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Cannabidiol Promotes Amyloid Precursor Protein Ubiquitination and Reduction of Beta Amyloid Expression in SHSY5Y^{APP+} Cells Through PPARγ Involvement

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The amyloidogenic cascade is regarded as a key factor at the basis of Alzheimer's disease (AD) pathogenesis. The aberrant cleavage of amyloid precursor protein (APP) induces an increased production and a subsequent aggregation of beta amyloid (Aβ) peptide in limbic and association cortices. As a result, altered neuronal homeostasis and oxidative injury provoke tangle formation with consequent neuronal loss. Cannabidiol (CBD), a *Cannabis* derivative devoid of psychotropic effects, has attracted much attention because it may beneficially interfere with several Aβ-triggered neurodegenerative pathways, even though the mechanism responsible for such actions remains unknown. In the present research, the role of CBD was investigated as a possible modulating compound of APP processing in SHSY5Y^{APP+} neurons. In addition, the putative involvement of peroxisome proliferator-activated receptor-γ (PPARγ) was explored as a candidate molecular site responsible for CBD actions. Results indicated the CBD capability to induce the ubiquitination of APP protein which led to a substantial decrease in APP full length protein levels in SHSY5Y^{APP+} with the consequent decrease in Aβ production. Moreover, CBD promoted an increased survival of SHSY5Y^{APP+} neurons, by reducing their long-term apoptotic rate. Obtained results also showed that all, here observed, CBD effects were dependent on the selective activation of PPARγ.

Keywords: cannabidiol; PPARγ; APP ubiquitination; SHSY5Y^{APP+}.

INTRODUCTION

Alzheimer's Disease (AD) is a complex neurodegenerative disorder characterized by a progressive cognitive impairment, a variety of neuropsychiatric and behavioural disturbances leading to severe restrictions in daily living activities (Jicha and Carr, 2010; Querfurth and LaFerla, 2010). Post-mortem visible pathognomonic features occurring in AD brains include the accumulation of beta amyloid (Aβ) fibrils in senile plaques (SP) and intraneuronal neurofibrillary tangles (NFT) (Blennow *et al*., 2006; Huang and Jiang, 2009). Such neuropathological features are putatively considered the result of the along the years prior formation of Aβ due to a misregulated cleavage of amyloid precursor protein (APP). Amyloidogenic cascade is commonly regarded as a key factor at the basis of AD pathogenesis (Walsh and Selkoe, 2007). According to this hypothesis, the cleavage of aberrant APP production by the protease β-secretase (BACE-1) occurs at the N-terminus of the Aβ domain leading to the formation of secreted sAPPβ as well as a C-terminal fragment of APP of 99 amino acids (C99). Subsequently, C99 is cleaved within its transmembrane domain by a γ-secretase, resulting in the secretion of Aβ peptide and in the generation of the APP intracellular domain (AICD) (Selkoe, 2001).

In other words, such hypothesis suggests that the aberrant cleavage of APP induces an increased production and a subsequent aggregation of Aβ peptide in limbic and association cortices leading to synaptic alterations and formation of diffuse plaques which, in turn, cause reactive gliosis. As a result, altered neuronal homeostasis and oxidative injury provoke tangle formation with subsequent neuronal loss (Crews and Masliah, 2010). The most important implied prediction deriving from this postulate is that the reduction of Aβ production would slow down AD progression and ameliorate its symptoms.

In the recent years, several studies demonstrated that cannabidiol (CBD), the main non psychoactive component of *Cannabis sativa*, displays a plethora of beneficial effects, all able to remarkably counteract Aβ induced pathology either in *in vitro* and *in vivo* AD models (Esposito *et al*., 2006; Esposito *et al*., 2007). Indeed, CBD decreased Aβ-induced neuronal cell death by virtue of its ability to scavenge reactive oxygen species and antagonized lipid peroxidation. Its antioxidant properties occur without any mediation of cannabinoid receptor activation (Hampson *et al*., 1998; Iuvone *et al*., 2004). CBD reverted tau hyperphosphorylation by antagonizing glycogen synthase kinase 3 beta (GSK-3β) activity, a tau protein kinase responsible for tau hyperphosphorylation in AD (Esposito *et al*., 2006).

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In addition, CBD exerted *in vivo* a wide range of antinflammatory effects in Aβ induced reactive gliosis, since it was reported to blunt synthesis and release of a number of pro-inflammatory molecules from activated glial cells, including cytokines, nitric oxide (NO), and GFAP protein (Esposito *et al*., 2007). However, although CBD molecular mechanism still remains under debate, it is commonly accepted that it might exert its effects by the interaction at a not yet recognized extra-cannabinoid binding site. Along this line, results recently provided suggest that CBD moves into nucleus to exert over there its activity through the interaction with peroxisome proliferator-activated receptors (PPARs) (O'Sullivan *et al*., 2009).

PPARs are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily, which includes three isoforms: alpha, beta/delta, and gamma (Desvergne and Wahli, 1999). They are key mediators of energy homeostasis, of glucose and lipid metabolism, and they have been also associated to other biological processes including inflammation, cell proliferation, and differentiation (PP1-2) (Heneka and Landreth, 2007; Kersten *et al*., 2000). PPARγ in the central nervous system (CNS) is expressed at low levels under physiological conditions, whereas its expression is reported to be enhanced in some pathological situations, such as AD (Kitamura *et al*., 1999). These results have arisen the hypothesis that PPARγ may have a substantial role in modulating pathophysiological features of AD and they have supported the notion that regulating PPARγ activity could be therapeutically effective in the disease treatment (Jiang *et al*., 2008).

To date, although it is evident that CBD may beneficially interfere with different Aβ triggered neurodegenerative

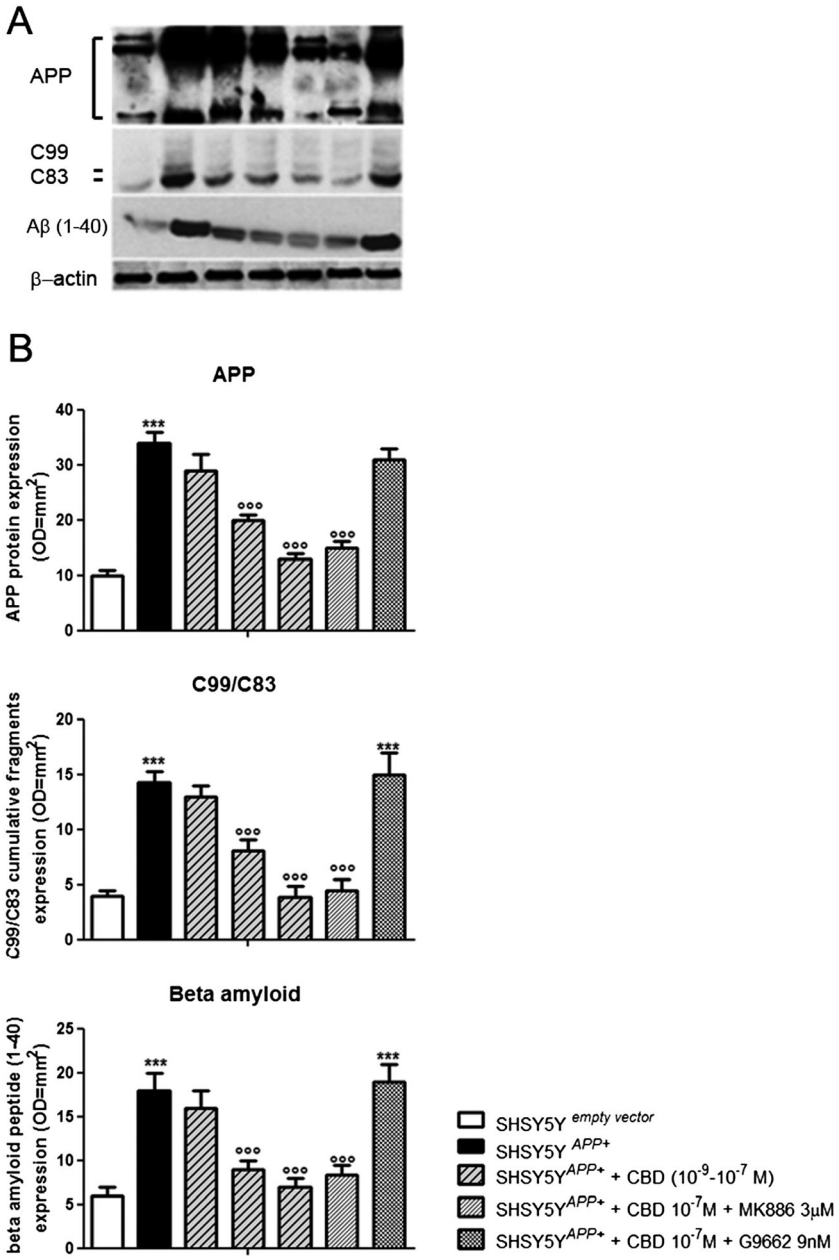

Figure 1. Effect of CBD on the expression of APP, its fragments C83 and C99, and Aβ (1–40). SHSY5Y^{APP+} were treated with CBD (10^{-9}–10^{-7} M) in the presence of PPARα (MK886, 3 μM) or PPARγ (GW9662, 9 nM) antagonist. Twenty-four hours later, protein expression was determined by Western blot analysis. Results are the mean ± S.E.M. of $n = 4$ separate experiments. ***$p < 0.001$ *vs. SHSY5Y$^{empty\ vector}$*; °°$p < 0.01$ *and* °°°$p < 0.001$ *vs. SHSY5Y^{APP+}*.

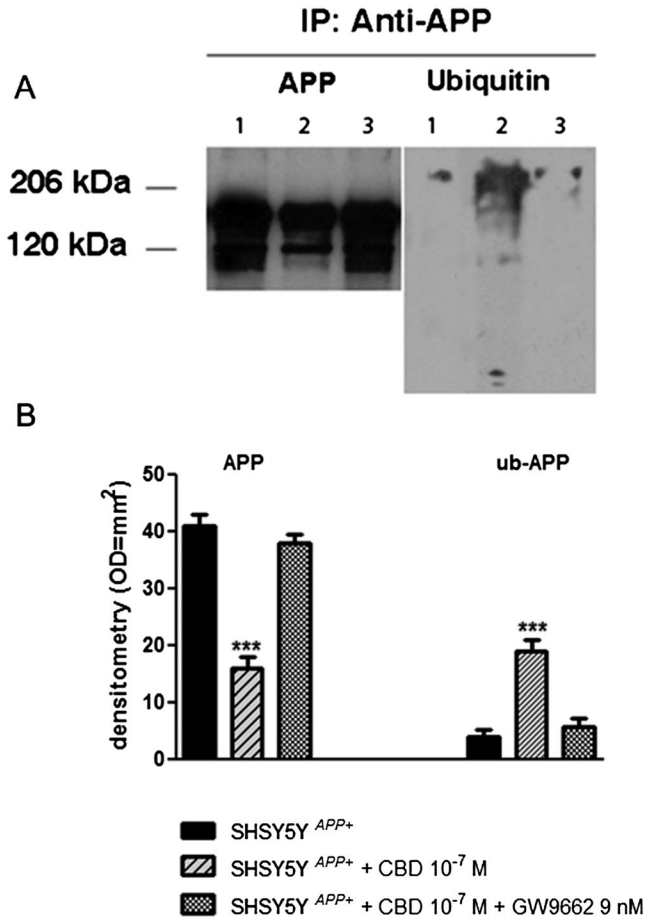

Figure 2. Effect of CBD on APP ubiquitination. SHSY5Y^{APP+} were treated with CBD (10^{-7} M) in the presence or absence of PPARγ antagonist (GW9662, 9 nM). APP was immunoprecipitated from the cell extracts. Bars represent the mean ± S.E.M. of $n = 3$ separate immunoblot experiments. ***$p < 0.001$ *vs. untreated SHSY5Y*$^{APP+}$.

pathways, the possible upstream effect of this phytocannabinoid on APP misregulation has not yet investigated. To this purpose, the present research was aimed at investigating the role of CBD as possible modulating compound of APP in APP-overexpressing SHSY5Y human neurons. Moreover, in this study, the possible involvement of PPARγ as putative key cellular site for CBD action was also investigated in the attempt to establish for this phytocannabinoid a possible molecular site of functioning.

MATERIALS AND METHODS

Materials. Medium, substances, and reagents for cell cultures and MTT assay were purchased from Sigma Aldrich, St. Louis, MO, USA except when specified in the text. Instruments, reagents, and materials for western blot analysis were from Biorad Laboratories, Milan, Italy.

Substances used for cell treatments, such as CBD, MK886, and GW9662, were purchased from Tocris Bioscience, Bristol, UK.

Cell cultures. Experiments were performed on SHSY5Y (obtained from the American Tissue Culture Collection), stably transfected with APP695 (SHSY5Y^{APP+}, obtained from Dr. Jie Lu, Harvard Medical School) or with empty vector, as internal control (SHSY5Y$^{empty\ vector}$). Both non transfected and APP transfected cells were plated onto 10 cm ø Petri dishes at a density of 1×10^6 cells/dish or onto eight chambers polystyrene culture slides at a density of 3×10^4 cells/chamber, depending upon the experimental procedure. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. When cells reached the confluence, they were treated with CBD (10^{-9}–10^{-6} M) in the presence or absence of MK886 (3 µM) and GW9662 (9nM), selective antagonists of PPARα and PPARγ, respectively.

The concentrations of the substances were chosen according to the results of a series of pilot experiments

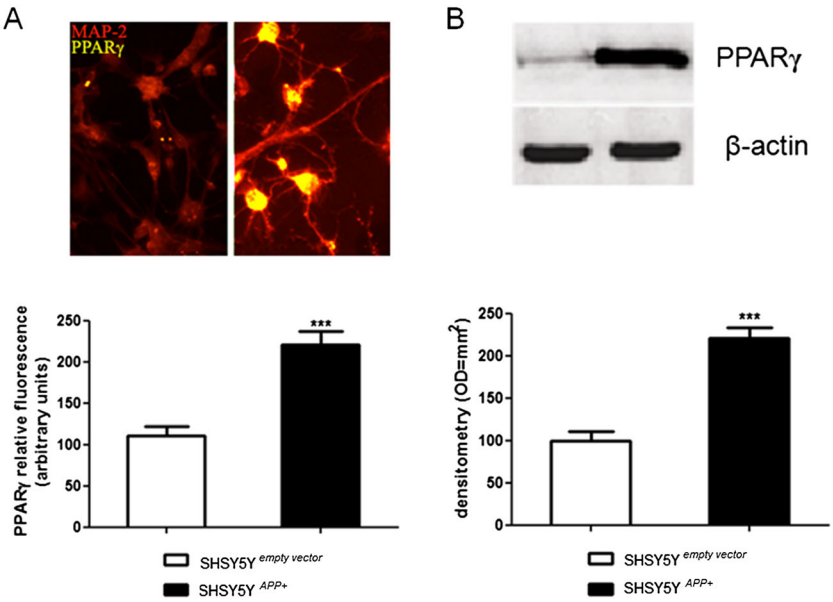

Figure 3. PPARγ expression in SHSY5Y and SHSY5Y^{APP+}. PPARγ expression was evaluated in SHSY5Y and SHSY5Y^{APP+} by immunofluorescence (a) and Western blot analysis (b). Bars represent the mean ± S.E.M. of $n = 4$ independent experiments. ***$p < 0.001$ *vs. SHSY5Y*$^{empty\ vector}$. This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

aimed at identifying the lowest effective concentration (data not shown). No significant variation versus control was observed when CBD, MK886, or GW9662 was given alone (data not shown).

Cell viability assay. Cell viability was measured both on both SHSY5Y$^{empty\ vector}$ and SHSY5Y^{APP+} cells by the MTT assay. This analysis is based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by mitochondrial dehydrogenase of viable cells to a purple formazon product (Mosman, 1983). Briefly, cells were seeded onto 96 wells plate at 1×10^4 cells/well density in 100 μL culture medium. Cells were incubated at 37 °C in a humidified atmosphere 5%CO_2/95% air and left to adhere. Afterwards, culture protocol schedule. MTT (5 mg/mL in DMEM) was added to cells 24 h after treatments. Three hours later, cells were lysed and dark blue crystals were solubilized with a solution containing 50% (v/v) *N*,*N*′-dimethylformamide, 20% (w/v) SDS with an adjusted pH of 4.5. The optical density (OD) of each well was measured at 620 nm with a microtiter plate reader (BioTek Instruments, Inc. Vermont, USA). Cell viability was calculated as percentage of cell viability (OD treated/OD control) × 100.

Immunoblotting. Twenty-four hours after treatments, cells were detached from Petri dishes and each pellet was suspended in ice-cold mammalian cell lysis buffer (CelLytic M, purchased from Sigma Aldrich, St. Louis, MO, USA) supplemented with the proper protease inhibitor cocktail (Roche, Mannheim, Germany). Protein concentration of each sample was determined performing Bradford assay. Equivalent amounts of protein extract (100 μg) were denatured in sample buffer at 95 °C for 3 min, separated by SDS-PAGE electrophoresis and transferred onto nitrocellulose membranes. The immunoblot was carried out blocking the membrane with a 5% albumin bovine serum (BSA) solution or 5% no fat dry milk solution, then incubated at 4 °C overnight with a solution of one of the following primary antibodies: anti-APP (Santa Cruz Biotechnology), anti-ubiquitin (Santa Cruz Biotechnology), anti-Abeta$_{1-40}$, anti-PPARγ, and anti β-actin (all purchased from Abcam, Cambridge, UK).

Both SHSY5Y$^{empty\ vector}$and SHSY5Y^{APP+} extracts were immunoprecipitated; proteins were analysed by immunoblotting with anti-APP (Zymed, San Francisco) or anti-ubiquitin antibody. After being extensively washed in TBS 1X supplemented with 0.1% Tween 20, each membrane was incubated for 2 h at room temperature with the proper secondary HRP-conjugated antibodies anti-mouse or anti-rabbit (both purchased from Abcam). Finally, it was developed using enhanced chemiluminescence substrate (ECL plus from Biorad). Bands were revealed through a Versadoc (Bio-Rad Laboratories) and corresponded digital images were analyzed with Quantity One Software (Bio-Rad Laboratories).

Immunofluorescence. Both SHSY5Y$^{empty\ vector}$ and SHSY5Y^{APP+} cells plated onto glass slide chambers coated with poly-D-lysine with a density of 3×10^4 cells/well were cultured and challenged as above described.

Twenty-four hours after treatments, cells were washed with PBS-Triton 0.1% (T-PBS) and fixed with 4% paraformaldehyde. Afterwards, cells were blocked in 10% albumin bovine serum 0.1% T-PBS solution for 90 min and subsequently incubated for 1 h with a 10% BSA 0.1% T-PBS solution of anti PPARγ antibody 1:100 (Abcam). Finally, cells were incubated for 1 h in the dark with fluorescein isothiocyanate (FITC) conjugated anti-rabbit antibody 1:100 (Abcam). Nuclei were stained with Hoechst 1:5000 (Sigma Aldrich) added in the secondary antibody solution.

Pictures were taken using a camera (Nikon DIGITAL SIGHT DS-U1) connected with a microscope (Nikon ECLIPSE 80i by Nikon Instruments Europe B.V., Kingston, UK). The analysis of RGB intensity was performed using NIH software.

Statistical analysis. Results are expressed as mean ± S.E.M. from four experimental determinations. Statistical analyses performed include one-way analysis of variance (ANOVA) and Bonferroni's test. Graphs were

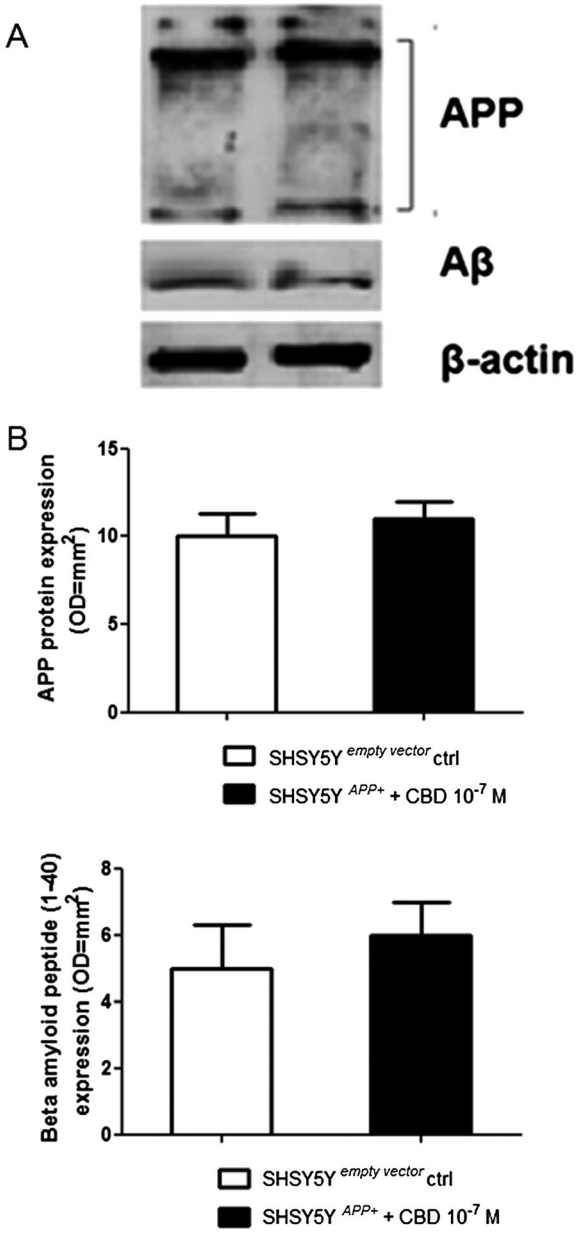

Figure 4. Effect of CBD on APP and Aβ expression in SHSY5Y^{wt}. SHSY5Y$^{empty\ vector}$ were treated with CBD (10^{-7} M). APP and Aβ expression was measured in cell lysates by Western blot. Bars represent the mean ± S.E.M. of $n = 3$ independent experiments. No significant differences were observed between two groups.

obtained using the software GraphPad 5. A value of $p < 0.05$ was considered significant.

RESULTS

CBD decreases APP expression and subsequently Aβ production.

As expected, SHSY5Y^{APP+} cells revealed significant increase in full length APP expression in comparison to the control ones. Such increase was dose dependently counteracted by CBD (10^{-9}–10^{-7} M). This result is paralleled by a CBD-mediated decrease in both the C83 and C99 fragments and consequently accompanied to a progressive reduction of Aβ peptide expression in cell lysates. All these findings demonstrated the selective involvement of PPARγ in mediating CBD activity because of the almost completely lack of any efficacy of this compound when it was co-administered with GW9662, the selective PPARγ antagonist (Fig. 1).

CBD promotes APP ubiquitination through PPARγ activation

SHSY5Y^{APP+} cell extracts were immunoprecipitated with the anti-APP antibody. Immunoprecipitated proteins were analysed by immunoblotting with anti-APP or anti-ubiquitin antibody. The results demonstrated that CBD significantly decreased APP expression and, at the same time, it caused a marked increase of APP ubiquitination in comparison to untreated SHSY5Y^{APP+} cells. Also in this case, such effect was dependent on the selective involvement of PPARγ activation (Fig. 2).

PPARγ is up-regulated in SHSY5Y^{APP+} cells

PPAR expression was evaluated in both SHSY5Y$^{empty\ vector}$ and SHSY5Y^{APP+} cells by immunofluorescence and Western blot analyses. Results clearly demonstrated that PPARγ expression is significantly higher in SHSY5Y^{APP+} cells versus the un-transfected ones (Fig. 3).

CBD does not alter APP and Aβ expression in SHSY5Y^{wt}

APP and Aβ expression was evaluated in by Western blot analysis 24 h later CBD treatments. Results indicated no significant differences in protein expression between two groups (Fig. 4).

CBD inhibits apoptosis in SHSY5Y^{APP+} through PPARγ involvement

Detection of apoptotic cell bodies was performed by Hoecst staining at 7th day of culture. Results indicated that cultured SHSY5Y^{APP+} showed more apoptotic cell bodies in comparison with SHSY5Y$^{empty\ vector}$. CBD (10^{-7} M) counteracted apoptotic events and such activity was dependent on the activation of PPARγ (Fig. 5).

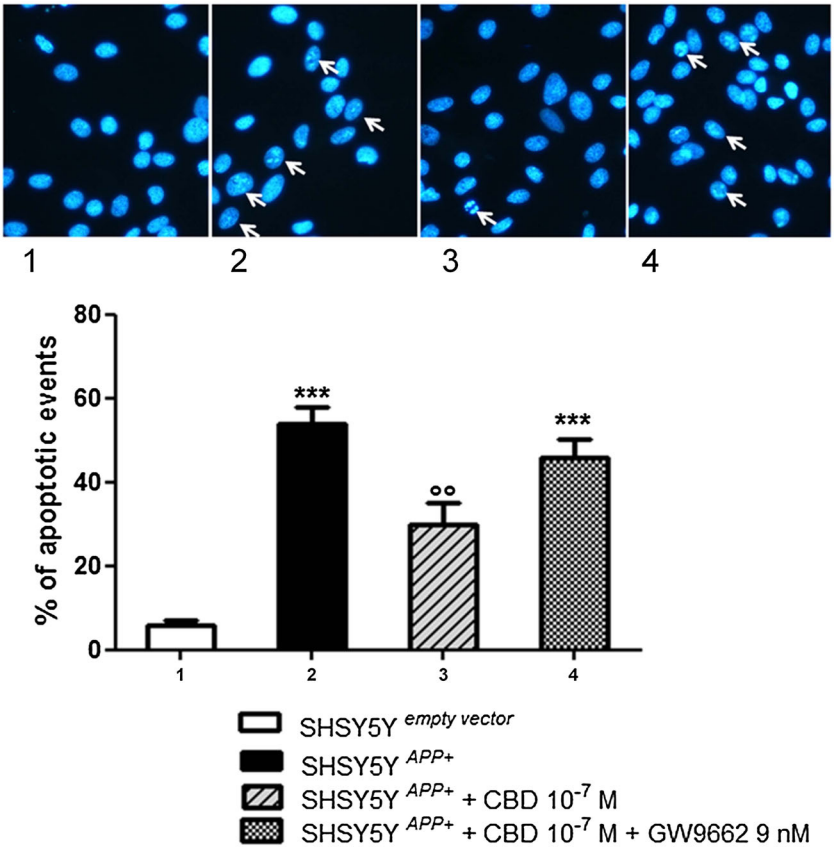

Figure 5. Photomicrographs showing the effect of CBD on neuronal apoptosis. SHSY5Y$^{empty\ vector}$ and SHSY5Y^{APP+} were treated with CBD (10^{-7} M) in the presence or absence of PPARγ antagonist (GW9662, 9 nM). Nuclei were immunostained with Hoecst at 7th culture day. Arrows indicate apoptotic cell bodies. Magnification 10×.

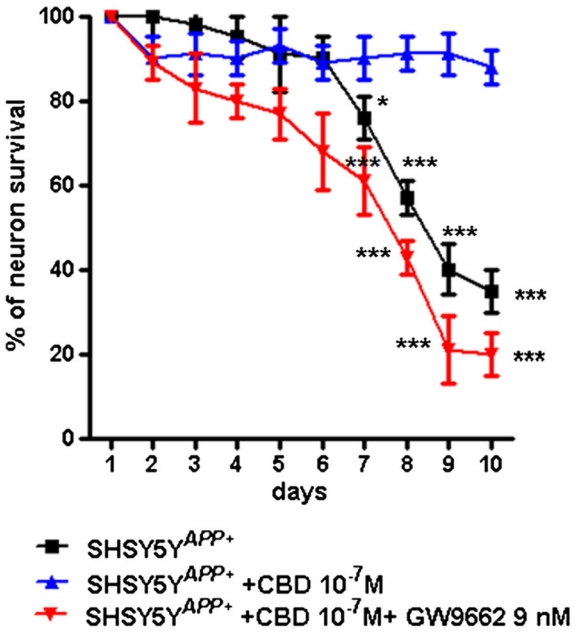

Figure 6. Effect of CBD on SHSY5Y^{APP+} survival. SHSY5Y^{APP+} were treated with CBD (10^{-7} M) in the presence or absence of PPARγ antagonist (GW9662, 9nM). Time course showing neuron survival measured by MTT formazan assay. Curves are referred to $n = 3$ separate experiments. * $p < 0.05$ *and* *** $p < 0.001$ *vs. CBD-treated SHSY5Y*$^{APP+}$. This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

Qualitative morphological analysis of apoptosis was confirmed by MTT formazan assay. As expected, starting from the 7th day of culture SHSY5Y^{APP+} survival was significantly reduced. CBD (10^{-7} M) was able to markedly counteract apoptosis through PPARγ interaction (Fig. 6). Such effect was long-lasting and it was particularly evident at the 10th day of culture.

DISCUSSION AND CONCLUSION

Results of the present study show that CBD is able to reduce APP over-expression and, consequently, Aβ overproduction and release from transfected human neuroblastoma SHSY5Y^{APP+} cells. Such activity is the result of a selective CBD-mediated PPARγ activation and, interestingly, is restricted to APP-overexpressing rather than SHSY5Y$^{empty\ vector}$ neuroblastoma cells. As consequence of APP down-regulation and reduced Aβ expression, CBD promotes an increased survival of SHSY5Y^{APP+} neurons reducing their apoptotic rate and increasing their survival in long-term period of cell culture.

Once transfected to overexpress APP, SHSY5Y neurons demonstrated significantly higher expression of PPARγ. Such observation may explain the CBD capability to affect only aberrant APP overproduction and its neuropathological consequences. This notion is reinforced by the evidence that PPARγ is not activated in normal condition but it seems to work 'on demand' in several pathological conditions, such as AD (Kitamura *et al*., 1999) and cancer (Ramer *et al*., 2013).

Several mechanisms have been proposed to elucidate PPARγ-dependent down-regulation of APP over-expression and consequent Aβ production. It has been reported that PPARγ activation results in a marked inhibition of APP expression (d'Abramo *et al*., 2005). Furthermore, additional explanations suggested that activation of endogenous PPARγ or over-expression of this nuclear receptor site leads to a dramatically enhanced clearance of Aβ from the media of both neuronal and non-neuronal cells. These studies rule out the possibility of a PPARγ-dependent transcriptional regulation of APP expression and they also exclude the activation of any known secretases or Notch processing involvement in lowering APP levels (Camacho *et al*., 2004).

Increased expression of C83 and C99 fragments is indicative of an increased BACE activity and the results of the present study indicate that, similarly to classical thiazolidinediones (TZDs) such as troglitazone, CBD resulted in a reduction in both C83 and C99 levels and in APP full length protein decrease, suggesting a lack of involvement of alpha and beta secretases. Since the present findings exclude alpha and beta secretase implication, consequently also the involvement of gamma secretase cannot be taken into account.

Ubiquitination of APP has been indicated as a post-trascriptional secretase-independent mechanism by which cells may down-regulate APP over-expression inducing a faster removal of over-released Aβ (d'Abramo *et al*., 2005).

Evidence has been also provided showing PPARγ up-regulation or agonists at this site may promote APP ubiquitination. Nicely fitting with this observation, the present results demonstrate that CBD may induce the ubiquitination of APP protein producing a substantial decrease in APP full length protein levels in SHSY5Y^{APP+} cells and, once again, prove that CBD ubiquitination activity is selectively mediated by PPARγ.

APP over-expression is a key feature in cellular changes in AD brain, even before florid dementia clinically arises in the affected subjects. Out of control APP production acts as a self-perpetuating stimulus for alzheimerian neurodegeneration since a huge accumulation of this protein conducts to a massive Aβ burden through the proteolytic activation operated by β- and γ- secretase. This, in turn, is responsible for free radicals over-production and oxidative damage in neurons, protracted reactive gliosis with consequent persistence of brain inflammation, Wnt pathway disruption, neuronal mitochondrial dysfunction, and Ca^{+2} homeostasis changes. All of these events converge in inducing neuronal death (Garrido *et al*., 2002; Inestrosa *et al*., 2002; Esposito *et al*., 2008; Inestrosa *et al*., 2005; Ohyagi *et al*., 2000; Caspersen *et al*., 2005).

Very interestingly, CBD may positively impact on all the above neuropathological pathways, and according to the present findings it appears able to exert a beneficial effect also in the amyloidogenic pathway, through a specific molecular mechanism involving PPARγ. In line with our findings, it has been postulated that CBD-PPARγ agonism is a promising tool to counteract the progression of other neurological disorders due to the unique combination of CBD anti-inflammatory and anti-oxidant properties (Fernández-Ruiz *et al*., 2013). The ability of CBD to reduce Aβ expression positively affects neuronal survival, as here observed in cultured SHSY5Y^{APP+}. In the view of CBD unique and promising capability to counteract AD neuropathology at different sites and with different mechanisms, this study encourages to further expand knowledge about this phytocannabinoid, suggesting it as an attractive compound useful to develop future and more effective treatments for AD.

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Conflicts of Interest

The authors declare no conflict of interest.

REFERENCES

Blennow K, de Leon MJ, Zetterberg H. 2006. Alzheimer's disease. *Lancet* **368**: 387–403.

Camacho IE, Serneels L, Spittaels K, *et al*. 2004. Peroxisome-proliferator-activated receptor gamma induces a clearance mechanism for the amyloid-beta peptide. *J Neurosci* **24**: 10908–10917.

Caspersen C, Wang N, Yao J, *et al*. 2005. Mitochondrial Abeta: a potential focal point for neuronal metabolic dysfunction in Alzheimer's disease. *FASEB J* **19**: 2040–2041.

Crews L, Masliah E. 2010. Molecular mechanisms of neurodegeneration in Alzheimer's disease. *Hum Mol Genet* **19**: R12–R20.

d'Abramo C, Massone S, Zingg JM, *et al*. 2005. Role of peroxisome proliferator-activated receptor gamma in amyloid precursor protein processing and amyloid beta-mediated cell death. *Biochem J* **391**: 693–698.

Desvergne B, Wahli W. 1999. Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr Rev* **20**: 649–688.

Esposito G, De Filippis D, Carnuccio R, *et al*. 2006. The marijuana component cannabidiol inhibits beta-amyloid-induced tau protein hyperphosphorylation through Wnt/beta-catenin pathway rescue in PC12 cells. *J Mol Med* **84**: 253–258.

Esposito G, Scuderi C, Lu J, *et al*. 2008. S100B induces tau protein hyperphosphorylation via Dickopff-1 up-regulation and disrupts the Wnt pathway in human neural stem cells. *J Cell Mol Med* **12**: 914–927.

Esposito G, Scuderi C, Savani C, *et al*. 2007. Cannabidiol in vivo blunts beta-amyloid induced neuroinflammation by suppressing IL-1beta and iNOS expression. *Br J Pharmacol* **151**: 1272–1279.

Fernández-Ruiz J, Sagredo O, Pazos MR, *et al*. 2013. Cannabidiol for neurodegenerative disorders: important new clinical applications for this phytocannabinoid? *Br J Clin Pharmacol* **75**: 323–33.

Garrido JL, Godoy JA, Alvarez A, *et al*. 2002. Protein kinase C inhibits amyloid beta peptide neurotoxicity by acting on members of the Wnt pathway. *FASEB J* **16**: 1982–1984.

Hampson AJ, Grimaldi M, Axelrod J, *et al*. 1998. Cannabidiol and (-)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. *Proc Natl Acad Sci U S A* **95**: 8268–8273.

Heneka MT, Landreth GE. 2007. PPARs in the brain. *Biochim Biophys Acta* **1771**: 1031–1045.

Huang HC, Jiang ZF. 2009. Accumulated Amyloid-beta Peptide and Hyperphosphorylated Tau Protein: Relationship and Links in Alzheimer's Disease. *J Alzheimers Dis* **16**: 15–27.

Inestrosa N, De Ferrari GV, Garrido JL, *et al*. 2002. Wnt signaling involvement in beta-amyloid-dependent neurodegeneration. *Neurochem Int* **41**: 341–344.

Inestrosa NC, Godoy JA, Quintanilla RA, *et al*. 2005. Peroxisome proliferator-activated receptor gamma is expressed in hippocampal neurons and its activation prevents beta-amyloid neurodegeneration: role of Wnt signaling. *Exp Cell Res* **304**: 91–104.

Iuvone T, Esposito G, Esposito R, *et al*. 2004. Neuroprotective effect of cannabidiol, a non-psychoactive component from Cannabis sativa, on beta-amyloid-induced toxicity in PC12 cells. *J Neurochem* **89**: 134–141.

Jiang Q, Heneka M, Landreth GE. 2008. The role of peroxisome proliferator-activated receptor-gamma (PPARgamma) in Alzheimer's disease: therapeutic implications. *CNS Drugs* **22**: 1–14.

Jicha GA, Carr SA. 2010. Conceptual evolution in Alzheimer's disease: implications for understanding the clinical phenotype of progressive neurodegenerative disease. *J Alzheimers Dis* **19**: 253–272.

Kersten S, Desvergne B, Wahli W. 2000. Roles of PPARs in health and disease. *Nature* **405**: 421–424.

Kitamura Y, Shimohama S, Koike H, *et al*. 1999. Increased expression of cyclooxygenases and peroxisome proliferator-activated receptor-gamma in Alzheimer's disease brains. *Biochem Biophys Res Commun* **254**: 582–586.

Mosman T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Meth* **65**: 55–63.

Ohyagi Y, Yamada T, Nishioka K, *et al*. 2000. Selective increase in cellular A beta 42 is related to apoptosis but not necrosis. *Neuroreport* **11**: 167–171.

O'Sullivan SE, Sun Y, Bennett AJ, *et al*. 2009. Time-dependent vascular actions of cannabidiol in the rat aorta. *Eur J Pharmacol* **612**: 61–68.

Querfurth HW, LaFerla FM. 2010. Alzheimer's disease. *N Engl J Med* **362**: 329–344.

Ramer R, Heinemann K, Merkord J, *et al*. 2013. COX-2 and PPAR-γ confer cannabidiol-induced apoptosis of human lung cancer cells. *Mol Cancer Ther* **12**: 69–82.

Selkoe DJ. 2001. Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev* **81**: 741–766.

Walsh DM, Selkoe DJ. 2007. A-beta oligomers - a decade of discovery. *J Neurochem* **101**: 1172–1184.