td>THC</td>
<td>Adult male Sprague-Dawley rat model (age: 12 weeks old)</td>
<td>5mg/kg/day; i.p.; 5 days</td>
<td>↓progression of striatal degeneration, ↓protect against oxidative stress</td>
<td>↑mRNA level of SOD-1, SOD-2</td>
<td>(Sagredo et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>THC</td>
<td>Adult male Sprague-Dawley rat model (age: 12 weeks old)</td>
<td>4.63 mg/kg (1:1 ratio of pure CBD and THC); i.p.</td>
<td>↓number of degenerating cells, ↓number of surviving cells, ↓edema and glial activation</td>
<td>↓JGF-1 and iNOS expression</td>
<td>(Valdeolivas et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>THC</td>
<td>Adult male Sprague-Dawley rat model (age: 12 weeks old)</td>
<td>4.63 mg/kg/day for 5 days (1:1 ratio of pure CBD and THC); i.p.</td>
<td>↓GABA and Nissl-stained neurons deficiency</td>
<td>↓CB1, SOD1 expression</td>
<td>(Sagredo et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>THC</td>
<td>Male R6/2 transgenic HD mouse model (age: 4 weeks)</td>
<td>4.5 mg/kg/day for 8 weeks (1:1 ratio of pure CBD and THC); i.p.</td>
<td>↓clamping behavior, ↓basal ganglial metabolism, ↓mitochondrial dysfunction, energy failure, excitotoxicity</td>
<td>↓PPARγ activation</td>
<td>(Scuderi et al., 2014)</td>
</tr>
<tr>
<td></td>
<td>THC</td>
<td>Human (age: &gt;18 years)</td>
<td>12 oral sprays/day for 12 weeks (2.7 mg CBD/2.5 mg THC)</td>
<td>no significant changes on the HD biomarker</td>
<td>↓ ROS production</td>
<td>(Harvey et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>THC</td>
<td>SH-SY5Y(APP+)-Cells</td>
<td>0.001μM to 0.1μM for 24 h</td>
<td>↓APF ubiquitination, ↓cell viability, ↓apoptosis, ↓inflammation</td>
<td>↓PPARγ activation</td>
<td>(Iuvone et al., 2004)</td>
</tr>
<tr>
<td></td>
<td>THC</td>
<td>MC65 cells</td>
<td>100 nM for 24 h</td>
<td>inhibits cell death, induces the degradation and removal of preformed Aβ aggregation</td>
<td>↓PPARγ activation</td>
<td>(Esposito et al., 2006b)</td>
</tr>
<tr>
<td></td>
<td>THC</td>
<td>PC12 cells</td>
<td>10 μM for 24 h</td>
<td>↓oxidative stress, ↓Aβ toxicity</td>
<td>↓PPARγ activation</td>
<td>(Esposito et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>THC</td>
<td>PC12 neuronal cells</td>
<td>0.1 μM–100 μM for 24 h</td>
<td>↓oxidative stress, ↓Aβ toxicity</td>
<td>↓PPARγ activation</td>
<td>(Esposito et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>THC</td>
<td>PC12 neuronal cells</td>
<td>1μM to 100 μM for 36 h</td>
<td>↓Aβ toxicity</td>
<td>↓PPARγ activation</td>
<td>(Esposito et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>THC</td>
<td>Male C57BL/6J mice model (age: 3-5 months)</td>
<td>2.5, 10 mg/kg for 7days; i.p. injection</td>
<td>↓protect against oxidative stress, ↓reactive gliosis, promotes hippocampal neurogenesis, ↑Aβ induced inflammation, ↑reactive gliosis</td>
<td>↑IL-6 expression</td>
<td>(Esposito et al., 2011)</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>THC</td>
<td>Male Sprague-Dawley rat model</td>
<td>5 mg/kg for 30 days, i.p. injection</td>
<td>↓microglial activation</td>
<td>↑IL-6 expression</td>
<td>(Esposito et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>THC</td>
<td>Male C57/B6 AD mice model (age: 3 months old)</td>
<td>20 mg/kg for 21days, i.p. injection</td>
<td>↓reverses spatial learning and social recognition, ↓hippocampal insoluble Aβ level</td>
<td>activates PPARγ</td>
<td>(Watt et al., 2020)</td>
</tr>
<tr>
<td></td>
<td>THC</td>
<td>APPswe/PS1ΔE9 (age: 12-month-old)</td>
<td>50 mg/kg, i.p. for 3 weeks</td>
<td>↓reactive gliosis</td>
<td>↓reactive gliosis, ↑microglial activation</td>
<td>(Watt et al., 2020)</td>
</tr>
<tr>
<td></td>
<td>THC</td>
<td>Male APPswe/PS1ΔE9 (age: 10 weeks)</td>
<td>20 mg/kg for 3 weeks, i.p. injections</td>
<td>↓reactive gliosis, promotes hippocampal neurogenesis, ↑Aβ induced inflammation, ↑reactive gliosis</td>
<td>↓IL-6 expression</td>
<td>(Watt et al., 2020)</td>
</tr>
<tr>
<td></td>
<td>THC</td>
<td>Male AβPP × PS1 (age: 2.5 months)</td>
<td>20 mg/kg daily for 8 months, p.o.</td>
<td>↓oxidative stress, prevents social recognition deficit</td>
<td>activates PPARγ</td>
<td>(Watt et al., 2020)</td>
</tr>
<tr>
<td></td>
<td>THC</td>
<td>Gingiva derived mesenchymal stem cells</td>
<td>5 μM for 24 h</td>
<td>↓tau hyperphosphorylation, ↓Aβ production, ↓</td>
<td>↓P38/Akt signaling cascade</td>
<td>(Watt et al., 2020)</td>
</tr>
<tr>
<td></td>
<td>THC</td>
<td>PC12 neuronal cells</td>
<td>0.1–10 μM for 24 h</td>
<td>↓tau hyperphosphorylation</td>
<td>↑IL-6 expression</td>
<td>(Watt et al., 2020)</td>
</tr>
<tr>
<td></td>
<td>THC</td>
<td>Male APPswe/PS1 transgenic mice (age: 6 months)</td>
<td>0.75 mg/kg each, for 5 weeks, i.p. injection</td>
<td>↓tau hyperphosphorylation</td>
<td>↓IL-6 expression</td>
<td>(Watt et al., 2020)</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Disease</th>
<th>Drug combination</th>
<th>Experimental model</th>
<th>Dose regimen</th>
<th>Cellular effects</th>
<th>Molecular pharmacology</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>THC</td>
<td>Male APPxPS1 transgenic mice (age: 12 months)</td>
<td>10 mL/kg (0.75 mg/kg each) for 5 weeks, i.p. injections</td>
<td>• alters plaque composition and Aβ processing</td>
<td>• IL-42 peptide levels</td>
<td>(Asó et al., 2016)</td>
</tr>
<tr>
<td></td>
<td>THC</td>
<td>Transgenic tauopathy male (PK−/−/TaNTm) mouse model</td>
<td>4.63 mg/kg (CBD: 1.5 mg/kg) for one month, i.p. injections/daily</td>
<td>• tau hyperphosphorylation and protect against oxidative stress</td>
<td>• iNOS level</td>
<td>(Casarejos et al., 2013)</td>
</tr>
<tr>
<td></td>
<td>THC</td>
<td>Human (female, average age: 79.5 years)</td>
<td>7.6 mg THC/13.2 mg CBD daily after two weeks, 8.8 mg THC/17.6 mg CBD after one month, and 9.0 mg THC/18.0 mg CBD after two months orally</td>
<td>• neurotic plaques and NFTs improve rigidity, daily care, and behavior problems</td>
<td>• IL-1 level</td>
<td>(Broers et al., 2019)</td>
</tr>
<tr>
<td></td>
<td>PC12 cells</td>
<td>1 μM for 72 h</td>
<td>• cell viability</td>
<td></td>
<td>• TrkB receptors</td>
<td>(Santos et al., 2015)</td>
</tr>
<tr>
<td></td>
<td>MPP + -induced dopaminergic neurons</td>
<td>10 μM for 48 h</td>
<td>• antioxidants properties</td>
<td></td>
<td>• ERK and AKT/mTOR pathway</td>
<td>(Moldzio et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>SH-SYSY cells of PD model</td>
<td>cells pretreated with 10 μM CBD for 24 h, and incubated 48 h with 1/2 mM MPP +</td>
<td>• loss of cell viability</td>
<td></td>
<td>• PARP-1 levels</td>
<td>(Gugliandolo et al., 2020)</td>
</tr>
<tr>
<td>Parkinson’s disease</td>
<td>6-OHDA -induced male Sprague-Dawley rat model (age: 8 weeks)</td>
<td>3 mg/kg for 2 weeks; i.p.</td>
<td>• dopaminergic transmission</td>
<td></td>
<td>• ROS</td>
<td>(Lastres-Becker et al., 2005)</td>
</tr>
<tr>
<td>Parkinson’s disease</td>
<td>6-OHDA induced male Sprague-Dawley rat model (age: 8 weeks)</td>
<td>3 mg/kg for 2 weeks, i.p.</td>
<td>• oxidative stress</td>
<td></td>
<td>• mRNA levels of tyrosine hydroxylase</td>
<td>(Garcia-Arencibia et al., 2007)</td>
</tr>
<tr>
<td>Parkinson’s disease</td>
<td>Reserpine-induced male Wistar rat model (age: 3 months)</td>
<td>0.5 mg/kg for 1 week; i.p.</td>
<td>• catalepsy, memory deficits</td>
<td></td>
<td>• 5-HT1A receptor activation</td>
<td>(Peres et al., 2016)</td>
</tr>
<tr>
<td></td>
<td>Human (4 men, 2 women, mean age 58.8 ± 14.9 years)</td>
<td>150 mg/day, p.o., for 4 weeks</td>
<td>• PD symptoms</td>
<td></td>
<td>• IL-1 level</td>
<td>(Zuardi et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>Human (age: 59 – 71 years, male)</td>
<td>300 mg/day, p.o. at night for 6 weeks</td>
<td>• improves patient’s life quality</td>
<td></td>
<td>• IL-17 and IL-17 levels</td>
<td>(Chagas et al., 2014a)</td>
</tr>
<tr>
<td></td>
<td>Human(age: &gt;45 years)</td>
<td>75 mg/day to 3 patients, and 300 mg/day to one patient for 6 weeks</td>
<td>• BEAM related sleep behavior disorder</td>
<td></td>
<td>• ILF-1 levels</td>
<td>(Chagas et al., 2014b)</td>
</tr>
<tr>
<td></td>
<td>SJL/J female mice model (age: 4 weeks old)</td>
<td>5 mg/kg; i.p. for 10 days</td>
<td>• improves motor deficit</td>
<td></td>
<td>• IL-1 level</td>
<td>(Mech et al., 2013)</td>
</tr>
<tr>
<td>Multiple</td>
<td>EAE-induced female C57BL/6 mice model (age: 6–8 weeks)</td>
<td>20 mg/kg; i.p. for 16 days</td>
<td>• immunogliosis</td>
<td></td>
<td>• IL-1 level</td>
<td>(Elliott et al., 2018)</td>
</tr>
<tr>
<td>Sclerosis</td>
<td>EAE-induced female Lewis rat model (age: 9–14 weeks)</td>
<td>215 mg/kg from day 6–18; p.o.</td>
<td>• myeloid-derived suppressor cell</td>
<td></td>
<td>• ILF-1 gene expression</td>
<td>(Zhou et al., 2019)</td>
</tr>
<tr>
<td></td>
<td>Male C57BL/6 mice (age: 12 weeks old)</td>
<td>10 mg/kg; i.p. for 14 days</td>
<td>• improves EAE illness</td>
<td></td>
<td>• phosphorylation of ERK p42/44</td>
<td>(Giacoppo et al., 2015c)</td>
</tr>
<tr>
<td></td>
<td>EAE-induced male C57BL/6 mice model (age: 12 weeks)</td>
<td>10 mg/kg; i.p. for 14 days</td>
<td>• improves MS characteristic symptoms</td>
<td></td>
<td>• activation of Fas pathway,</td>
<td>(Giacoppo et al., 2017)</td>
</tr>
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 Known to be involved in ALS (Giacoppo and Mazzon, 2016; Zarei et al., 2015). Having antioxidant, anti-inflammatory, and neuroprotective potentials, CBD has shown to attenuate ALS pathology in various experimental evidence (Giacoppo and Mazzon, 2016; Raman et al., 2004).

As an in vitro ALS model, treatment of human ginviva-derived mesenchymal stromal cells with CBD (5 μM) modulated the expression of genes associated with ALS pathologies, including OS, mitochondrial dysfunction, and excitotoxicity (Rajan et al., 2017). Rajan et al. found that CBD upregulates the expression of Nrf2, SDHC1, NDUFV3, and NDUFV3, and downregulates CABIN1, PPP3CC, HTRA2, and TARP2 expression (Rajan et al., 2017). The expression of CB2 receptors was reported in activated microglia from the spinal cord of human ALS patients, which suggests the possibility of CBD as therapeutic in ALS at the clinical level. A recent phase 2 trial (CANAFLS study) demonstrated that THC. CBD (a dose of 2.7 mg THC and 2.5 mg CBD) could alleviate the spasticity in ALS without any adverse effect (Riva et al., 2019). The treatment satisfaction of the patients has been studied in which 84 % of patients experienced positive effects on THC. CBD treatment in ALS (Meyer et al., 2019). A randomized, double-blind, placebo-controlled trial has shown that the CBD oil (CBD: THC = 25:2) is effective in slowing down the ALS progression (Urbi et al., 2019).

### 9. Concluding remarks and future perspectives

OS and neuroinflammation affect the integrity of the proteostasis network and thereby play a decisive role in the pathogenesis of age-related neurodegenerative disorders by affecting the integrity of the proteostasis network. Due to the involvement of endocannabinoid systems in OS modulation, CBD may be considered as an attractive molecule, as it has shown to provide antioxidant and anti-inflammatory effects in various preclinical models (Table 2). However, the precise mechanism of CBD remains to be elucidated, especially in the activation of protein aggregates clearance systems.

Although multidirectional studies provide clear evidence of CBD mediated autophagy induction, however, neuronal system-based studies are limited, and the molecular pharmacology is also poorly understood. Furthermore, studies showed that autophagy as a double edge sword might induce either death or survival mechanism, suggesting the need for future study, as the phenomenon of the activation and activating receptors are unlikely with different cell types.

Recently, Scott et al. showed that CBD treatment modulated HSPs expression, notably upregulated HSP70 and HSP90 in glioma cell lines (Scott et al., 2015). Substantial evidence highlighted the critical roles of HSP70 and HSP90 in the quality control and clearance of the misfolded and aggregated proteins by the UPS and chaperone machinery (Gupta et al., 2020). Additional observation by Rodríguez-Muñoz et al. stated that CBD acts as an antagonist of δ1R, which has been identified as a master regulator of proteostasis system (Christ et al., 2020). The antagonist of δ1R is responsible for the induction of unfolded protein response and autophagy in a dose-dependent manner (Schrock et al., 2013), and also modulate HSP70 regulations (Sánchez-Blaquez et al., 2014). Furthermore, CBD promoted cytosolic degradation of BACH1, which, according to another study, is mediated through the UPS (Zenke-Kawasaki et al., 2007). Thus, these findings provide a clue that CBD can regulate both the UPS and chaperone machinery. Maintenance of intracellular Ca^{2+} homeostasis is an essential function of ER, if disturb, stress in ER induced that endorsed misfolded protein accumulation. Since CBD regulates both glutamate release and NMDAR activation, it can protect neurons from glutamate excitotoxicity and ER stress-mediated injuries. Although several comprehensive reviews (Atalay et al., 2020; Cabral and Griffin-Thomas, 2009; Campos et al., 2016; Peres et al., 2018) suggest that CBD regulation in OS and inflammations is likely to mediate by CB1, CB2, TRPV1 or PPAR-γ.
receptors, but none of the receptors is reported to implicate when studies showed CBD effect in mitigating OS in ER stress response (Ligresti et al., 2006). Furthermore, the molecular insights into the activation and regulation of PERK, IRE1, and ATF6 pathways are still unclear. Thus, understanding the ROS regulation by CBD is still far away and deserves more studies, especially in neuronal models.

As illustrated in Table 1, CBD and its combination with THC provide therapeutic benefits for various neurodegenerative disorders. However, most of the findings were based on short term effects, and the preclinical studies barely used transgenic models, and future studies should, therefore, be designed to analyze long term effects analysis, as well as using transgenic mouse models. Although studies discussed in this review suggest therapeutic benefits of combination therapy with CBD, care must be taken when choosing drug combination, as the study showed induction of serum ALT and AST, and also inactivation of cytochrome P450 3A and P450 2C in CBD therapy (Samara et al., 1990). Furthermore, combination therapy may facilitate the drug interaction that could be antagonistic to reduce the pharmacological efficacy of the compounds.

Finally, this review suggests a “proof of principle” of CBD regulation in the proteostasis network, especially in the activation of protein clearance systems. The research findings highlighted in this communication are promising. However, in vivo studies of CBD effects on various disease models are perhaps encouraging, but not in humans. Like other approved or proposed therapeutic agents in NDDs till to date, CBD may not be able to reverse the “core” disease pathophysiology significantly, because the pathophysiological spectrum of these NDDs is more complicated than the only aberrant accumulation of disease-specific protein aggregates. However, CBD can determine some clinical improvements, such as lessening behavior problems in AD, pain or spasms in PD, seizures in TLE, reduction in ALS or MS, and or of daily care. Therefore, we suggest advanced research to investigate the CBD effects in clearing protein aggregates in the various disease models of neurodegeneration, which could offer a therapeutic window in proteinopathies.

Author contributions

R.D contributes to review designing, manuscript writing, table, and figure mapping and figure design. M.C.A., I.J, Y.A.M., and S.M contribute to manuscript writing, revision, and summary table preparation. M.A.H., B.T., D.F.O., and H.J.C contribute to manuscript writing and revision. I.S.M. contributes to review planning and supervision and manuscript revision. All the authors read and approved this manuscript.

Declaration of Competing Interest

The authors report no declarations of interest.

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References


