

RESEARCH ARTICLE

WIN 55,212-2, Agonist of Cannabinoid Receptors, Prevents Amyloid β_{1-42} Effects on Astrocytes in Primary Culture

Diana Aguirre-Rueda, Sol Guerra-Ojeda, Martin Aldasoro, Antonio Iradi, Elena Obrador, Maria D. Mauricio, Jose M^a Vila, Patricia Marchio, Soraya L. Valles*

Department of Physiology, School of Medicine, University of Valencia, Valencia, Spain

* lilian.valles@uv.es



OPEN ACCESS

Citation: Aguirre-Rueda D, Guerra-Ojeda S, Aldasoro M, Iradi A, Obrador E, Mauricio MD, et al. (2015) WIN 55,212-2, Agonist of Cannabinoid Receptors, Prevents Amyloid β_{1-42} Effects on Astrocytes in Primary Culture. PLoS ONE 10(4): e0122843. doi:10.1371/journal.pone.0122843

Academic Editor: Partha Mukhopadhyay, National Institutes of Health, UNITED STATES

Received: December 22, 2014

Accepted: February 18, 2015

Published: April 13, 2015

Copyright: © 2015 Aguirre-Rueda et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper.

Funding: Generalitat Valenciana AP-073/09. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Alzheimer's disease (AD), a neurodegenerative illness involving synaptic dysfunction with extracellular accumulation of $A\beta_{1-42}$ toxic peptide, glial activation, inflammatory response and oxidative stress, can lead to neuronal death. Endogenous cannabinoid system is implicated in physiological and physiopathological events in central nervous system (CNS), and changes in this system are related to many human diseases, including AD. However, studies on the effects of cannabinoids on astrocytes functions are scarce. In primary cultured astrocytes we studied cellular viability using MTT assay. Inflammatory and oxidative stress mediators were determined by ELISA and Western-blot techniques both in the presence and absence of $A\beta_{1-42}$ peptide. Effects of WIN 55,212-2 (a synthetic cannabinoid) on cell viability, inflammatory mediators and oxidative stress were also determined. $A\beta_{1-42}$ diminished astrocytes viability, increased TNF- α and IL-1 β levels and p-65, COX-2 and iNOS protein expression while decreased PPAR- γ and antioxidant enzyme Cu/Zn SOD. WIN 55,212-2 pretreatment prevents all effects elicited by $A\beta_{1-42}$. Furthermore, cannabinoid WIN 55,212-2 also increased cell viability and PPAR- γ expression in control astrocytes. In conclusion cannabinoid WIN 55,212-2 increases cell viability and anti-inflammatory response in cultured astrocytes. Moreover, WIN 55,212-2 increases expression of anti-oxidant Cu/Zn SOD and is able to prevent inflammation induced by $A\beta_{1-42}$ in cultured astrocytes. Further studies would be needed to assess the possible beneficial effects of cannabinoids in Alzheimer's disease patients.

Introduction

Alzheimer's disease (AD) is a common neurodegenerative disease implicated in the aging process, affecting nearly 50% of people over 75 [1,2]. It involves neurofibrillary degeneration, extracellular accumulation of beta-amyloid peptide ($A\beta$) and synaptic dysfunction, resulting in neural cell death in the hippocampus and cerebral cortex, and in activation of glial cells [3,4].

A β can interact with different cellular components producing Ca²⁺ deregulation, oxidative stress and inflammation [5,6].

Astrocytes are specialized neural cells serving as a structural and metabolic support and trophic help to the brain [7]. Astrocytes also release cytokines and chemokines involved both in protective and toxic roles in neuroinflammatory processes [8]. However, released cytokines in neuroinflammation may induce deleterious effects on the viability and functionality of astrocytes [9]. Furthermore, in pathological situations such as hypoxia, cytokines induce activation of vascular endothelial cells thereby modulating inflammatory responses [10]. In AD, astrocytes are found around senile plaques producing phagocytosis, and cleaning up toxic compounds such as A β [11]. Moreover, when stimulated with compounds such as genistein or estradiol, astrocytes block the release of pro-inflammatory mediators and induce the synthesis of anti-inflammatory proteins [12].

Endocannabinoids have been implicated in various physiopathological events in different organs, including the peripheral and central nervous system (CNS) [13], and changes in the endocannabinoid system have been related to many human diseases, such as metabolic syndrome [14], neurodegeneration [15], inflammatory diseases [16], psychiatric disorders [17] and cancer [18]. The endocannabinoid signaling system is composed of anandamide and 2-arachidonoyl glycerol interacting with CB1 and CB2 cannabinoid receptors. Receptor signaling may involve mechanisms such as adenylyl cyclase blockade or activation of mitogen-activated protein kinases or ceramide signaling [13].

Different authors have proposed cannabinoids as preventive treatment in AD [19] due to their anti-inflammatory and neuroprotective properties [16]. In this sense, cannabinoids prevented microglial activation and cognitive impairment in A β -treated rats [19]. In mice exposed to A β , cannabinoids also suppress neuroinflammation by inhibiting inducible nitric oxide synthase (iNOS) expression and interleukin-1 β generation [20]. However, the effects of cannabinoids on astrocytes functions have been poorly investigated. Therefore, we investigated the role of WIN 55,212-2 (WIN) as a neuroprotective agent against lesions induced by A β_{1-42} on cultured astrocytes.

Material and Methods

Materials

Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco (Gibco Invitrogen Corporation, Barcelona, Spain). The oligomers A β (40-1 and 1-42), were prepared following manufacture instructions (Sigma-Aldrich biotechnology). Briefly, the peptides were dissolved in H₂O, and, for assembly the oligomers, preparations were heated for 24 h at 37°C. WIN and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (St Louis, MO). Enzyme-linked immunosorbent assay (ELISA) kits for interleukin 1 β (IL-1 β) and tumor necrosis factor (TNF- α) from Pierce Biotechnology, Inc. (Rockford, USA). Western Blot Chemiluminescent Detection System (ECL) was from Amersham (Amersham Biosciences, Barcelona, Spain). Monoclonal anti-peroxisome proliferator-activated receptor antibody (PPAR- γ) (1:250) and polyclonal anti-cyclooxygenase-2 antibody (COX-2) (1:250) from Sigma Aldrich (Madrid, Spain). Monoclonal p65 antibody (p65) (1:250) and monoclonal anti-Mn superoxide dismutase antibody (Mn-SOD) (1:250) from Santa Cruz Biotechnology (Madrid, Spain). Polyclonal anti-Cu/Zn superoxide dismutase antibody (Cu/Zn SOD) (1:250) from Assay Designs (Madrid, Spain). Monoclonal inducible nitric oxide synthetize (iNOS) (1:250) and anti-tubuline (1:1000) antibodies from Cell Signaling (Beverly, MA, USA). All other reagents are analytical or culture grade purity.

Primary culture of cortical astrocytes

All animals were handled according to the recommendations of the Bioethics Committee of the School of Medicine of the University of Valencia, Spain. Ethics committee specifically approved this study. Cortical astrocytes were isolated from rat fetuses of 21 days gestation. Fetuses were obtained by cesarean section and decapitated. Cerebral cortices were removed and triturated 10–15 times through a Pasteur pipette with 10 ml DMEM. The cell suspension was filtered through nylon mesh with a pore size of 90 μ m and re-suspended in DMEM containing 20% fetal bovine serum (FBS), supplemented with L-glutamine (1%), HEPES (10 mM), fungizone (1%), and antibiotics (1%). Cells were plated on T75 culture flask and maintained in a humidified atmosphere of 5% CO₂/95% air at 37°C during 15 days. After 4 days of culture, the FBS was maintained at 20% and after 1 week of culture, the FBS content was reduced to 10%, and the medium was changed twice a week. The purity of astrocytes was assessed by immunofluorescence using anti-gial fibrillary acidic protein (astrocyte marker; Sigma-Aldrich), anti-CD-68 (microglial marker; Serotec), anti-myelin basic protein (oligodendroglial marker; Sigma-Aldrich) and anti-microtubule-associated protein 2 (neuronal marker; Sigma-Aldrich). The astrocyte cultures were found to be at least 99% gial fibrillary acidic protein positive. No cells were found to express CD-68, myelin basic protein, or microtubule-associated protein-2.

Cell treatments

Ten days after seeding, WIN (10 μ M) was added to culture flasks. Twenty-four hours later, 10 μ M A β_{1-42} (toxic peptide) or A β_{40-1} (control peptide) (Sigma-Aldrich) were added to the flasks. A β_{1-42} concentration used in our study is in the range of toxic concentrations of the peptide [21,22]. Before incubation, the peptides were diluted in 100 μ M of phosphate-buffered saline (PBS) and incubated for 24 h at 37°C. Assays were performed 24 h after peptide addition.

MTT assay

Cell viability was determined by MTT assay. The MTT assay is a well-established, widely used and easily reproducible method for the assessment of cell viability and cytotoxicity [23,24]. Astrocytes were plated in 96-well culture plate and incubated with WIN during 24h. Subsequently, A β_{40-1} (control) and A β_{1-42} peptides were added to wells for another 24h. After cell treatments, the medium was removed and cells were incubated with red free medium and MTT solution [0.5 mg/ml, prepared in phosphate buffer saline (PBS) solution] for 4 h at 37°C. Finally, the medium was removed and formazan particles were dissolved in dimethyl sulfoxide. Cell viability, defined as the relative amount of MTT reduction, was determined by spectrophotometry at 570 nm.

Cytokine determination, IL-1 and TNF α

Astrocytes were seeded as previously published [12]. At the time of assay, the red phenol medium was removed and replaced by PBS containing 1 mg/ml bovine serum albumin, either in the presence or absence of A β_{1-42} (10 μ M). IL-1 β and TNF- α concentration (pg/ml) were ascertained using ELISA kits (Pierce Biotechnology, Inc.).

Western blot analysis

Cultured cells were treated with lysis buffer and mechanically degraded to release the proteins. Protein concentration was determined using modified Lowry method [25]. Loading buffer (0.125 M Tris-HCl, pH 6.8, 2% SDS, 0.5% (v/v) 2-mercaptoethanol, 1% bromophenolblue and 19% glycerol) was added to protein sample and heated for 5 min at 95°C. Proteins were

separated on SDS-PAGE gels and transferred to nitrocellulose membranes in a humid environment using a transfer buffer (25mM Tris, 190mM glycine, 20% methanol). Membranes were blocked with 5% milk in TBS-T (0.05% Tween-20) and incubated with primary antibodies overnight at 4°C. Membranes were washed 3 times with wash buffer TBS-T and incubated with a secondary anti-rabbit IgG or anti-mouse IgG (Cell Signalling Technologies Danvers, MA) antibody conjugated to the enzyme horseradish peroxidase (HRP) for 1 h. Membranes were washed three times and proteins were detected using the ECL method as specified by the manufacturer. Autoradiography signals were assessed using digital image system ImageQuant LAS 4000 (GE Healthcare).

Statistical analyses

Values are expressed as mean \pm S.D. Differences between groups were assessed by one-way analysis of variance (ANOVA). Statistical significance was accepted at $P \leq 0.05$. Data sets in which F was significant were examined by a modified t -test.

Results

Protective role of WIN on cell viability

The role of WIN on cell viability was studied using MTT conversion assay. [Fig 1A](#) shows that incubation with WIN at different concentrations induced a significant increase in cell viability at 10 μ M. Consequently, that concentration was used in future experiments. Astrocytes previously incubated with 10 μ M A β_{1-42} for 24 h significantly decreased cell viability compared to control cells ([Fig 1B](#)). Furthermore, pretreating astrocytes during 48 h with WIN (10 μ M) prevented the decrease in cell viability induced by A β_{1-42} (WIN + A β), conversely WIN (1, 2, 5 μ M) did not have any effect ([Fig 1B](#)).

WIN prevents IL-1 β and TNF- α increase elicited by A β_{1-42}

Cultured astrocytes were incubated with 10 μ M A β_{1-42} and proinflammatory mediators TNF- α and IL-1 β were detected by ELISA. A β_{1-42} increased 4.5-fold IL-1 β release (480.4 \pm 150.3 pg/ml) compared with control (103.9 \pm 82.9 pg/ml) ([Fig 2A](#)) and 2.4 fold TNF- α release (605.3 \pm 103.4 pg/ml vs 210.5 \pm 85.3 pg/ml in control group) ([Fig 2B](#)). Furthermore, WIN pre-treatment (10 μ M) prevented the increase in pro-inflammatory mediators induced by A β_{1-42} ([Fig 2A and 2B](#)).

Effect of A β_{1-42} and WIN on p65 protein expression

Nuclear factor κ B (NF- κ B), the pro-inflammatory transcription factor is formed by different subunits. We measured p65 protein expression by western-blot. Incubation with A β_{1-42} increased p65 protein expression compared with control astrocytes ([Fig 3](#)), which was prevented by WIN pretreatment. ($p < 0.05$ compared with A β_{1-42} treated astrocytes).

WIN prevents COX-2 and iNOS protein increase induced by A β_{1-42} peptide

Incubation with A β_{1-42} significantly increased inflammatory proteins COX-2 ([Fig 4A](#)) and iNOS ([Fig 4B](#)) expressions compared to control. Furthermore, pretreating astrocytes with WIN prevented the effects produced by A β_{1-42} .

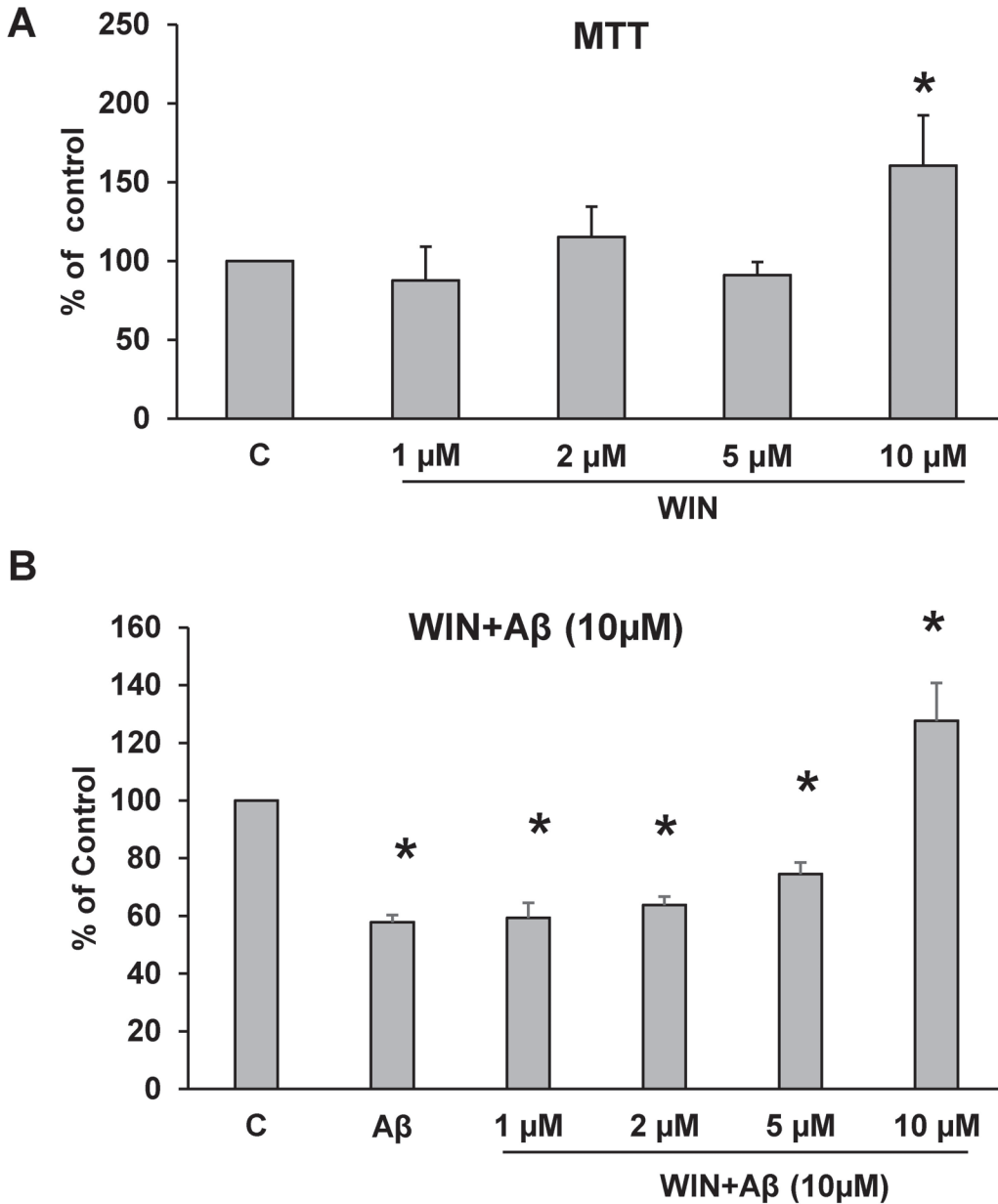


Fig 1. Astrocytes viability. (A) Astrocytes viability induced by WIN. Concentration-dependent viability of WIN (1, 2, 5, 10 μM) was determined by MTT assay for 24 h. Data are means ± SD for 4 independent experiments. **p*<0.04 comparing WIN vs control cells. (B) Astrocytes viability in cells treated during 24 h with 10 μM Aβ₄₀₋₁ (control peptide, C), 10 μM Aβ₁₋₄₂ (Aβ) and WIN (1, 2, 5, 10 μM) + 10 μM Aβ₁₋₄₂ (WIN + Aβ). Data are means ± SD of 3 independent experiments. **p*<0.05 vs control cells.

doi:10.1371/journal.pone.0122843.g001

Effect of Aβ₁₋₄₂ and WIN on PPAR-γ protein expression

Pro-inflammatory gene expression is downregulated by PPARs family [26]. We found that pretreatment with WIN (10 μM) increased PPAR-γ expression compared to control cells (Fig 5). Incubation with Aβ₁₋₄₂ significantly decreased PPAR-γ expression that was prevented by WIN pretreatment.

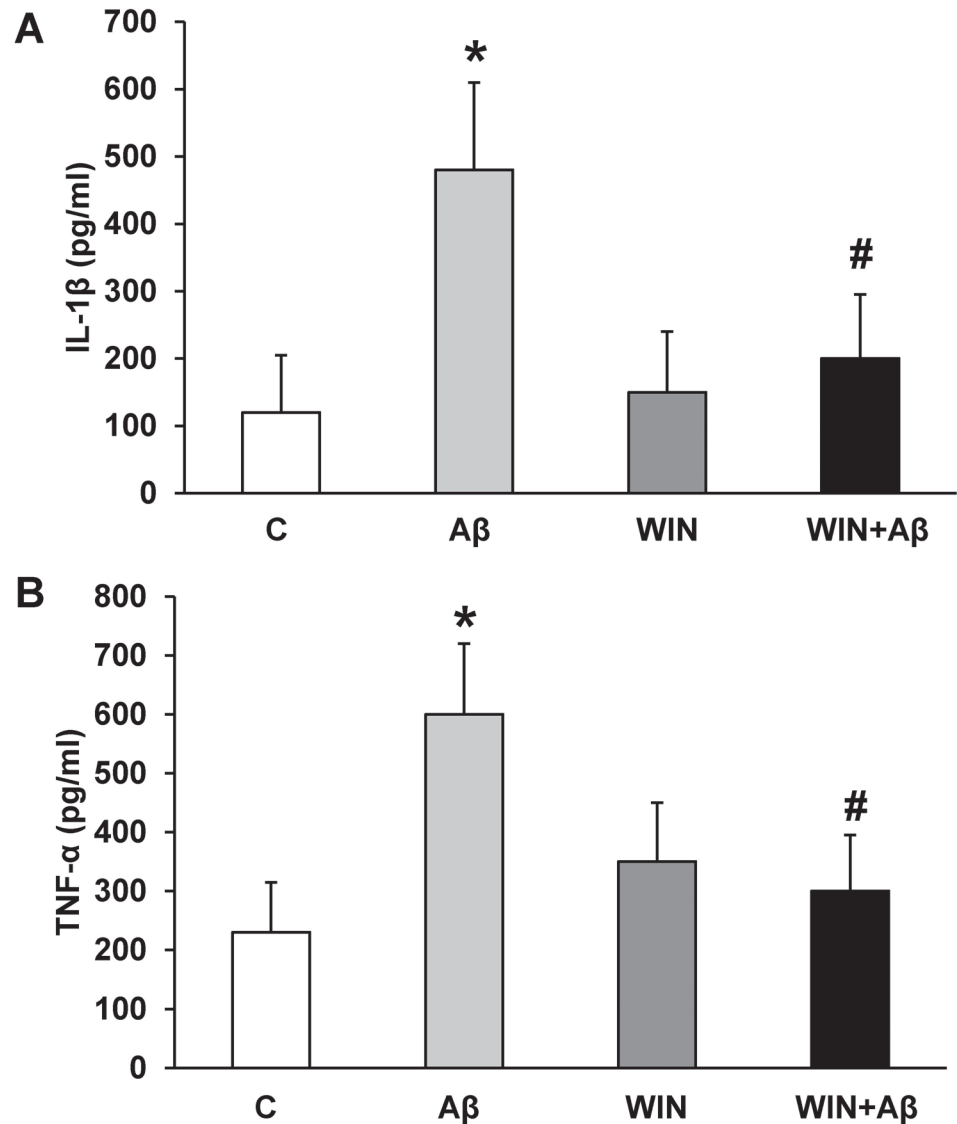


Fig 2. IL-1 β and TNF- α secretion. WIN prevents the increase of IL-1 β and TNF- α secretion caused by A β_{1-42} in astrocytes. Cells were incubated with 10 μ M A β_{40-1} (control peptide, C), 10 μ M A β_{1-42} (A β), 10 μ M WIN + 10 μ M control peptide (WIN) and 10 μ M WIN + 10 μ M A β_{1-42} (WIN + A β). Cell culture supernatants were harvested, and IL-1 β (panel A) and TNF- α (panel B) secretion were determined by ELISA. Values are means \pm SD of replicate experiments from 4 independent astrocytes cultures. * p <0.05 vs control astrocytes. # p <0.05 vs A β_{1-42} treated cells.

doi:10.1371/journal.pone.0122843.g002

Effect of A β_{1-42} and WIN on Cu/Zn SOD and Mn SOD protein expression

Superoxide dismutase is a key antioxidant enzyme. In our study, incubation with A β_{1-42} decreased Cu/Zn SOD expression in astrocytes in primary culture which was prevented by WIN pretreatment, evidencing that WIN could play a neuro-protective role against oxidative stress induced by A β_{1-42} peptide (Fig 6A). On the other hand, our results indicated that Mn SOD protein expression is increased in presence of A β_{1-42} . Pretreatment with WIN did not prevent Mn SOD increase induced by A β_{1-42} (Fig 6B).

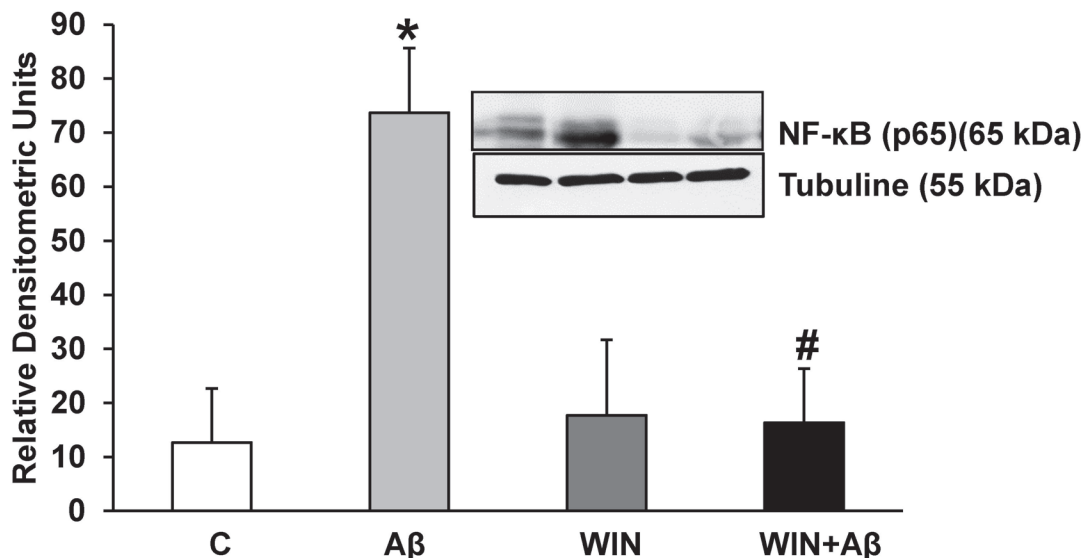


Fig 3. p65 protein expression. WIN 55, 212–2 prevents p65 expression induced by A β_{1-42} in astrocytes in primary culture. p65 and α -tubulin expressions were determined by Western-blot in astrocytes treated for 24 h with 10 μ M A β_{40-1} (control peptide, C), 10 μ M A β_{1-42} (A β), 10 μ M WIN + 10 μ M control peptide (WIN) and 10 μ M WIN + 10 μ M A β_{1-42} (WIN + A β). A representative immunoblot of each protein is shown and tubulin was used as control amount of protein. Data are means \pm SD of 5 independent experiments. * p <0.05 vs control cells. # p <0.05 vs A β_{1-42} .

doi:10.1371/journal.pone.0122843.g003

Discussion

Oxidative stress and inflammation are the main mechanisms in the progression of various neurodegenerative diseases, including AD [27–30]. In our study, we determined different markers involved in inflammation and oxidative stress induced by the A β_{1-42} peptide in primary cultures of astrocytes, with the aim to assess the antioxidant and anti-inflammatory effects of cannabinoid WIN. We found that WIN significantly increased astrocytes viability compared to control cells. Furthermore, WIN prevented the decrease in astrocytes viability induced by A β_{1-42} .

It has been shown that cannabinoids preserve neurons from A β exposure by activating MAP kinase cascade [31] and by anti-oxidative and anti-apoptotic effects [32]. Moreover, some studies demonstrated that cannabinoids protect glial cells from death [33,34]. Nevertheless, in cancer, where cells are highly proliferative and undifferentiated, treatment with cannabinoids can block cell proliferation in a dose dependent manner [35–38], demonstrating that the effects of cannabinoids on cell viability are probably dependent on cell type [39] and developmental stage [40].

Expression of CB1 [41] and CB2 [42] receptors in rat culture astrocytes have been published and also dual activation of both cannabinoid receptors by WIN (the mixed non-selective CB1/CB2 agonist) in rat cortical astrocytes have been detected [41]. On the other hand, WIN confers its protective and anti-inflammatory effects against A β injury through both CB1 and CB2 receptors [43]. Given that our results there is expression of both types of cannabinoid receptors, CB1 and CB2, it is likely that the effect of WIN observed in our study is due to the interaction with both types of receptors, consistent with published results by Fakhfour and cols [44].

We found that WIN prevented the increase of inflammatory mediators IL-1 β , TNF- α , NF- κ B, iNOS and COX-2, as well as the decrease of the anti-inflammatory mediator PPAR- γ induced by A β_{1-42} in astrocytes in primary culture. The inflammatory process is a characteristic mechanism in the development of AD, and pro-inflammatory agents are involved in the progression of cell damage [45,46,47,12]. Moreover, it is known that astrocytes participate in the

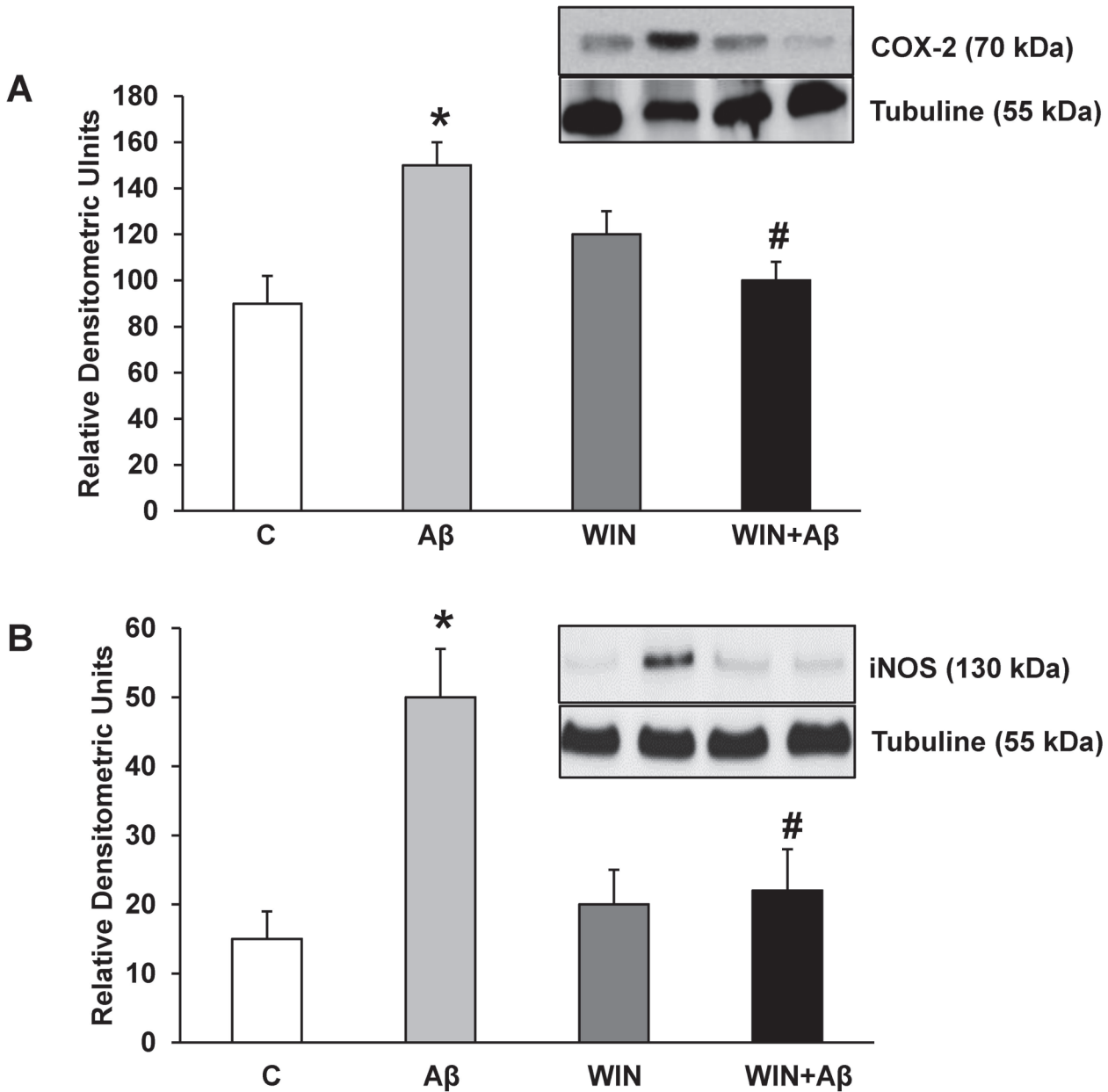


Fig 4. COX-2 and iNOS protein expression. WIN prevents COX-2 and iNOS expression induced by A β_{1-42} . COX-2 (panel A), iNOS (panel B) and α -tubulin expressions were determined by Western-blot in astrocytes treated for 24 h with 10 μ M A β_{40-1} (control peptide, C), 10 μ M A β_{1-42} (A β), 10 μ M WIN + 10 μ M control peptide (WIN) and 10 μ M WIN + 10 μ M A β_{1-42} (WIN + A β). A representative immunoblot of each protein is shown and tubulin was used as control amount of protein. Data are means \pm SD of 6 independent experiments. * p <0.05 vs control cells. # p <0.05 vs A β_{1-42} .

doi:10.1371/journal.pone.0122843.g004

inflammatory process induced by A β_{1-42} [27,28,48]. Initially, inflammation is beneficial since it produces pro-inflammatory substances involved in tissue protection, limiting the survival and proliferation of cells exposed to toxic agents, such as A β_{1-42} [49,50]. However, sustained inflammatory response could lead to neurotoxic damage or cell death [12,51,8]. NF- κ B proteins are up-regulated in inflammation conditions such as astroglial activation induced by A β_{1-42} oligomers [52]. In this regard, we found an increase in NF- κ B/p-65 expression in astrocytes after addition of A β_{1-42} that was prevented by WIN pretreatment. Valles and collaborators

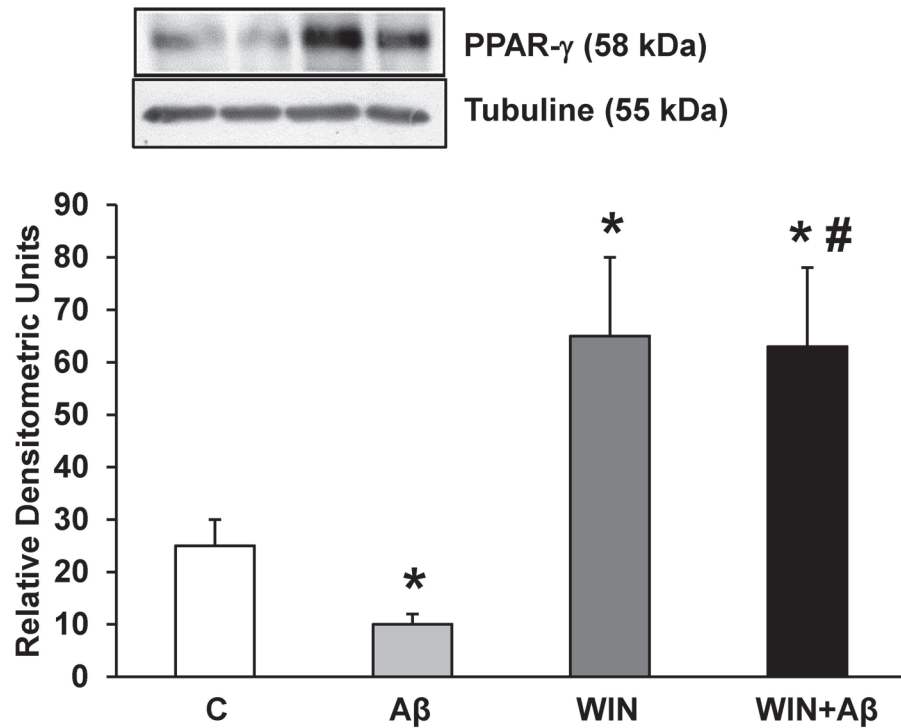


Fig 5. PPAR- γ protein expression. WIN induces PPAR- γ expression in astrocytes in primary culture treated with $A\beta_{1-42}$. PPAR- γ and α -tubulin expressions were determined by Western-blot in astrocytes treated for 24 h with 10 μ M $A\beta_{40-1}$ (control peptide, C), 10 μ M $A\beta_{1-42}$ ($A\beta$), 10 μ M WIN + 10 μ M control peptide (WIN) and 10 μ M WIN + 10 μ M $A\beta_{1-42}$ (WIN + $A\beta$). A representative immunoblot of each protein is shown and tubulin was used as control amount of protein. Data are means \pm SD of 4 independent experiments. * $p < 0.05$ vs control cells. # $p < 0.05$ vs $A\beta_{1-42}$.

doi:10.1371/journal.pone.0122843.g005

[53] found that the cytokine-receptor complex is able to bind to cytokines and other proteins of the extracellular matrix, producing inflammatory signals which could be important in pathologies such as Alzheimer's disease [53,54]. In agreement with our results, different authors have reported that cannabinoids mitigate neural cell activation in the neuroinflammatory response induced by $A\beta_{1-42}$, reducing the levels of pro-inflammatory molecules such as IL-1 β , TNF- α , COX-2 and iNOS [55,56,57]. Likewise, the activation of cannabinoid receptors diminishes the release of IL-1 β , IL-6 and TNF- α in microglial cells [58,59,60] as well as COX-2 and iNOS [61]. Studies conducted in rats pretreated with the $A\beta$ peptide found that WIN prevented cognitive impairment, glial activation and neuronal loss [19,62,63], and also reduced COX-2, iNOS and TNF- α levels [63,64].

Kainu et al. [65] demonstrated for the first time the presence of mRNA and protein PPAR- γ in CNS cells. Subsequent studies have detected PPAR- γ expression in microglial and astrocytic cells [66,12]. PPAR- γ agonists protect against $A\beta$ -induced inflammatory and neuronal damage [67,68], thus making neurons and astrocytes potential therapeutic targets for PPAR- γ ligands [69,12]. Astrocytes also express the largest levels of PPAR- γ in the neural tissues [70,71]. As other authors [72,73,74], we found a decreased expression of PPAR- γ in astrocytes treated with $A\beta_{1-42}$. Esposito et al. [56] showed in neurons that cannabinoids may act as neuro-protective agents by PPAR- γ activation. In this study, we demonstrate an increase in this protein expression in astrocytic cells previously incubated with WIN. Furthermore, we found for the first time that WIN prevents PPAR- γ expression decrease induced by $A\beta_{1-42}$ peptide in astrocytes in primary culture. There is strong evidence to suggest that some cannabinoids can act on

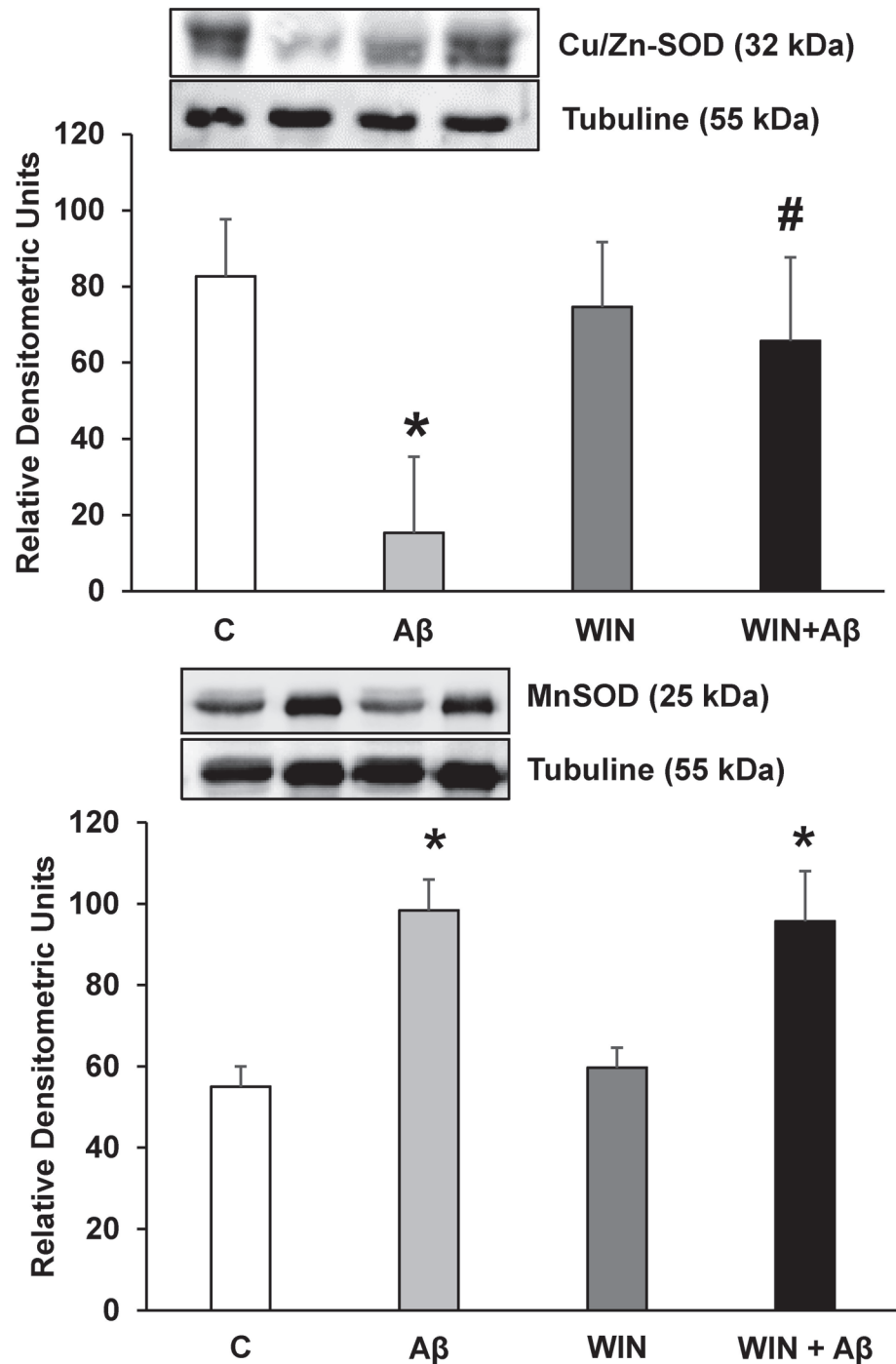


Fig 6. Cu/Zn-SOD and Mn-SOD protein expressions. WIN prevents Cu/Zn-SOD expression decrease in astrocytes in primary culture treated with A β_{1-42} . Cu/Zn-SOD, Mn-SOD and α -tubulin expressions were determined by Western-blot in astrocytes treated for 24 h with 10 μ M A β_{40-1} (control peptide, C), 10 μ M A β_{1-42} (A β), 10 μ M WIN + 10 μ M control peptide (WIN) and 10 μ M WIN + 10 μ M A β_{1-42} (WIN + A β). A representative immunoblot of each protein is shown and tubulin was used as control amount of protein. Data are means \pm SD of 4 independent experiments. * p <0.05 vs control cells. # p <0.05 vs A β_{1-42} .

doi:10.1371/journal.pone.0122843.g006

PPARs through either direct or indirect pathways. In order to directly act on nuclear transcriptional factors PPARs, exogenous cannabinoids need to pass through plasma membrane and be transported into nucleus which may involve certain membrane and intracellular transporters. However, we still cannot rule out that cannabinoids effects could be indirect through the binding of other cellular targets which in turn induces PPARs activation [75]. In fact, WIN attenuates amyloid-beta-induced neuroinflammation in rats through activation of cannabinoid receptors and PPAR- γ pathway [44].

Different authors have demonstrated the role of oxidative stress in AD [76–79]. The cumulative damage caused by free radicals induces alterations in the activity or expression of antioxidant enzymes like catalase or SOD. These enzymes were found to be decreased in both CNS and peripheral tissues of AD patients [80,81]. In this sense, we demonstrate that Cu/Zn SOD is decreased in astrocytes treated with A β_{1-42} . Our results are consistent with those reported by other authors, highlighting the role of oxidative stress in the development of AD [82]. New substances are under research to reduce damage caused by oxidative stress in this disease. Widely distributed in the body, cannabinoids receptors were discovered few decades ago and are still under research [57]. Few studies address the effect of cannabinoids on oxidative stress. For instance, cannabinoids were found to prevent or antagonize oxidative stress toxicity in cortical neurons in cultures [83,84], and in lymphoblastic cells [85]. Studies with PC12 cells exposed to A β_{1-42} peptide demonstrated that cannabinoids reduced reactive oxygen species production and membrane lipid oxidation [86,32]. Our results provide evidence that A β_{1-42} decreases Cu/Zn SOD expression in astrocytes in primary culture, and pretreatment with WIN increases Cu/Zn SOD expression, preventing the decrease caused by A β_{1-42} . These findings indicate that cannabinoids could act as a protective agent against oxidative stress caused by A β_{1-42} . In Fig 7 the set of results are summarized. However our results indicate that A β_{1-42} elevated Mn SOD protein expression, increasing mitochondrial biogenesis mechanism, such as we previously published [74]. Pretreatment with WIN did not prevent Mn SOD overexpression induced by A β_{1-42} . Mn SOD plays a role in the adaptive response which protects brain cells from damage, as in the case of AD. In fact, Mn SOD preserves neurons against oxidative stress [87] and protects developing neurons from β -amyloid toxicity [88]. This enzyme catalyzes the conversion of superoxide radicals to molecular oxygen and H₂O₂, whereas glutathione peroxidase, peroxiredoxin reductase and catalase neutralize H₂O₂. Overexpression of Mn SOD induces cognitive recovery and reduces A β levels in AD animal models [89]. Furthermore, Mn SOD deficiency increases β -amyloid levels and amyloid plaque burden, promoting the development of behavioural disturbances [90].

Preclinical data suggest a beneficial role of some cannabinoids for treatment of different diseases. Dronabidol, an oil-based solution of Δ^9 -THC, is used as anti-emetic and appetite stimulant [91]. Δ^9 -THC also decreases agitation present in the advanced stage of AD [92]. In 2003, the FDA granted the patent for cannabinoids as antioxidants and neuro-protectants (U.S. Department of Health and Human Services). Despite these promising preliminary results, the clinical utility of cannabinoids in AD is still to be determined [93].

Conclusions

Taken together, our findings show that cannabinoid WIN increases cell viability and anti-inflammatory response in astrocytes in primary culture and prevents cell death induced by A β_{1-42} . Furthermore, WIN increases expression of anti-oxidant Cu/Zn SOD and is able to prevent inflammation induced by A β_{1-42} in astrocytes. In this sense, clinical studies are needed to evaluate the neuro-protective effects of cannabinoids in Alzheimer's disease.

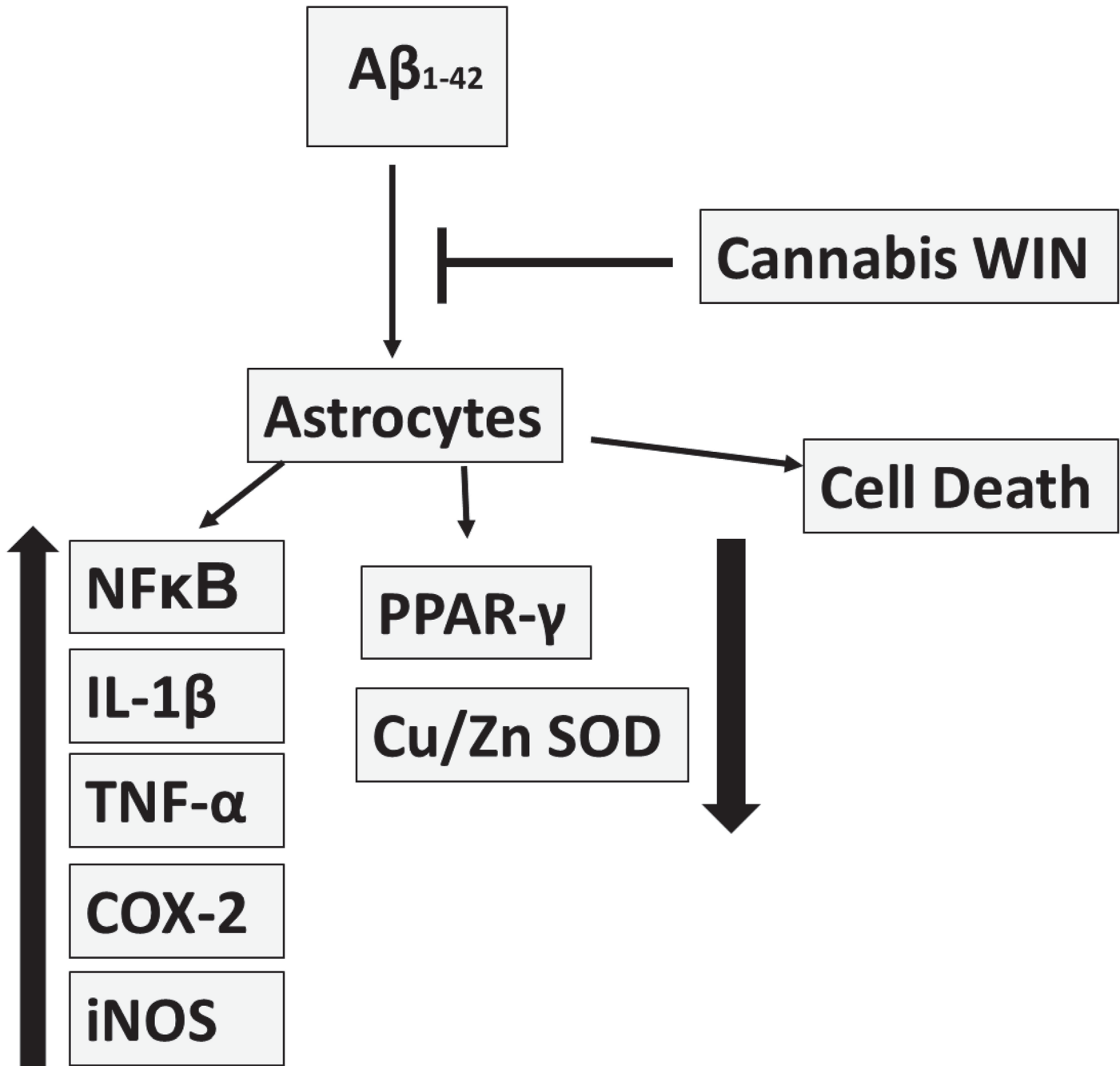


Fig 7. Preventive function of cannabinoid WIN on Aβ₁₋₄₂-induced toxic effects in astrocytes in primary culture. Cannabinoid WIN 55,212-2 increases cell viability and anti-inflammatory response in cultured astrocytes and prevents inflammatory effects induced by Aβ₁₋₄₂.

doi:10.1371/journal.pone.0122843.g007

Author Contributions

Conceived and designed the experiments: SLV MA. Performed the experiments: DAR SGO MDM EO. Analyzed the data: AI JMV. Contributed reagents/materials/analysis tools: PM. Wrote the paper: SLV MA.

References

1. Selkoe DJ. Alzheimer's disease: genotypes, phenotypes, and treatments. *Science*. 1977; 275: 630–631.
2. Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, et al. Alzheimer's Disease International. Global prevalence of dementia: a Delphi consensus study. *Lancet*. 2005; 366: 2112–2117. PMID: [16360788](#)
3. Heneka MT, O'Banion MK. Inflammatory processes in Alzheimer's disease. *J Neuroimmunol*. 2007; 184: 69–91. PMID: [17222916](#)
4. Pratico D. Evidence of oxidative stress in Alzheimer's disease brain and antioxidant therapy: lights and shadows. *Ann N Y Acad Sci*. 2008; 1147: 70–78. doi: [10.1196/annals.1427.010](#) PMID: [19076432](#)
5. Inestrosa NC, Reyes AE, Chacón MA, Cerpa W, Villalón A, Montiel J, et al. Human-like rodent amyloid-beta-peptide determines Alzheimer pathology in aged wild-type *Octodon degu*. *Neurobiol Aging*. 2005; 26: 1023–1028. PMID: [15748782](#)
6. Vallés SL, Borrás C, Gambini J, Furriol J, Ortega A, Sastre J, et al. Oestradiol or genistein rescues neurons from amyloid beta-induced cell death by inhibiting activation of p38. *Aging Cell*. 2008; 7: 112–118. PMID: [18031570](#)
7. Sofroniew MV and Vinters HV. Astrocytes: biology and pathology. *Acta Neuropathol*. 2010; 119: 7–35. doi: [10.1007/s00401-009-0619-8](#) PMID: [20012068](#)
8. Choi SS, Lee HJ, Lim I, Satoh J, Kim SU. Human astrocytes: secretome profiles of cytokines and chemokines. *PLoS One*. 2014;1: 9(4):e92325. doi: [10.1371/journal.pone.0092325](#)
9. van Kralingen C, Kho DT, Costa J, Angel CE, Graham ES. Exposure to inflammatory cytokines IL-1 β and TNF α induces compromise and death of astrocytes; implications for chronic neuroinflammation. *PLoS One*. 2013;19; 8(12): e84269. doi: [10.1371/journal.pone.0084269](#)
10. Zhang W, Smith C, Howlett C, Stanimirovic D. Inflammatory activation of human brain endothelial cells by hypoxic astrocytes in vitro is mediated by IL-1beta. *J Cereb Blood Flow Metab*. 2000; 20: 967–978. PMID: [10894180](#)
11. Li C, Zhao R, Gao K, Wei Z, Yin MY, Lau LT et al. Astrocytes: implications for neuroinflammatory pathogenesis of Alzheimer's disease. *Curr Alzheimer Res*. 2011; 8: 67–80. PMID: [21143158](#)
12. Valles SL, Dolz-Gaiton P, Gambini J, Borrás C, Lloret A, Pallardo FV. Estradiol or genistein prevent Alzheimer's disease-associated inflammation correlating with an increase PPAR gamma expression in cultured astrocytes. *Brain Res*. 2010; 1312: 138–144. doi: [10.1016/j.brainres.2009.11.044](#) PMID: [19948157](#)
13. Pacher P, Kunos G. Modulating the endocannabinoid system in human health and disease-successes and failures. *FEBS J*. 2013; 280: 1918–1943. doi: [10.1111/febs.12260](#) PMID: [23551849](#)
14. Kunos G and Tam J. The case for peripheral CB $_1$ receptor blockade in the treatment of visceral obesity and its cardiometabolic complications. *Br J Pharmacol*. 2011; 163: 1423–1431. doi: [10.1111/j.1476-5381.2011.01352.x](#) PMID: [21434882](#)
15. Centonze D, Finazzi-Agrò A, Bernardi G, Maccarrone M. The endocannabinoid system in targeting inflammatory neurodegenerative diseases. *Trends Pharmacol Sci*. 2007; 28: 180–187. PMID: [17350694](#)
16. Klein WK. Cannabinoid-based drugs as anti-inflammatory therapeutics. *Nat Rev Immunol*. 2005; 5: 400–411. PMID: [15864274](#)
17. Hillard CJ, Weinlander KM, Stuhr KL. Contributions of endocannabinoid signaling to psychiatric disorders in humans: genetic and biochemical evidence. *Neuroscience*. 2012; 1: 207–229.
18. Guindon J, Hohmann AG. The endocannabinoid system and cancer: therapeutic implication. *Br J Pharmacol*. 2011; 163: 1447–1463. doi: [10.1111/j.1476-5381.2011.01327.x](#) PMID: [21410463](#)
19. Ramírez BG, Blázquez C, Gómez del Pulgar T, Guzmán M, de Ceballos ML. Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *J Neurosci*. 2005; 25: 1904–1913. PMID: [15728830](#)
20. Esposito G, Scuderi C, Savani C, Steardo L Jr, De Filippis D, Cottone P, et al. Cannabidiol in vivo blunts beta-amyloid induced neuroinflammation by suppressing IL-1beta and iNOS expression. *Br J Pharmacol*. 2007; 151: 1272–1279. PMID: [17592514](#)
21. Blanchard BJ, Chen A, Rozeboom LM, Stafford KA, Weigele P, Ingram VM. Efficient reversal of Alzheimer's disease fibril formation and elimination of neurotoxicity by a small molecule. *Proc Natl Acad Sci*. 2004; 101: 14326–14332. PMID: [15388848](#)
22. McGeer PL, McGeer EG. The amyloid cascade-inflammatory hypothesis of Alzheimer disease: implications for therapy. *Acta Neuropathol*. 2013; 126: 479–497. doi: [10.1007/s00401-013-1177-7](#) PMID: [24052108](#)

23. Rönicke R, Klemm A, Meinhardt J, Schröder UH, Fändrich M, Reymann KG. Abeta mediated diminution of MTT reduction—an artefact of single cell culture?. *PLoS One*. 2008; 3: e3236. doi: [10.1371/journal.pone.0003236](https://doi.org/10.1371/journal.pone.0003236) PMID: [18800168](https://pubmed.ncbi.nlm.nih.gov/18800168/)
24. Palanca JM, Aguirre-Rueda D, Granell MV, Aldasoro M, Garcia A, Iradi A. Sugammadex, a neuromuscular blockade reversal agent, causes neuronal apoptosis in primary cultures. *Int J Med Sci*. 2013; 10: 1278–1285. doi: [10.7150/ijms.6254](https://doi.org/10.7150/ijms.6254) PMID: [23983586](https://pubmed.ncbi.nlm.nih.gov/23983586/)
25. Peterson GL. A simplification of the protein assay method of Lowry et al. which is more generally applicable. *Anal Biochem*. 1977; 83: 346–356. PMID: [603028](https://pubmed.ncbi.nlm.nih.gov/603028/)
26. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature*. 1998; 391: 79–82. PMID: [9422508](https://pubmed.ncbi.nlm.nih.gov/9422508/)
27. Eikelenboom P, van Gool WA. Neuroinflammatory perspectives on the two faces of Alzheimer's disease. *J Neural Transm*. 2004; 111: 281–294. PMID: [14991455](https://pubmed.ncbi.nlm.nih.gov/14991455/)
28. Zhu X, Smith MA, Perry G, Aliev G. Mitochondrial failures in Alzheimer's disease. *Am J Alzheimers Dis Other Demen*. 2004; 19: 345–352. PMID: [15633943](https://pubmed.ncbi.nlm.nih.gov/15633943/)
29. Jiménez-Jiménez FJ, Alonso-Navarro H, Ayuso-Peralta L, Jabbour-Wadiah T. Oxidative stress and Alzheimer's disease. *Rev Neurol*. 2006; 42: 419–427. PMID: [16602059](https://pubmed.ncbi.nlm.nih.gov/16602059/)
30. Quintanilla RA, Orellana JA, von Bernhardi R. Understanding risk factors for Alzheimer's disease: interplay of neuroinflammation, connexin-based communication and oxidative stress. *Arch Med Res*. 2012; 43: 632–644. doi: [10.1016/j.arcmed.2012.10.016](https://doi.org/10.1016/j.arcmed.2012.10.016) PMID: [23142264](https://pubmed.ncbi.nlm.nih.gov/23142264/)
31. Milton NG. Anandamide and noladin ether prevent neurotoxicity of the human amyloid-beta peptide. *Neurosci Lett*. 2002; 332: 127–130. PMID: [12384227](https://pubmed.ncbi.nlm.nih.gov/12384227/)
32. Iuvone T, Esposito G, Esposito R, Santamaria R, Di Rosa M, Izzo AA. Neuroprotective effect of cannabidiol, a non-psychoactive component from Cannabis sativa, on beta-amyloid-induced toxicity in PC12 cells. *J Neurochem*. 2004; 89: 134–141. PMID: [15030397](https://pubmed.ncbi.nlm.nih.gov/15030397/)
33. Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, et al. The endogenous cannabinoid system controls extinction of aversive memories. *Nature*. 2002; 1: 530–534.
34. Gómez Del Pulgar T, De Ceballos ML, Guzmán M, Velasco G. Cannabinoids protect astrocytes from ceramide-induced apoptosis through the phosphatidylinositol 3-kinase/protein kinase B pathway. *J Biol Chem*. 2002; 277: 36527–36533. PMID: [12133838](https://pubmed.ncbi.nlm.nih.gov/12133838/)
35. Pourkhalili N, Ghahremani MH, Farsandaj N, Tavajohi S, Majdzadeh M, Parsa M, et al. Evaluation of anti-invasion effect of cannabinoids on human hepatocarcinoma cells. *Toxicol Mech Methods*. 2013; 23: 120–126. doi: [10.3109/15376516.2012.730559](https://doi.org/10.3109/15376516.2012.730559) PMID: [22978792](https://pubmed.ncbi.nlm.nih.gov/22978792/)
36. De Petrocellis L, Ligresti A, Schiano Moriello A, Iappelli M, Verde R, Stott CG, et al. Non-THC cannabinoids inhibit prostate carcinoma growth in vitro and in vivo: pro-apoptotic effects and underlying mechanisms. *Br J Pharmacol*. 2013; 168: 79–102. doi: [10.1111/j.1476-5381.2012.02027.x](https://doi.org/10.1111/j.1476-5381.2012.02027.x) PMID: [22594963](https://pubmed.ncbi.nlm.nih.gov/22594963/)
37. Vara D, Salazar M, Olea-Herrero N, Guzmán M, Velasco G, Díaz-Laviada I. Anti-tumoral action of cannabinoids on hepatocellular carcinoma: role of AMPK-dependent activation of autophagy. *Cell Death Differ*. 2011; 18: 1099–1111. doi: [10.1038/cdd.2011.32](https://doi.org/10.1038/cdd.2011.32) PMID: [21475304](https://pubmed.ncbi.nlm.nih.gov/21475304/)
38. Rocha FC, Dos Santos JG Júnior, Stefano SC, da Silveira DX. Systematic review of the literature on clinical and experimental trials on the antitumor effects of cannabinoids in gliomas. *J Neurooncol*. 2014; 116: 11–24. doi: [10.1007/s11060-013-1277-1](https://doi.org/10.1007/s11060-013-1277-1) PMID: [24142199](https://pubmed.ncbi.nlm.nih.gov/24142199/)
39. Sánchez C, Galve-Roperh I, Canova C, Brachet P, Guzmán M. Delta9-tetrahydrocannabinol induces apoptosis in C6 glioma cells. *FEBS Lett*. 1998; 436: 6–10. PMID: [9771884](https://pubmed.ncbi.nlm.nih.gov/9771884/)
40. Guzmán M, Sánchez C, Galve-Roperh I. Cannabinoids and cell fate. *Pharmacol Ther*. 2002; 95: 175–184. PMID: [12182964](https://pubmed.ncbi.nlm.nih.gov/12182964/)
41. Stella N. Cannabinoid signaling in glial cells. *Glia*. 2004; 48: 267–277. PMID: [15390110](https://pubmed.ncbi.nlm.nih.gov/15390110/)
42. Sheng WS, Hu S, min X, Cabral GA, Lokensgard JR, Peterson P. Synthetic cannabinoid WIN55,212–2 inhibits generation of inflammatory mediators by IL-1beta-stimulated human astrocytes. *Glia*. 2005; 49: 211–219. PMID: [15390091](https://pubmed.ncbi.nlm.nih.gov/15390091/)
43. Palmer SL, Thakur GA, Makriyannis A. Cannabinergic ligands. *Chem Phys Lipids*. 2002; 121: 3–19. PMID: [12505686](https://pubmed.ncbi.nlm.nih.gov/12505686/)
44. Fakhfouri G, Ahmadiani A, Rahimian R, Grolla AA, Moradi F, Haeri A. WIN55212-2 attenuates amyloid-beta-induced neuroinflammation in rats through activation of cannabinoid receptors and PPAR- γ pathway. *Neuropharmacology*. 2012; 63: 653–666 doi: [10.1016/j.neuropharm.2012.05.013](https://doi.org/10.1016/j.neuropharm.2012.05.013) PMID: [22634229](https://pubmed.ncbi.nlm.nih.gov/22634229/)
45. Strohmeyer R, Rogers J. Molecular and cellular mediators of Alzheimer's disease inflammation. *J Alzheimers Dis*. 2001; 3: 131–157. PMID: [12214082](https://pubmed.ncbi.nlm.nih.gov/12214082/)

46. Wyss-Coray T, Mucke L. Inflammation in neurodegenerative disease-A doubleedged sword. *Neuron*. 2002; 35: 419–432. PMID: [12165466](#)
47. Griffin WS, Mrak RE. Interleukin-1 in the genesis and progression of and risk for development of neuronal degeneration in Alzheimer's disease. *J Leukoc Biol*. 2002; 72: 233–238. PMID: [12149413](#)
48. McGeer PL, Rogers J, McGeer EG. Inflammation, anti-inflammatory agents and Alzheimer disease: the last 12 years. *J Alzheimers Dis*. 2006; 9: 271–276. PMID: [16914866](#)
49. Allan SM, Rothwell NJ. Cytokines and acute neurodegeneration. *Nat Rev Neurosci*. 2001; 2: 734–744. PMID: [11584311](#)
50. Allan SM, Rothwell NJ. Inflammation in central nervous system injury. *Philos Trans R Soc Lond B Biol Sci*. 2003; 358: 1669–1677. PMID: [14561325](#)
51. Burkert K, Moodley K, Angel CE, Brooks A, Graham ES. Detailed analysis of inflammatory and neuro-modulatory cytokine secretion from human NT2 astrocytes using multiplex bead array. *Neurochem Int*. 2012; 60: 573–580. doi: [10.1016/j.neuint.2011.09.002](#) PMID: [21939706](#)
52. Carrero I, Gonzalo MR, Martin B, Sanz-Anquela JM, Arévalo-Serrano J, Gonzalo-Ruiz A. Oligomers of β -amyloid protein (A β _{1–42}) induce the activation of cyclooxygenase-2 in astrocytes via an interaction with interleukin-1 β , tumour necrosis factor- α , and a nuclear factor κ -B mechanism in the rat brain. *Exp Neurol*. 2012; 236: 215–227. doi: [10.1016/j.expneurol.2012.05.004](#) PMID: [22617488](#)
53. Vallés S, Tsoi C, Huang WY, Wyllie D, Carlotti F, Askari JA, et al. Recruitment of a heparan sulfate subunit to the interleukin-1 receptor complex. Regulation by fibronectin attachment. *J Biol Chem*. 1999; 274: 20103–20109. PMID: [10400621](#)
54. Bondareff W. Age-related changes in brain extracellular space affect processing of amyloid- β peptides in Alzheimer's disease. *J Alzheimers Dis*. 2013; 35: 1–6. doi: [10.3233/JAD-122305](#) PMID: [23364138](#)
55. Sheng WS, Hu S, Min X, Cabral GA, Lokensgard JR, Peterson PK. Synthetic cannabinoid WIN55,212–2 inhibits generation of inflammatory mediators by IL-1 β -stimulated human astrocytes. *Glia*. 2005; 49: 211–219. PMID: [15390091](#)
56. Esposito G, Scuderi C, Valenza M, Togna GI, Latina V, De Filippis D, et al. Cannabidiol reduces A β -induced neuroinflammation and promotes hippocampal neurogenesis through PPAR γ involvement. *PLoS One*. 2011; 6:e28668. doi: [10.1371/journal.pone.0028668](#) PMID: [22163051](#)
57. Scuderi C, Esposito G, Blasio A, Valenza M, Arietti P, Steardo L Jr, et al. Palmitoylethanolamide counteracts reactive astrogliosis induced by β -amyloid peptide. *J Cell Mol Med*. 2011; 15: 2664–2674. doi: [10.1111/j.1582-4934.2011.01267.x](#) PMID: [21255263](#)
58. Puffenbarger RA, Boothe AC, Cabral GA. Cannabinoids inhibit LPS-inducible cytokine mRNA expression in rat microglial cells. *Glia*. 2000; 29: 58–69. PMID: [10594923](#)
59. Facchinetti F, Del Giudice E, Furegato S, Passarotto M, Leon A. Cannabinoids ablate release of TNF α in rat microglial cells stimulated with lipopolysaccharide. *Glia*. 2003; 41: 161–168. PMID: [12509806](#)
60. Ehrhart J, Obregon D, Mori T, Hou H, Sun N, Bai Y, et al. Stimulation of cannabinoid receptor 2 (CB2) suppresses microglial activation. *J Neuroinflammation*. 2005; 12: 2–29.
61. Castillo A, Tolón MR, Fernández-Ruiz J, Romero J, Martínez-Orgado J. The neuroprotective effect of cannabidiol in an in vitro model of newborn hypoxic-ischemic brain damage in mice is mediated by CB (2) and adenosine receptors. *Neurobiol Dis*. 2010; 37: 434–440. doi: [10.1016/j.nbd.2009.10.023](#) PMID: [19900555](#)
62. Martín-Moreno AM, Reigada D, Ramírez BG, Mechoulam R, Innamorato N, Cuadrado A, et al. Cannabidiol and other cannabinoids reduce microglial activation in vitro and in vivo: relevance to Alzheimer's disease. *Mol Pharmacol*. 2011; 79: 964–973. doi: [10.1124/mol.111.071290](#) PMID: [21350020](#)
63. Martín-Moreno AM, Brera B, Spuch C, Carro E, García-García L, Delgado M, et al. Prolonged oral cannabinoid administration prevents neuroinflammation, lowers β -amyloid levels and improves cognitive performance in Tg APP 2576 mice. *J Neuroinflammation*. 2012; Jan 16 9:8. doi: [10.1186/1742-2094-9-8](#) PMID: [22248049](#)
64. Esposito G, De Filippis D, Carnuccio R, Izzo AA, Iuvone T. The marijuana component cannabidiol inhibits β -amyloid-induced tau protein hyperphosphorylation through Wnt/ β -catenin pathway rescue PC12 cells. *J Mol Med*. 2006; 84: 253–258. PMID: [16389547](#)
65. Kainu T, Wikström AC, Gustafsson JA, Peltö-Huikko M. Localization of the peroxisome proliferator-activated receptor in the brain. *Neuroreport*. 1994; 5: 2481–2485. PMID: [7696585](#)
66. Luna-Medina R, Cortes-Canteli M, Alonso M, Santos A, Martínez A, Perez-Castillo A. Regulation of inflammatory response in neural cells in vitro by thiazolidinones derivatives through peroxisome proliferator-activated receptor gamma activation. *J Biol Chem*. 2005; 280: 21453–21462. PMID: [15817469](#)
67. Bright JJ, Kanakasabai S, Chearwae W, Chakraborty S. PPAR Regulation of Inflammatory Signaling in CNS Diseases. *PPAR Res*. 2008;658520. doi: [10.1155/2008/658520](#)

68. Kapadia R, Yi JH, Vemuganti R. Mechanisms of anti-inflammatory and neuroprotective actions of PPAR- γ agonists. *Front Biosci.* 2008; 13: 1813–1826. PMID: [17981670](#)
69. Landreth G, Jiang Q, Mandrekar S, Heneka M. PPARgamma agonists as therapeutics for the treatment of Alzheimer's disease. *Neurotherapeutics.* 2008; 5: 481–489. doi: [10.1016/j.nurt.2008.05.003](#) PMID: [18625459](#)
70. Bodles AM, Barger SW. Secreted β -amyloid precursor protein activates microglia via JNK and p38-MAPK. *Neurobiol Aging.* 2005; 26: 9–16. PMID: [15585341](#)
71. Ho GJ, Drego R, Hakimian E, Masliah E. Mechanisms of cell signaling and inflammation in Alzheimer's disease. *Curr Drug Targets Inflamm Allergy.* 2005; 4: 247–256. PMID: [15853747](#)
72. Wang HM, Zhao YX, Zhang S, Liu GD, Kang WY, Tang HD, et al. PPARgamma agonist curcumin reduces the amyloid-beta-stimulated inflammatory responses in primary astrocytes. *J Alzheimers Dis.* 2010; 20: 1189–1199. doi: [10.3233/JAD-2010-091336](#) PMID: [20413894](#)
73. Chen YC, Wu JS, Tsai HD, Huang CY, Chen JJ, Sun GY, et al. Peroxisome proliferator-activated receptor gamma (PPAR- γ) and neurodegenerative disorders. *Mol Neurobiol.* 2012; 46: 114–124. doi: [10.1007/s12035-012-8259-8](#) PMID: [22434581](#)
74. Aguirre-Rueda D, Guerra-Ojeda S, Aldasoro M, Iradi A, Obrador E, Ortega A, et al. Astrocytes protect neurons from A β _{1–42} peptide-induced neurotoxicity increasing TFAM and PGC-1 and decreasing PPAR- γ and SIRT-1. *Int J Med Sci.* 2015; 12: 48–56. doi: [10.7150/ijms.10035](#) PMID: [25552918](#)
75. Sun Y, Bennett A. Cannabinoids: a new group of agonists of PPARs. *PPAR Res.* 2007; 23513. doi: [10.1155/2007/23513](#)
76. Wallace DC. Mitochondrial defects in cardiomyopathy and neuromuscular disease. *Am Heart J.* 2000; 139: S70–S85. PMID: [10650320](#)
77. Lesnefsky EJ, Moghaddas S, Tandler B, Kerner J, Hoppel CL. Mitochondrial dysfunction in cardiac disease: ischemia—reperfusion, aging, and heart failure. *J Mol Cell Cardiol.* 2001; 33: 1065–1089. PMID: [11444914](#)
78. Chen Q, Vazquez EJ, Moghaddas S, Hoppel CL, Lesnefsky EJ. Production of reactive oxygen species by mitochondria: central role of complex III. *J Biol Chem.* 2003; 278: 36027–36031. PMID: [12840017](#)
79. Abeti R, Duchon MR. Activation of PARP by Oxidative Stress Induced by β -Amyloid: Implications for Alzheimer's Disease. *Neurochem Res.* 2012; 37: 2589–2596. doi: [10.1007/s11064-012-0895-x](#) PMID: [23076628](#)
80. Marcus DL, Thomas C, Rodriguez C, Simberkoff K, Tsai JS, Strafaci JA, et al. Increased peroxidation and reduced antioxidant enzyme activity in Alzheimer's disease. *Exp Neurol.* 1998; 150: 40–44. PMID: [9514828](#)
81. Padurariu M, Ciobica A, Hritcu L, Stoica B, Bild W, Stefanescu C. Changes of some oxidative stress markers in the serum of patients with mild cognitive impairment and Alzheimer's disease. *Neuroscience Letters.* 2010; 469: 6–10. doi: [10.1016/j.neulet.2009.11.033](#) PMID: [19914330](#)
82. Pappolla MA, Chyan YJ, Omar RA, Hsiao K, Perry G, Smith MA, et al. Evidence of oxidative stress and in vivo neurotoxicity of beta-amyloid in a transgenic mouse model of Alzheimer's disease: a chronic oxidative paradigm for testing antioxidant therapies in vivo. *Am J Pathol.* 1998; 152: 871–877. PMID: [9546346](#)
83. Hampson AJ, Grimaldi M, Axelrod J, Wink D. Cannabidiol and (-)- Δ 9-tetrahydrocannabinol are neuroprotective antioxidants. *Proc Natl Acad Sci.* 1998; 95: 8268–8273. PMID: [9653176](#)
84. Hampson AJ, Grimaldi M. Cannabinoid receptor activation and elevated cyclic AMP reduce glutamate neurotoxicity. *Eur J Neurosci.* 2001; 13: 1529–1536. PMID: [11328347](#)
85. Chen Y, Buck J. Cannabinoids protect cells from oxidative cell death: a receptor-independent mechanism. *J. Pharmacol. Exp. Ther.* 2000; 293: 807–812. PMID: [10869379](#)
86. Praticò D, MY Lee V, Trojanowski JQ, Rokach J, Fitzgerald GA. Increased F2-isoprostanes in Alzheimer's disease: evidence for enhanced lipid peroxidation in vivo. *FASEB J.* 1998; 12: 1777–1783. PMID: [9837868](#)
87. Keller JN, Kindy MS, Holtzman FW, St Clair DK, Yen HC, Germeyer A, et al. Mitochondrial manganese superoxide dismutase prevents neural apoptosis and reduces ischemic brain injury: suppression of peroxynitrite production, lipid peroxidation, and mitochondrial dysfunction. *J Neurosci.* 1998; 18: 687–697. PMID: [9425011](#)
88. Sompol P, Ittarat W, Tangpong J, Chen Y, Doubinskaia I, Batinic-Haberle I, et al. A neuronal model of Alzheimer's disease: an insight into the mechanisms of oxidative stress-mediated mitochondrial injury. *Neuroscience.* 2008; 153: 120–130. doi: [10.1016/j.neuroscience.2008.01.044](#) PMID: [18353561](#)
89. Dumont M, Wille E, Stack C, Calingasan NY, Beal MF, Lin MT. Reduction of oxidative stress, amyloid deposition, and memory deficit by manganese superoxide dismutase overexpression in a transgenic

mouse model of Alzheimer's disease. *FASEB J.* 2009; 23: 2459–2466. doi: [10.1096/fj.09-132928](https://doi.org/10.1096/fj.09-132928)
PMID: [19346295](https://pubmed.ncbi.nlm.nih.gov/19346295/)

90. Esposito L, Raber J, Kekoni L, Yan F, Yu GQ, Bien-Ly N, et al. Reduction in mitochondrial superoxide dismutase modulates Alzheimer's disease-like pathology and accelerates the onset of behavioral changes in human amyloid precursor protein transgenic mice. *J Neurosci.* 2006; 26: 5167–5179. PMID: [16687508](https://pubmed.ncbi.nlm.nih.gov/16687508/)
91. Volicer L, Stelly M, Morris J, McLaughlin J, Volicer BJ. Effects of dronabinol on anorexia and disturbed behavior in patients with Alzheimer's disease. *Int J Geriatr Psychiatry.* 1997; 12: 913–919. PMID: [9309469](https://pubmed.ncbi.nlm.nih.gov/9309469/)
92. Walther S, Mahlberg R, Eichmann U, Kunz D. 9-tetrahydrocannabinol for nighttime agitation in severe dementia. *Psychopharmacology.* 2006; 185: 524–528. PMID: [16521031](https://pubmed.ncbi.nlm.nih.gov/16521031/)
93. Krishnan S, Cairns R, Howard R. (2009) Cannabinoids for the treatment of dementia. *Cochrane Database Syst Rev.* 15;(2): CD007204. doi: [10.1002/14651858](https://doi.org/10.1002/14651858)