Biomedicine & Pharmacotherapy 138 (2021) 111405

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Original article

A nuclear factor-kappa B inhibiting peptide suppresses innate immune receptors and gliosis in a transgenic mouse model of Alzheimer's disease

Alison Lindsay [a], Deborah Hickman [b], Mythily Srinivasan [a,c,*]

[a] Department of Oral Pathology, Medicine and Radiology, Indiana University School of Dentistry, United States
[b] Laboratory of Animal Care and Research, Indiana University School of Medicine, Indiana University–Purdue University Indianapolis, United States
[c] Provaidya LLC, Indianapolis, IN, United States

ARTICLE INFO

Keywords:
Alzheimer's disease
NF-κB p65 blockade
GILZ
Peptide therapeutics

ABSTRACT

A disproportionate increase in activated nuclear factor-kappa B (NF-κB) has been shown to drive the Aβ deposition, neuroinflammation and neurodegeneration in Alzheimer's disease (AD). Hence, selective targeting of activated p65 represents an attractive therapeutic approach for AD. Glucocorticoid induced leucine zipper (GILZ) is a NF-κB interactant that binds and sequesters the activated p65 in the cytoplasm. The p65 binding domain of GILZ adopts a polyproline type II helical conformation, a motif that acts as an adaptable glove in the interface with the binding partner and constitutes an excellent template for drug design. Previously, peptide analogs of the p65 binding domain of GILZ, referred to as GA have been shown to suppress pathology in the lipopolysaccharide induced model of neuroinflammation. In this study, we investigated the CNS delivery of labeled GA administered intraperitoneally in adult mice for a period of upto 24 h. Further, we evaluated the suppressive potential of GA in 5xFAD mice, an aggressive model with five genetic mutations closely associated with human AD. Groups of 5xFAD mice administered GA or control peptide intraperitoneally on alternate days for six weeks were evaluated for Aβ deposition, microglia, inflammation and innate immune responses by immunohistochemistry and real time polymerase reaction. GA was observed in proximity with NeuN positive neurons suggesting that the compound crossed the blood brain barrier to reach the brain parenchyma. Further, GA treatment decreased Aβ load, reduced Iba1 + microglia and glial fibrillary acidic protein (GFAP)+ astrocytes, inhibited inflammatory cytokines and suppressed toll like receptor (TLR-2, TLR-4) expressions in 5xFAD mice.

1. Introduction

Neuroinflammation is increasingly recognized as the third core pathological feature in Alzheimer's disease (AD) along with the characteristic accumulations of amyloid beta (Aβ) plaques and neurofibrillary tangles. Considerable evidence suggests that the increased activation of the transcription factor nuclear factor-kappa B (NF-κB), drives the self-sustaining cycle of neuroinflammation leading to neurodegeneration in AD [1–3]. However, constitutive expression of NF-κB in the central nervous system (CNS) is also critical for the physiological process of neurogenesis and synaptic activity [4,5]. Such dichotomous effects in the cellular responses has been attributed to the composition of the activated NF-κB subunits [6,7].

Amongst the NF-κB family of proteins, Rel-B, c-rel and p65 subunits possess the transactivation domain and are endowed with the transactivating ability [8]. Together with the other two members of the family, p50 and p52, the five NF-κB proteins combine diversely to form homo or hetero dimers. In resting cells, the NF-κB dimers remain in an inactive complex with the inhibitory proteins. Upon activation, the dimers translocate to the nucleus and mediate transactivation of target genes [6,7]. Dissecting the effect of different NF-κB components, it was shown that the neuroprotective stimuli such as the interleukin-1β, S100B and agonists at metabotropic glutamate type-5 (mGlu5) receptor preferentially activate the c-rel containing dimers and mediate transactivation of anti-apoptotic and neurotrophic factors [6,7,9,10]. In contrast, noxious stimuli such as the excitotoxic glutamate or the Aβ increase activation of the p65/p50 dimers and upregulate transactivation of pro-apoptotic and neurotoxic factors [6,11,12]. A highly regulated balance between the c-rel containing and the p65/p50 dimers maintains the NF-κB homeostasis in the CNS.

* Correspondence to: Oral Pathology, Radiology and Medicine, Indiana University School of Dentistry and Provaidya LLC, Indiana Center for Biomedical Innovation, 1800 North Capitol Av, Indianapolis, IN 46202, United States.
E-mail addresses: mysriniv@iupui.edu, Provaidya12@gmail.com (M. Srinivasan).

https://doi.org/10.1016/j.biopha.2021.111405
Received 13 October 2020; Received in revised form 5 February 2021; Accepted 10 February 2021
Available online 20 March 2021

Induced activation of p65 in response to a variety of stimuli is typically transient but lasts sufficiently long enough to upregulate transactivation of target genes. The process is tightly controlled by intermolecular interactions in the cytoplasm that modulate the nuclear import of activated p65 or interfere with the DNA binding in the nucleus [4,6,13]. Increased activation of the p65/p50 dimers contributes to the sustained neuroinflammation, cell death and the resultant neurodegeneration [7,10,14]. This is supported by the observations of p65-mediated upregulation of Aβ producing beta secretase enzyme-1 (BACE-1) and the increased *Bax (pro-apoptotic)* to *Bcl-2 (anti-apoptotic)* ratio in Aβ stimulated neuronal cells. Upregulated p65 expression is also observed in the neurons and their processes in the hippocampus, entorhinal cortex and in the nucleus basalis of Meynert of AD brains [15–17]. Hence, targeting activated p65 is an attractive approach to inhibit neuroinflammation and progression to neurodegeneration. Indeed, targeted disruption of NF-κB p65 or indirect inhibition of p65 by diterpenoids or stilbene has been shown to improve cognition in AD models [18,19].

Glucocorticoid induced leucine zipper (GILZ) is a transcriptional regulatory protein that sequesters the activated p65 in the cytoplasm by binding its transactivation domain [20,21]. Structurally, the p65 binding domain of GILZ adopts an extended polyproline type II (PPII) helical conformation [20,22–24]. Often found in transient interfaces, PPII helices act as adaptable gloves in the binding interface and constitute excellent templates for drug design [25–28]. The sequence of the motif determines the specificity of the interaction and the flexibility promotes efficacious binding. Incorporating rational amino acid substitutions in the p65 binding motif, we designed low molecular weight GILZ analogs (GA) that exhibit increased propensity to form stable PPII helical conformation in the context of the p65-transactivation domain. Previously, we showed that the treatment with select GA suppressed pathology in the lipopolysaccharide induced model of neuroinflammation. The objective of this study is to investigate the delivery of GA to the brain and evaluate its suppressive potential in 5xFAD, an aggressive mouse model that closely mimics human AD and exhibits five specific mutations implicated in the disease pathology.

2. Materials and methods

2.1. Animals

Female B6SJL-Tg (APPSwFlLon,Psen1*M146L*L286V) 6799Vas/J transgenic (5xFAD) (N = 44) and wild type female B6SJL mice (N = 8) were provided by the Indiana University/Jackson Laboratory Alzheimer's Disease Precision Models (Model AD, Stark Neuroscience Research). Mice were housed at the laboratory for animal care and research, fed with water and food ad libitum, and maintained in a ventilated room and a 12 h diurnal light cycle. All animal experiments were performed in accordance with the rules and regulations approved by the institutional animal care and use committee of the Indiana University Purdue University at Indianapolis.

2.2. GA peptides

Fluorescein isothicyanate (FITC) labeled GA peptide amides, unlabeled end groups blocked GA and control peptides [sequence control (CP-1) and conformation control-peptide with no PPII potential (CP-2)] were commercially synthesized (GenScript, Piscataway, NJ) and the purity confirmed by mass spectrometry.

2.3. Entry of GA peptide into the CNS

Previously, we showed that select GA covalently synthesized with a cell penetrating peptide (CPP) exhibited suppressive potential in a neuroinflammation mouse model [27,29]. Here, we investigated the ability of GA to enter the brain in the absence of CPP. 5–8 weeks old 5xFAD mice were injected i.p. 0.2 mg/kg of the fluorescein labeled GA. 5xFAD and wild type B6 mice injected saline served as controls. Groups of mice (n = 3 in each group) were perfused with PBS and paraformaldehyde at different time points after GA or saline administration (immediately, 30 min, 1 h, 6 h, 24 h). One half of the brain was drop-fixed in 4% paraformaldehyde in PBS and cryoprotected in 30% sucrose and the second half was snap frozen in liquid nitrogen. Coronal sections of cryopreserved tissues (30 μm) permeabilized in 0.5% Triton X-100 for 5 min, and blocked in 10% normal serum, were then incubated overnight at 4 °C with NeuN antibody (ab128886:1:500, Abcam, Cambridge, MA) followed by Alexa Fluor 555 (Abcam) conjugated secondary antibody. Nuclei were stained with 4′, 6–diamidino-2–phenylindole, dilactate (DAPI, Sigma, St. Louis, MO). Immunostained sections were scanned with a NIKON multiphoton microscope attached with a DS Ri2 Camera (NIKON Instruments Ind, Melville, USA).

2.4. GA treatment

For treatment, groups of female 6–8 weeks old 5xFAD mice (N = 5/group) were used for uniformity. Previous dose response studies using increasing concentrations of GA-1, CP-1 and CP-2 from 25 μM to 250 μM determined their LD_{50} values 18.66 mg/kg, 10.2 mg/kg and 7.9 mg/kg respectively. Groups of mice (N = 5) were administered intraperitoneally GA or CP-1 or CP-2 in 100 μl of sterile saline on alternate days for six weeks [27]. Groups of (N = 5) 5xFAD and wild type mice treated with saline alone were included as controls. One mouse died in the control peptide treated group early (day 2 in the first week) in the study and was replaced. There was no other loss of animals throughout the study. GA treated and control groups of mice were sacrificed at the end of the treatment period by intra-cardial perfusion of 25 ml of 0.9% saline. Blood was collected prior to infusion. Lungs, liver and brain from each mouse was removed. The cerebral hemispheres of the brain were separated and one-half was immediately frozen and the other half fixed in 4% paraformaldehyde for paraffin embedding. All tissues were numbered sequentially and de-identified with reference to the treatment group prior to molecular investigations.

2.5. Immunohistochemistry (IHC)

Serial 8 μ thick coronal sections were immunostained for markers of microglial cells and astrocytes and for NF-κB p65 [30]. Briefly, after deparaffinization and hydration, sections were subjected to heat induced antigen retrieval (Prod. no. ENZ-ACC113) followed by sequential incubation in hydrogen peroxide and blocking buffer to reduce nonspecific binding using the Multiview IHC kit (ENZ--KIT181-0150, Enzo biosciences, Farmingdale, NY) following the manufacturer's protocol. The sections were then incubated overnight at 4 °C with the following primary mouse monoclonal antibodies: anti-Iba-1 (1:1000, AIF1 clone: Cat. # MABN92) or anti-glial fibrillary acidic protein (GFAP) (1:1000, clone GA5: Cat. # MAB3402) or anti-NF-κB p65 subunit (1:500, clone 12H11:Cat. #. MAB3026) (EMD Millipore Corporation, Temecula, CA, USA). After washing the sections were incubated with the horseradish peroxidase conjugated anti-mouse secondary antibody followed by detection with diaminobenzidine (DAB) chromogenic substrate as per the recommended protocol (ENZ-Kit 181–0150). The immunostained sections were counterstained with hematoxylin. For each marker, the specificity of staining was confirmed by incubating a separate set of sections with secondary antibodies alone.

For measuring Aβ, 8 μ sections were deparaffinized and antigen retrieval was performed by heating the sections in 0.1 M sodium citrate (pH 3.0, pH 7.2, and pH 10.0) [31]. Sections were then incubated in rabbit polyclonal primary antibody NBP2–25093 (1:1000, Novus Biologicals, Centennial CO) overnight at 4 °C. After washing the sections were stained with anti-rabbit Alexa flour 555 (1:500, Jackson Immuno Research Inc., PA) conjugated secondary antibody.

2.6. Quantitative analysis of staining intensity

Images of the IHC stained sections were captured using the NIKON Multiphoton microscope with attached DS Ri2 camera and analyzed using the ImageJ Software version 1.52P. All image analyses were performed by two individuals. The H-DAB stained sections were deconvoluted into separate colors using the IHC-tool box[32]. For each brain image, regions of interest encompassing the hippocampus were identified and staining above the background intensity threshold was measured as the relative optical density of Iba1+ and GFAP+ glial cells. The number of p65+cells was determined using the multi-point tool. Six consecutive coronal sections were assessed for each mouse and the average value was used for group comparisons.

2.7. Enzyme linked immunosorbent assay

Frozen cerebral cortical tissue harvested at the end of the treatment from each mouse was homogenized in RIPA lysis buffer (50 mmol/LTris–HCl, pH 6.8, 150 mmol/L NaCl, 5 mmol/L EDTA,0.5% sodium deoxycholate, 0.5% NP-40) supplemented with a cocktail containing protease and phosphatase inhibitors (Chemicon, Millipore) on ice at 1:25 ratio and then centrifuged at 16,000xg for 30 min. Supernatant was stored at −80 °C until further analysis. For each sample, 10 μl of tissue lysate was used for measuring the cytokines IL-6, IL-12, IFN-γ, and IL-17 using specific OptEIA kits (BD Biosciences, CA). IL-1β was measured using the single step ELISA kit (Catalog # ab100705, Abcam, Cambridge, MA). For measuring $A\beta_{1-42}$, a sandwich ELISA kit (Invitrogen, CA. Catalog number KMB3441) was used following the manufacturer's instruction for colorimetric detection [33–35]. For measuring NF-κB, the PathScan Phospho-NF-κB p65 (Ser^{536}) Sandwich ELISA kit was used following manufacturer's protocol (Cell Signaling Technology, Boston, MA) [29]. For all ELISA assays, absorbance at 450 nM was measured using a microplate reader (Model 680; Bio-Rad Laboratories, CA, USA). Scatchard plot analysis of known concentrations of the specific cytokine, purified recombinant $A\beta_{1-42}$ or p65 protein was used to calculate the concentration of the select analyte in the tissue homogenates.

2.8. Real time polymerase chain reaction

Total cellular RNA isolated from the frozen cerebral cortical tissue harvested from each mouse at the end of the treatment using Qiagen kit (Invitrogen, Carlsbad, CA) was reverse transcribed using iScript cDNA kit (Biorad, CA) [27,29]. Equal amount of cDNA was used for amplification of β-actin, IL-1β, IL-12, CD14, TLR-4 and CD4 by quantitative real time PCR using SYBR green/ROX qPCR master mix (SA Biosciences, Frederick, MD) on the ABI Prism 7000 sequence detection system (Applied Biosystem, Foster City, CA). The primers (gene accession number) used were: β-actin (NM_007393): F-5'TCATGAAGTGTGACGTTGACAT CCGTA3'; R-5'CCTAGAAGCATTT GCGCTGCACGATGG3'(286bp); IL1β (NM_008361) F-5'AGCTGATGGCCCTA AACAGA3'; R-5'GGTCGGAGATTCGTAGCTGG3' (89bp); CD14 (NM_009841) F-5'GAGCTAGACGA GGAAAGTTGT3'; R-5'ACCGT AAGCCGCTTTAAG GACAGA3' (206bp); IL-12 (NM_001303244) F-5'GGAAGCACGGCAGCAGAATA3'; R-5'AACTTGAGGGAGAAGTA GGAATGG3' (179bp), TLR4 (NM_021297) F-5'CAGTCG GTCAGCAAACGCCTTCTTC; R-5'TGTAACTGGTGGCAGCGCA3' (216bp) and TLR2 (NM_011905) F-5'CAAACTGGAG ACTCTGGAAG3'; R-5'GT CAACCAGGATTTGAGC CA3'(103bp). The gene specific threshold cycle (Ct) was corrected by subtracting the Ct for the housekeeping gene β-actin. The magnitude of change in each gene was determined by the $2^{-\Delta\Delta}$Ct method. Measurement of each sample was performed in duplicates and the experiments were repeated at least two times [29,36].

2.9. Statistical analysis

Differences in the evaluated parameters between treatment groups were analyzed by one-way analysis of variance and Tukey's post-hoc analysis. p values < 0.05 and < 0.01 were considered significant.

3. Results

3.1. CNS delivery of GA

Computational design of interface peptide mimics followed by functional evaluation represents an efficient method in the drug discovery. GA are rationally designed mimics of the p65 binding motif of GILZ that exhibit near native docking with the p65 transactivation domain [24,27]. An often-mentioned criticism of peptides as drugs is rapid clearance and poor penetration [37]. Hence, we first determined the potential of CNS delivery of GA. We tracked the fluorescein labeled GA (0.2 mg/kg) administered intraperitoneally in different regions of the brain of 5xFAD mice for upto 24 h. Image analysis showed that the GA was observed in close proximity with NeuN positive neurons in different regions of the hippocampus, and cortex (Fig. 1 and Supplementary Figure 1) suggesting that the compound crossed the blood brain barrier to reach the brain parenchyma.

In addition, the stability of GA was assessed in mouse serum, human serum and brain homogenates by HPLC (Supplementary file 1). GA exhibits a relatively pure single peak following 4 h incubation in human serum (Supplementary Figure 2A). Further, the ratio of percent response at each time point to time 0 suggests that nearly 70% of GA remains stable at 24 h in human serum and that 25% of GA was stable after 2 h of incubation in cyan brain homogenate (Supplementary Figure 2 B,C).

3.2. GA treatment reduces Aβ plaque burden in 5xFAD mouse brains

Inflammation in the brain of young 5xFAD mice has been correlated with the degree of Aβ burden, the hallmark of AD pathology [38,39]. To investigate whether the GA effects Aβ burden, we measured the amyloid deposits and the soluble Aβ in the brain tissues of 5xFAD mice treated with GA. Consistent with similar studies evaluating the effects of potential inhibitory agents in AD, we tested the longitudinal effects of GA treatment [40,41]. Image analysis showed that the mean area of amyloid plaques in the hippocampus was significantly lower in the GA treated (13.08 ± 1.52) mice as compared with the area of plaques in vehicle ($63.71 +/-6.5$) or CP-1 ($37.02 +/-5.44$) or CP-2 ($37.11 +/-2.26$) treated mice (Fig. 2A,B). The soluble fraction of Aβ was significantly reduced in the cerebral cortical tissue homogenates of GA ($8.62 +/-2$ pg/ml) treated mice as compared with that in the vehicle ($43.6 +/-5.7$ pg/ml) or CP-1 ($34.7 +/-9.4$ pg/ml) or CP-2 ($30.06 +/-5.2$ pg/ml) treated mice (Fig. 2C).

3.3. GA treatment inhibits the NF-κB activation in the hippocampus and suppressed inflammatory cytokines in 5xFAD mice

The 5xFAD mice have been shown to exhibit increased inflammation as early as 2 months of age [38,42]. Hence, the potential of any anti-inflammatory agent is likely to be beneficial when administered early [43,44]. Since activation of NF-κB is integral for inflammation, we investigated the effect of GA treatment on NF-κB p65 expression in young 5xFAD mice. Immunohistochemistry and image analysis showed that the hippocampus of the mice treated with GA ($19 +/-1.1$) exhibited fewer p65 positive cells as compared to that in the hippocampus of the vehicle ($69.3 +/-12.1$) or CP-1($67 +/-7.5$) or CP-2 ($69.5 +/-0.7$) treated mice (Fig. 3A,B). Elevated levels of phosphorylated p65 protein in cortical tissues has been reported in human AD brain and in mouse models [15,18,43,45]. To determine further the potential of GA in suppressing activated NF-κB, we measured phosphorylated NF-κB p65 protein in tissue homogenates by ELISA. The cerebral cortical tissues of 5xFAD mice treated with GA exhibited significantly lowered activated p65 protein expression as compared with that in the tissue homogenates of vehicle or control peptide treated mice

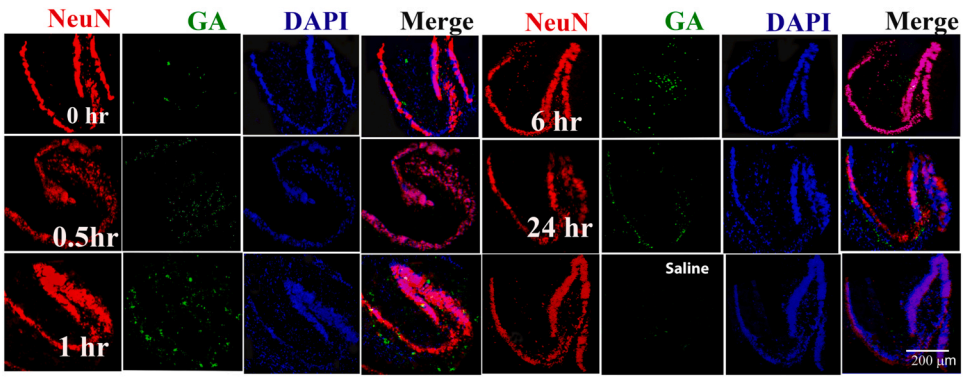

Fig. 1. Entry of GA into the hippocampus of 5xFAD Tg mice. Tg 5xFAD mice (5–8 weeks old) were injected i.p. 0.2 mg/kg of the fluorescein labeled GA (green) peptide. 5xFAD mice injected saline served as controls. Groups of mice (n = 3 in each group) were perfused with PBS and paraformaldehyde at specific time points after GA administration [immediate (0 h), 30 min, 1 h, 6 h, 24 h]. Coronal sections of the brain stained with NeuN followed by Alexa Flour 555 (red) and DAPI (blue) for nuclei. Images were captured using the NIKON Multiphoton microscope with attached DS Ri2 camera. Shown are represented images of the hippocampus of mice in the indicated group sacrificed at the indicated time point. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 3C).

We next examined whether the reduction in activated NF-κB p65 by GA is reflected in suppression of inflammatory mediators. The cerebral cortical homogenates of the mice treated with GA had significantly lower IL-6, IFN-γ and IL-1β (138.5 +/−15.5 pg/ml, 51.9 +/−9.8 pg/ml and 1218.72 +/−395.9 pg/ml respectively) cytokines as compared with that in the tissue homogenates of vehicle (251.4 +/−44.6, 95.9 +/−12.8 and 2802.3 +−562.01 pg/ml respectively) treated mice (Fig. 4A). However, no significant difference was observed in IL-12 and IL-17 levels in the tissue homogenates between the groups (Fig. 4B). Assessment by quantitative PCR showed that the message for IL-1β and IL-12 was significantly reduced in the cerebral cortical tissue of 5xFAD mice treated with GA (2.06 +/−1.26 and 2.28 +/−0.09 respectively) as compared with the tissue of vehicle (7.64 +/−1.4 and 5.9 +/−0.24 respectively) or CP-1 (6.15 +/−0.98) treated mice (Fig. 4C). As opposed to the decreased IL-1β protein in the cerebral cortical tissues in GA treated mice, the difference in the IL-1β mRNA was not significant between the GA and CP-2 peptide treated groups. Similar to the protein, no significant difference was observed in the IL-12 transcript between the GA and either of the CP treated groups.

3.4. GA suppresses gliosis in 5xFAD mice

Microglia are the innate immune cells of the central nervous system that protect the brain by eliminating cell debris and pathogens [46]. Activated microglia and astrocytes that exhibit increased NF-κB p65 have been consistently observed around the accumulating Aβ plaques and the damaged neurons in AD brain tissues [5,34,47]. To investigate whether the GA treatment modulated gliosis, we analyzed the expressions of activated microglia and astrocytes in the brain tissues of GA treated 5xFAD mice by immunohistochemistry. Fewer Iba1 positive microglia were observed in the hippocampus of GA treated mice as compared to that in the vehicle or CP-1 or CP-2 treated mice (Fig. 5A). Similarly, GFAP+ astrocytes were fewer in the hippocampus of GA treated mice as compared to that in the vehicle or CP-1 or CP-2 (Fig. 6 A) treated mice. Image analysis showed that the mean intensity of Iba1+ staining in microglia (Fig. 5B,C) and GFAP+ staining in astrocytes (Fig. 6B,C) was significantly lower in the CA1 region (5048.1 +/−1103.6 and 1706.7 +/−392.08 respectively) of hippocampus in mice treated with GA as compared with that in mice treated with vehicle (11074.5 +/−1286.9, 4047.3 +/−696.7 respectively) or CP-1 (15885.95 +/−1800.59, 6809.1 +/−22.9 respectively) or CP-2 (9013 +/−5008.9, 4967.95 +/−408 respectively). Similarly, the staining intensity of Iba1 + microglia and GFAP+ astrocytes was significantly lower in the CA3 region (7441.5 +/−248.4 and 1345.9 +/−143.2 respectively) of the hippocampus in GA treated mice as compared with that of the cells in the CA3 region of vehicle treated (18426.7 +/- 3891 and 4737 +/−254 respectively) or CP-1 (10232 +/−3239.4 and 6704.4 +/−220.9 respectively) or CP-2 (15177.1 +/−506.8 and 5985.6 +/−478 respectively) treated mice.

3.5. GA suppressed innate immune responses

The pattern recognition receptors TLR2 and TLR-4, in association with the co-receptor CD14, have been implicated in Aβ mediated microglial activation and immune responses [48,49]. While engagement of TLR-2 or TLR-4 stimulated NF-κB signaling in microglia [50–52], inhibition of CD14, TLR-4, and TLR-2 prevented Aβ-stimulated NF-κB dependent gene transactivation [47,53–55]. In order to determine whether the suppression of NF-κB by GA affected TLR gene transcription, we evaluated the expressions of CD14, TLR-2 and TLR-4 transcripts in the brain tissues of 5xFAD mice. We observed that the amount of CD14, TLR-2 and TLR-4 mRNA transcripts was significantly reduced in the cerebral cortical tissues of mice treated with GA as compared with the expression level in vehicle or control peptide treated 5xFAD mice (Fig. 7A, B, C).

4. Discussion

Neuroinflammation constitutes the molecular mechanism linking the pathognomonic Aβ deposition and NFT formation to neurodegeneration in AD pathogenesis [1,3]. Increased activation of NF-κB p65, the transcription factor integral to the process of inflammation has been consistently observed in the brain both in preclinical models and in human AD [2,5]. Here, we report that the administration of GA suppressed NF-κB p65 and ameliorated pathology in 5xFAD transgenic mice.

Previously, several direct or indirect inhibitors of NF-κB have been evaluated for the potential to reduce Aβ deposition and the associated pathology [56,57]. For instance, indirect suppression of NF-κB activation by non-steroidal anti-inflammatory agents and polyphenols such as resveratrol, and curcumin analogs have been shown to reduce Aβ load in AD models [58,59]. Direct inhibition of the NF-κB complex by the IKK targeting synthetic compounds (AS602686) have also been to suppress inflammation and improve memory in preclinical models of AD [57,60].

The advantages of peptides as therapeutic agents over small molecules include high target specificity, minimal potential for drug interactions, no accumulation in tissues and low toxicity. Hence, despite the perceived hurdles of rapid clearance and poor bioavailability increasing number of peptide drugs are currently in clinical development [37,61]. Interestingly, multiple potential peptide therapeutics have been shown to cross the blood brain barrier and reach the brain parenchyma. Many of these are rich in proline and include an octapeptide derived from presenilin, a peptide derived from the prolyl

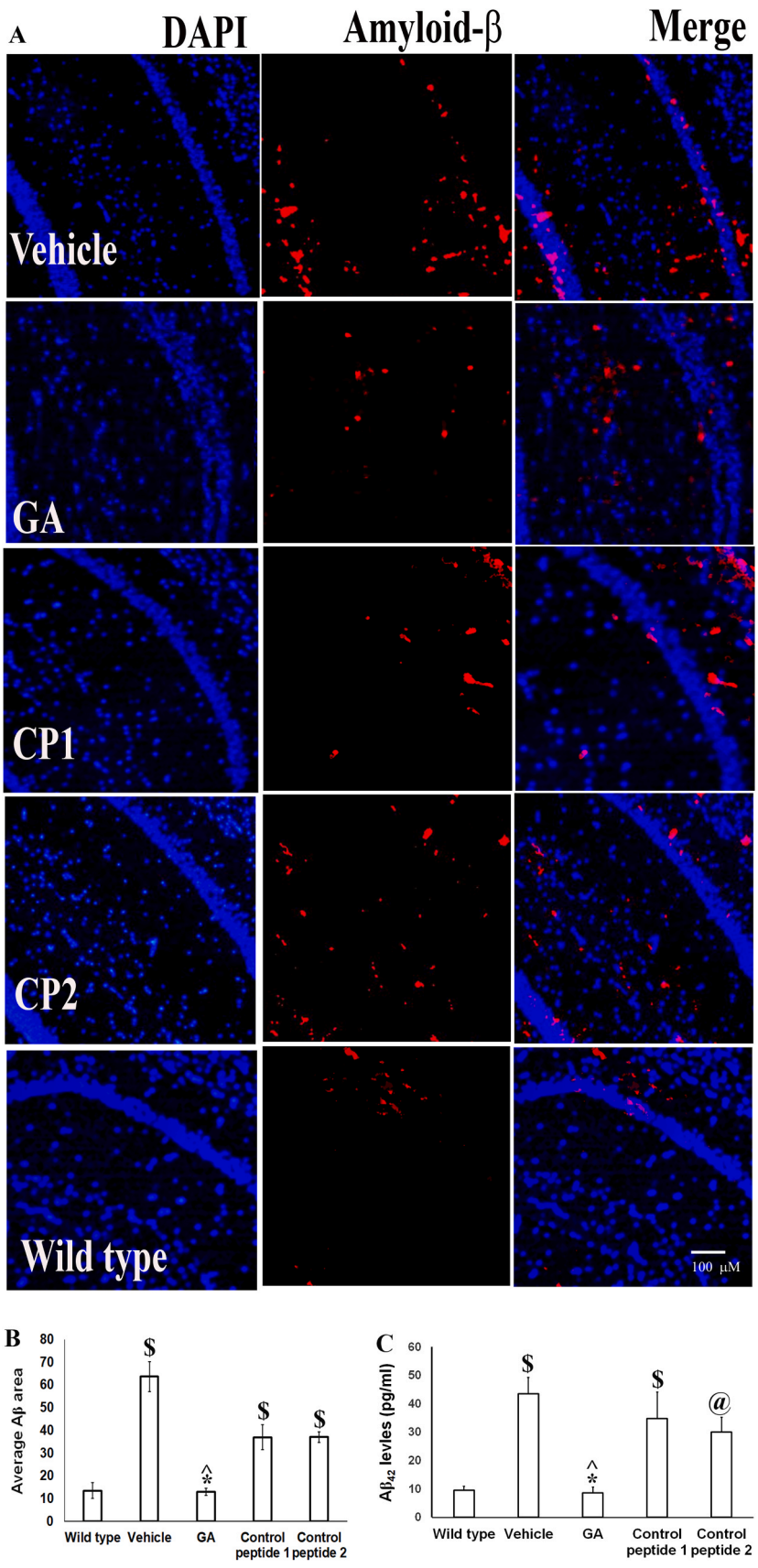

Fig. 2. GA treatment reduces Aβ plaque burden in 5xFAD mouse brains. Groups of 6–8 weeks old Tg 5xFAD mice were administered i.p. vehicle or GA or control peptides (CP-1 or CP2) on alternate days for six weeks. Vehicle only treated wild type (Wt) mice were included as control. (A) Brain tissue harvested at the end of the treatments were immunostained with NBP2-25093 to detect Aβ. Images captured were analyzed for Aβ-positive plaques in regions of interest outlining the hippocampus using the Image J software. (B) Six consecutive sections of each tissue were examined for Aβ staining. (C) Cerebral cortical tissue homogenates were analyzed for Aβ protein levels by ELISA. Data shown are mean+ /-SEM of two experiments of five mice per group. $=$p < 0.01$ and @=$p < 0.05$ versus vehicle treated Wt mice; *=$p < 0.01$ and ^=$p < 0.05$ versus vehicle treated or CP-1 or CP-2 Tg 5xFAD mice.

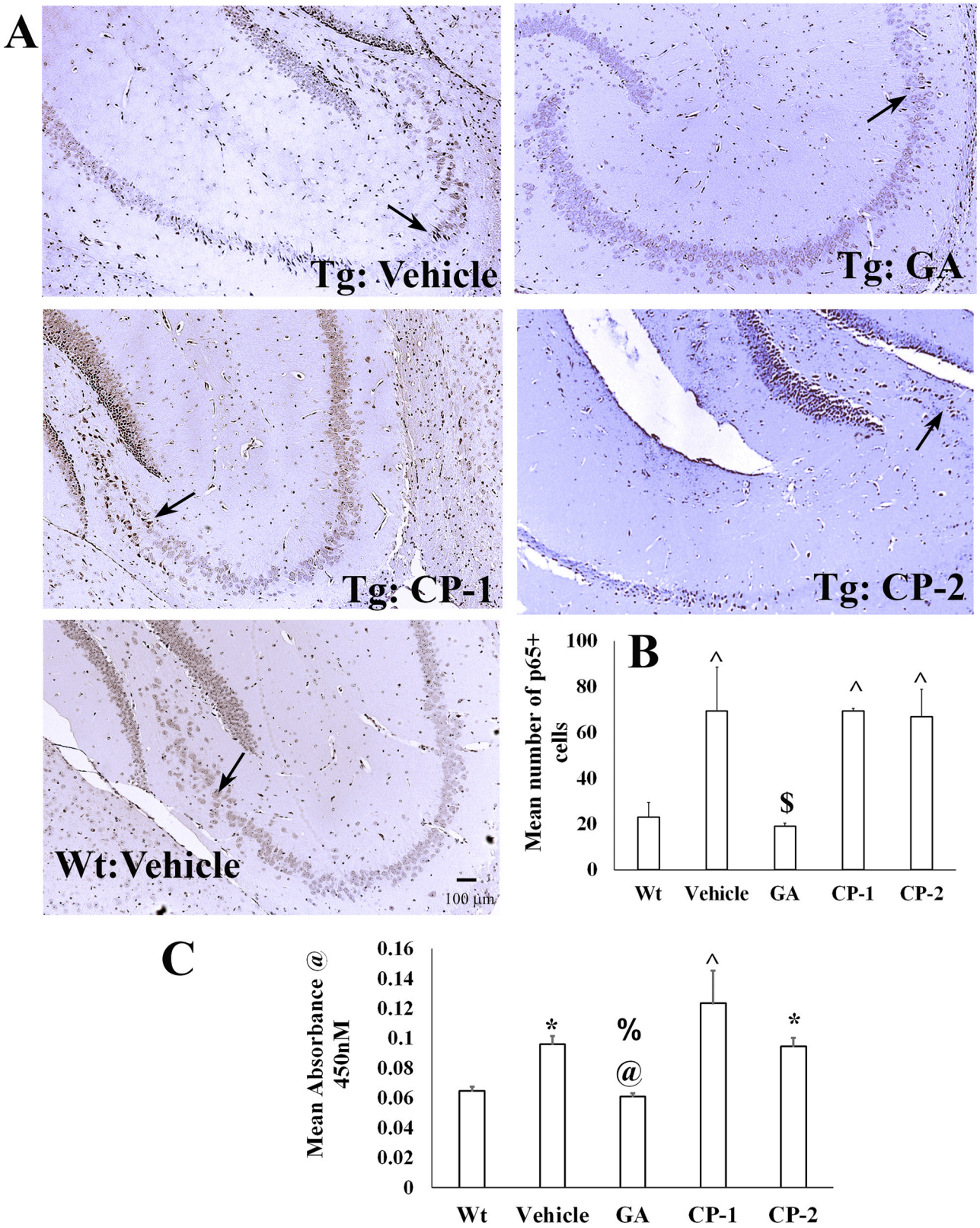

Fig. 3. GA treatment reduced NF-κB p65 in 5xFAD mouse brains. Groups of 6–8 weeks old Tg 5xFAD mice were administered i.p. vehicle or GA or control peptides (CP-1 or CP2) on alternate days for six weeks. Vehicle only treated wild type (Wt) mice were included as control. (A) Brain tissue harvested at the end of the treatments were immunostained for NF-κB p65. (B) The number of p65 positive cells (arrows) in regions of interest outlining the hippocampus were counted using the multipoint tool in the Image J software. Six consecutive sections of each tissue were assessed. (C) Cerebral cortical tissue homogenates were analyzed for phosphorylated NF-κB p65 protein by ELISA. Data shown are mean+ /-SEM of two experiments of five mice per group. ^=$p < 0.01$ and *=$p < 0.05$ versus vehicle treated Wt mice, $ =$p < 0.01$ versus vehicle or CP-1 or CP-1 treated Tg:5xFAD mice, @ =$p < 0.05$ versus vehicle or CP-2 treated and % =$p < 0.01$ compared with CP-1 treated Tg 5xFAD mice.

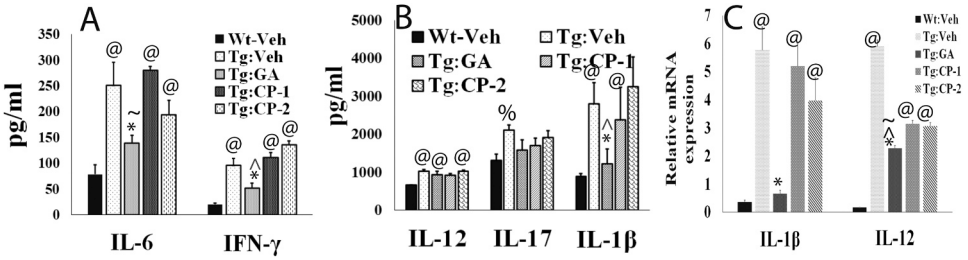

Fig. 4. GA treatment inhibits cytokine responses in 5xFAD mice. Groups of 6–8 weeks old Tg 5xFAD mice were administered i.p. vehicle or GA or control peptides (CP-1 or CP2) on alternate days for six weeks. Vehicle only treated wild type (Wt) mice were included as control. Cerebral cortical tissue harvested at the end of the treatments was assessed for the indicated cytokines (A, B) and transcript (C) by ELISA and RT-PCR respectively. Data shown are mean+ /-SEM of two experiments of five mice per group. @=$p < 0.01$ and %=$p < 0.05$ compared with the vehicle treated Wt group, *=$p < 0.01$ versus vehicle treated Tg 5xFAD mice, ^=$p < 0.05$ and ~ =$p < 0.05$ compared with the CP-1 or CP-2 treated Tg 5xFAD mice respectively.

endopeptidase, a peptide ligand from the lipoprotein receptor related protein and delta sleep inducing peptide [62–65]. The extended polyproline conformation of the proline or glutamine rich peptides has been suggested to facilitate passive penetration of biological barriers [28,66]. We observed that the GA mimicking the proline rich region of GILZ crossed the blood brain barrier to reach the CNS parenchyma.

Previously peptides derived from the proteins of the p65/p50:IKK complex have been shown to inhibit inflammatory responses. For example, peptides derived from the NF-κB essential modifier-binding domain (NBD) of IκB kinase α (IKKα) or the nuclear localization sequences of p50 (SN50) or the p65 phosphorylation epitope or the amino terminal residues of p65 (nt-p65) have been shown to be functional in preventing nuclear translocation and inhibiting transactivation of inflammatory and cytotoxic mediators [45,60,67]. In particular, the NBD peptide and the nt-p65 have been shown to cross the blood brain barrier and inhibit gliosis in models of AD and post-operative cognitive dysfunction respectively [60]. Our data show that the selective suppression of p65 by long-term (six weeks) treatment with GA reduced the Aβ burden, gliosis and suppressed inflammation in 5xFAD mice. Although the reduced number of p65 positive cells in the hippocampus of mice treated with GA supports its potential to suppress inflammation in the brain, individual CNS cell type may be variably inhibited.

In AD pathogenesis, signaling via TLR-2 and TLR-4 along with the co-receptor CD14 in microglia and astrocytes has been shown to be critical not only for Aβ phagocytosis but also for Aβ or tau induced inflammatory responses depending on the disease stage [49,52]. While reduced or lack of CD14/TLR-2 expression inhibited intracellular signaling and phagocytosis of fibrillar Aβ, increased TLR expression resulting from Aβ

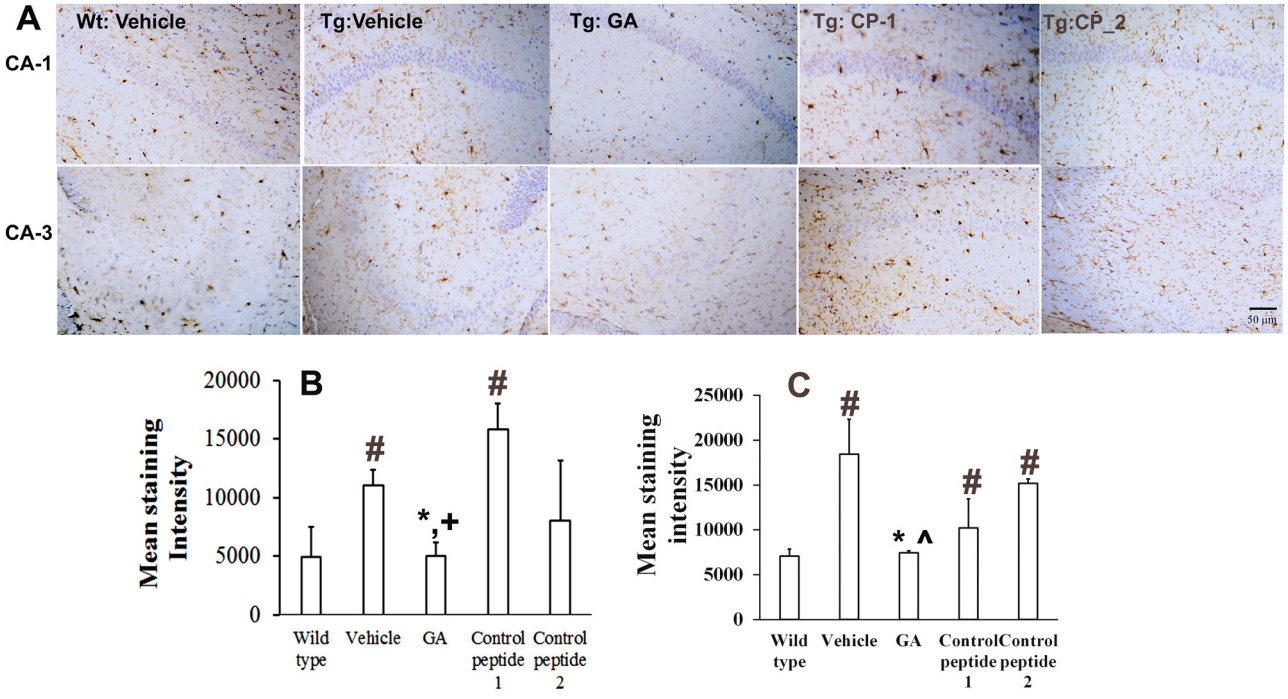

Fig. 5. GA treatment reduces microgliosis in 5xFAD mice. Groups of 6–8 weeks old Tg 5xFAD mice were administered i.p. vehicle or GA or control peptides (CP-1 or CP2) on alternate days for six weeks. Vehicle only treated wild type (Wt) mice were included as control. Brain tissue harvested at the end of the treatments were immunostained with anti-mouse Iba1 mAb. (A) Shown are representative images of Iba1 positive microglia in CA1 and CA3 regions of the hippocampus of mice treated as indicated. Six consecutive sections of each brain tissue were used for quantifying the Iba1 staining intensity in CA1 (B) and CA3 (C) regions using Image J software. Differences between the groups were analyzed by one-way ANOVA and Tukey's post-hoc test. Data shown are mean staining intensity + /-SEM of two experiments of five mice per group. *=$p < 0.01$ versus vehicle treated Tg 5xFAD mice and + = $p < 0.01$ versus CP-1 treated Tg 5xFAD mice, ^ =$p < 0.05$ versus CP2 treated Tg 5xFAD mice and # =$p < 0.01$ versus vehicle treated Wt mice.

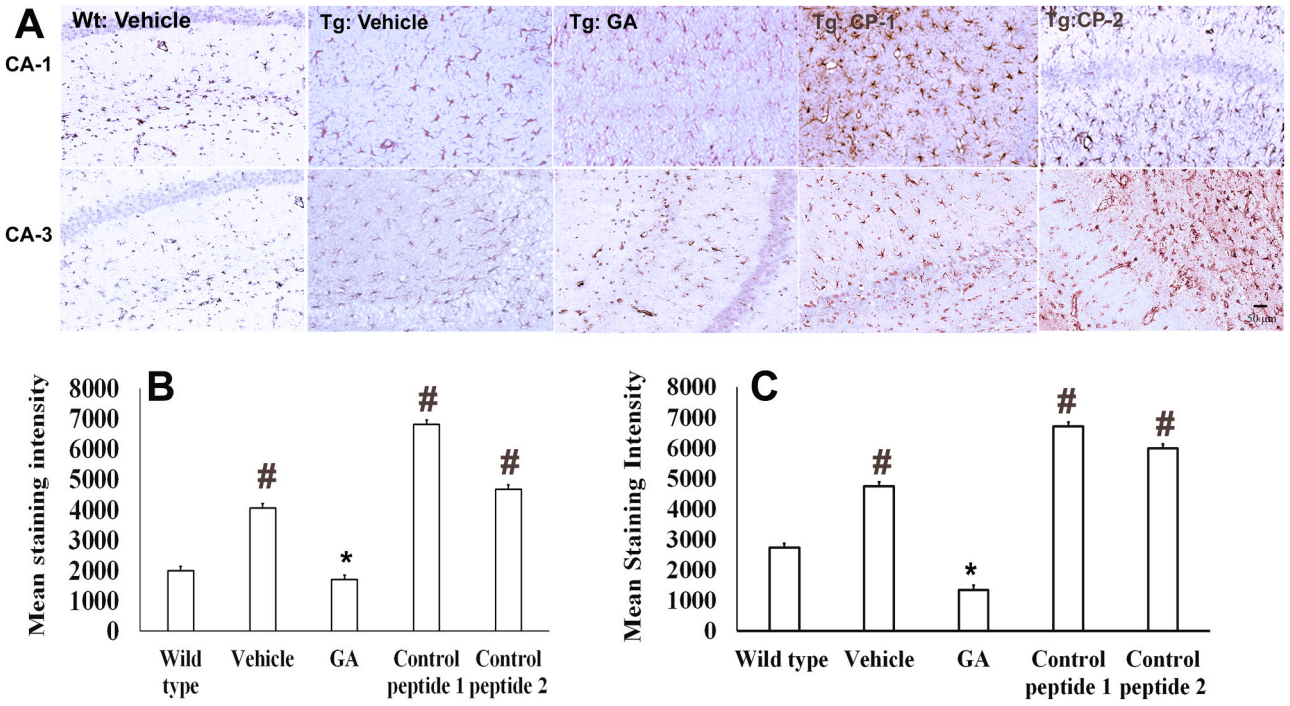

Fig. 6. GA treatment reduces astrogliosis in 5xFAD mice. Groups of 6–8 weeks old Tg 5xFAD mice were administered i.p. vehicle or GA or control peptides (CP-1 or CP2) on alternate days for six weeks. Vehicle only treated wild type (Wt) mice were included as control. Brain tissue harvested at the end of the treatments were immunostained with anti-mouse GFAP mAb. (A) Shown are representative images of GFAP positive astrocytes in CA1 and CA3 regions of the hippocampus of mice treated as indicated. Six consecutive sections of each brain tissue were used for quantifying the GFAP+ staining intensity in CA1 (B) and CA3 (C) regions using Image J software. Differences between the groups were analyzed by one-way ANOVA and Tukey's post-hoc test. Data shown are mean staining intensity + /-SEM of two experiments of five mice per group. *= $p < 0.01$ compared with vehicle treated and control peptide treated Tg 5xFAD transgenic mice; # is $p < 0.01$ compared with Wt or GA treated Tg 5xFAD mice.

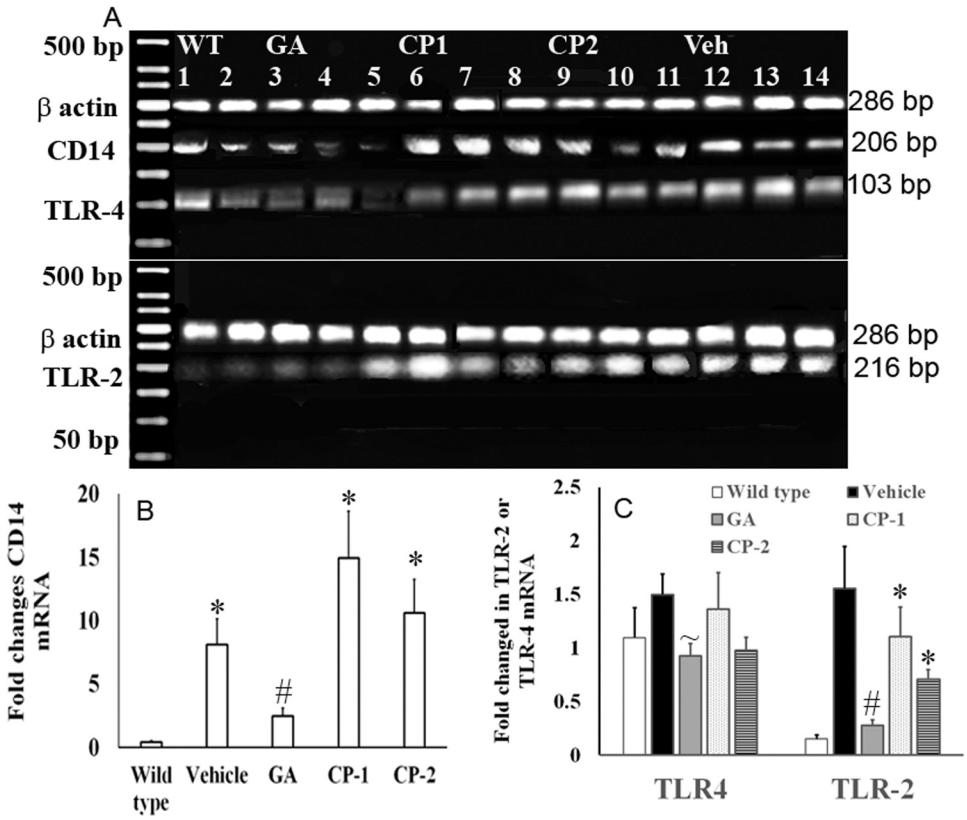

Fig. 7. GA treatment suppressed innate immune responses in 5xFAD mice. Groups of 6–8 weeks old Tg 5xFAD mice were administered i.p. vehicle or GA or control peptides (CP-1 or CP2) on alternate days for six weeks. Vehicle only treated wild type (WT) mice were included as control. Brain tissue harvested at the end of the treatments and from two WT mice were assessed for message for the innate immune associated molecules CD14, TLR2 and TLR4 by RT-PCR. (A) shows gel images of the indicated PCR product (lanes 1–2:WT, 3–5:GA, 6–8:CP-1, 9–11:CP-2 and 12–14:vehicle). (B) Fold changes in CD14 mRNA and (C) TLR-2 and TLR-4 mRNA. Differences between the groups were analyzed by one way ANOVA and Tukey's post-hoc test. Data shown are mean + /-SEM of two experiments of five mice per group. # is $p < 0.01$ versus vehicle or CP-1 or CP-2 treated Tg 5xFAD mice, * is $p < 0.01$ versus vehicle treated wt mice, ~ is < 0.05 versus vehicle treated Tg 5xFAD mice.

stimulation has been correlated with increased activation of NF-κB p65, gliosis and inflammatory cytokines[49,51,52,54]. Suppression of NF-κB and TLR-4 signaling, suppressed Aβ induced inflammatory responses [54,55]. We observed that the inhibition of NF-κB p65 by GA significantly reduced both TLR-2 and TLR-4 mRNA in the cerebral cortical tissues of 5xFAD mice and this reduction was reflected in the decreased gliosis. As a potential therapeutic agent, GA differs from other NF-κB targeting peptide agents in that it is derived from a protein extraneous to the NF-κB or the associated inhibitory complex (graphic abstract). GA are designed to selectively bind the transactivation domain of p65 exposed only in activated cells. Hence, treatment with GA is likely to exhibit minimal adverse effects. In this context, we observed that the liver and lungs of mice treated with GA did not exhibit any histologic evidence of adverse effects (Supplementary Figure 3).

In summary, we have demonstrated that the GA enters the CNS, blocks NF-κB p65, reduces Aβ load, inhibits the activation microglia in the hippocampus and attenuates inflammatory and innate immune responses. Although experiments with the 5xFAD mouse model have limitations in extrapolating to human AD, our results provide evidence that the administration of these peptides may be used either alone or in combination with other Aβ or tau targeting therapies in AD and other neurodegenerative disorders where inflammation within the CNS plays an important role in the disease pathogenesis.

Conflict of interest statement

None.

Acknowledgements

The authors sincerely appreciate the grant support from NIH (1R41 AG053117) to Mythily Srinivasan.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2021.111405.

References

[1] S. Amor, F. Puentes, D. Baker, P. van der Valk, Inflammation in neurodegenerative diseases, Immunology 129 (2010) 154–169.
[2] K.R. Bales, Y. Du, R.C. Dodel, G.M. Yan, E. Hamilton-Byrd, S.M. Paul, The NF-kappaB/Rel family of proteins mediates Abeta-induced neurotoxicity and glial activation, Brain Res. Mol. Brain Res. 57 (1998) 63–72.
[3] H.M. Gao, J.S. Hong, Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression, Trends Immunol. 29 (2008) 357–365.
[4] M. Grilli, M. Memo, Nuclear factor-kappaB/Rel proteins: a point of convergence of signalling pathways relevant in neuronal function and dysfunction, Biochem. Pharm. 57 (1999) 1–7.
[5] M. Srinivasan, D.K. Lahiri, Significance of NF-kappaB as a pivotal therapeutic target in the neurodegenerative pathologies of Alzheimer's disease and multiple sclerosis, Expert Opin. Ther. Targets 19 (2015) 471–487.
[6] M. Pizzi, F. Goffi, F. Boroni, M. Benarese, S.E. Perkins, H.C. Liou, P. Spano, Opposing roles for NF-kappa B/Rel factors p65 and c-Rel in the modulation of neuron survival elicited by glutamate and interleukin-1beta, J. Biol. Chem. 277 (2002) 20717–20723.
[7] I. Sarnico, A. Lanzillotta, F. Boroni, M. Benarese, M. Alghisi, M. Schwaninger, I. Inta, L. Battistin, P. Spano, M. Pizzi, NF-kappaB p50/RelA and c-Rel-containing dimers: opposite regulators of neuron vulnerability to ischaemia, J. Neurochem. 108 (2009) 475–485.
[8] I. Sarnico, A. Lanzillotta, M. Benarese, M. Alghisi, C. Baiguera, L. Battistin, P. Spano, M. Pizzi, NF-kappaB dimers in the regulation of neuronal survival, Int. Rev. Neurobiol. 85 (2009) 351–362.
[9] M. Pizzi, I. Sarnico, F. Boroni, M. Benarese, N. Steimberg, G. Mazzoleni, G.P. Dietz, M. Bahr, H.C. Liou, P.F. Spano, NF-kappaB factor c-Rel mediates neuroprotection elicited by mGlu5 receptor agonists against amyloid beta-peptide toxicity, Cell Death Differ. 12 (2005) 761–772.
[10] Z.H. Qin, L.Y. Tao, X. Chen, Dual roles of NF-kappaB in cell survival and implications of NF-kappaB inhibitors in neuroprotective therapy, Acta Pharm. Sin. 28 (2007) 1859–1872.
[11] K.T. Akama, C. Albanese, R.G. Pestell, L.J. Van Eldik, Amyloid beta-peptide stimulates nitric oxide production in astrocytes through an NFkappaB-dependent mechanism, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 5795–5800.
[12] L. Qin, X. Wu, M.L. Block, Y. Liu, G.R. Breese, J.S. Hong, D.J. Knapp, F.T. Crews, Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration, Glia 55 (2007) 453–462.
[13] S.J. Listwak, P. Rathore, M. Herkenham, Minimal NF-kappaB activity in neurons, Neuroscience 250 (2013) 282–299.
[14] A. Valerio, F. Boroni, M. Benarese, I. Sarnico, V. Ghisi, L.G. Bresciani, M. Ferrario, G. Borsani, P. Spano, M. Pizzi, NF-kappaB pathway: a target for preventing beta-amyloid (Abeta)-induced neuronal damage and Abeta42 production, Eur. J. Neurosci. 23 (2006) 1711–1720.
[15] G. Ashabi, S.Z. Alamdary, M. Ramin, F. Khodagholi, Reduction of hippocampal apoptosis by intracerebroventricular administration of extracellular signal-regulated protein kinase and/or p38 inhibitors in amyloid beta rat model of Alzheimer's disease: involvement of nuclear-related factor-2 and nuclear factor-kappaB, Basic Clin. Pharm. Toxicol. 112 (2013) 145–155.
[16] F. Boissiere, S. Hunot, B. Faucheux, C. Duyckaerts, J.J. Hauw, Y. Agid, E.C. Hirsch, Nuclear translocation of NF-kappaB in cholinergic neurons of patients with Alzheimer's disease, Neuroreport 8 (1997) 2849–2852.
[17] K. Terai, A. Matsuo, P.L. McGeer, Enhancement of immunoreactivity for NF-kappa B in the hippocampal formation and cerebral cortex of Alzheimer's disease, Brain Res. 735 (1996) 159–168.
[18] J. Chen, Y. Zhou, S. Mueller-Steiner, L.F. Chen, H. Kwon, S. Yi, L. Mucke, L. Gan, SIRT1 protects against microglia-dependent amyloid-beta toxicity through inhibiting NF-kappaB signaling, J. Biol. Chem. 280 (2005) 40364–40374.
[19] J. Jiao, B. Xue, L. Zhang, Y. Gong, K. Li, H. Wang, L. Jing, J. Xie, X. Wang, Triptolide inhibits amyloid-beta1-42-induced TNF-alpha and IL-1beta production in cultured rat microglia, J. Neuroimmunol. 205 (2008) 32–36.
[20] E. Ayroldi, C. Riccardi, Glucocorticoid-induced leucine zipper (GILZ): a new important mediator of glucocorticoid action, FASEB J. 23 (2009) 3649–3658.
[21] L. Cannarile, O. Zollo, F. D'Adamio, E. Ayroldi, C. Marchetti, A. Tabilio, S. Bruscoli, C. Riccardi, Cloning, chromosomal assignment and tissue distribution of human GILZ, a glucocorticoid hormone-induced gene, Cell Death Differ. 8 (2001) 201–203.
[22] F. D'Adamio, O. Zollo, R. Moraca, E. Ayroldi, S. Bruscoli, A. Bartoli, L. Cannarile, G. Migliorati, C. Riccardi, A new dexamethasone-induced gene of the leucine zipper family protects T lymphocytes from TCR/CD3-activated cell death, Immunity 7 (1997) 803–812.
[23] B. Di Marco, M. Massetti, S. Bruscoli, A. Macchiarulo, R. Di Virgilio, E. Velardi, V. Donato, G. Migliorati, C. Riccardi, Glucocorticoid-induced leucine zipper (GILZ)/NF-kappaB interaction: role of GILZ homo-dimerization and C-terminal domain, Nucleic Acids Res. 35 (2007) 517–528.
[24] M. Srinivasan, C. Blackburn, D.K. Lahiri, Functional characterization of a competitive peptide antagonist of p65 in human macrophage-like cells suggests therapeutic potential for chronic inflammation, Drug Des. Dev. Ther. 8 (2014) 2409–2421.
[25] T.P. Creamer, Left-handed polyproline II helix formation is (very) locally driven, Proteins 33 (1998) 218–226.
[26] B.K. Kay, M.P. Williamson, M. Sudol, The importance of being proline: the interaction of proline-rich motifs in signaling proteins with their cognate domains, FASEB J. 14 (2000) 231–241.
[27] M. Srinivasan, B. Bayon, N. Chopra, D.K. Lahiri, Novel nuclear factor-KappaB targeting peptide suppresses β-amyloid induced inflammatory and apoptotic responses in neuronal cells, PLoS One 11 (2016), e0160314.
[28] M. Srinivasan, A.K. Dunker, Proline rich motifs as drug targets in immune mediated disorders, Int. J. Pept. 2012 (2012) 1–14.
[29] M. Srinivasan, N. Lahiri, A. Thyagarajan, E. Witek, D. Hickman, D.K. Lahiri, Nuclear factor-kappa B: glucocorticoid-induced leucine zipper interface analogs suppress pathology in an Alzheimer's disease model, Alzheimers Dement. Transl. Res. Clin. Interv. 4 (2018) 488–498.
[30] B. Ray, J.A. Bailey, S. Sarkar, D.K. Lahiri, Molecular and immunocytochemical characterization of primary neuronal cultures from adult rat brain: differential expression of neuronal and glial protein markers, J. Neurosci. Methods 184 (2009) 294–302.
[31] B.J. Cummings, A.J. Mason, R.C. Kim, P.C. Sheu, A.J. Anderson, Optimization of techniques for the maximal detection and quantification of Alzheimer's-related neuropathology with digital imaging, Neurobiol. Aging 23 (2002) 161–170.
[32] K.L. Low, C. Ma, K.K. Soma, Tyramide signal amplification permits immunohistochemical analyses of androgen receptors in the rat prefrontal cortex, J. Histochem. Cytochem. 65 (2017) 295–308.
[33] G. Garcia-Rojo, F. Gamiz, E. Ampuero, D. Rojas-Espina, R. Sandoval, C. Rozas, B. Morales, U. Wyneken, F. Pancetti, In vivo sub-chronic treatment with dichlorvos in young rats promotes synaptic plasticity and learning by a mechanism that involves acylpeptide hydrolase instead of acetylcholinesterase inhibition. Correlation with endogenous β-amyloid levels, Front. Pharm. 8 (2017) 483.
[34] F. Zhang, R. Zhong, S. Li, Z. Fu, C. Cheng, H. Cai, W. Le, Acute hypoxia induced an imbalanced M1/M2 activation of microglia through NF-κB signaling in Alzheimer's disease mice and wild-type littermates, Front. Aging Neurosci. 9 (2017) 282.
[35] S.D. Schmidt, M.J. Mazzella, R.A. Nixon, P.M. Mathews, Abeta measurement by enzyme-linked immunosorbent assay, Methods Mol. Biol. 849 (2012) 507–527.
[36] E. Witek, D. Hickman, D.K. Lahiri, M. Srinivasan, Glucocorticoid induced leucine zipper in lipopolysaccharide induced neuroinflammation, Front. Aging Neurosci. 10 (2019) 432.
[37] C. Adessi, C. Soto, Converting a peptide into a drug: strategies to improve stability and bioavailability, Curr. Med. Chem. 9 (2002) 963–978.

[38] V. Landel, K. Baranger, I. Virard, B. Loriod, M. Khrestchatisky, S. Rivera, P. Benech, F. Feron, Temporal gene profiling of the 5XFAD transgenic mouse model highlights the importance of microglial activation in Alzheimer's disease, Mol. Neurodegener. 9 (2014) 33.
[39] T.P. O'Leary, Characterization of Age-related Changes in Motor Ability and Learning and Memory in the 5xFAD Mouse Model of Alzheimer's Disease, Department of Psychology and Neuroscience, Dalhousie University, Halifax, Nova Scotia, 2013, p. 162.
[40] P. Scheltens, N. Prins, A. Lammertsma, M. Yaqub, A. Gouw, A.M. Wink, H.M. Chu, B.N.M. van Berckel, J. Alam, An exploratory clinical study of p38alpha kinase inhibition in Alzheimer's disease, Ann. Clin. Transl. Neurol. 5 (2018) 464–473.
[41] A. Miller, V. Spada, D. Beerkircher, R.R. Kreitman, Long-term (up to 22 years), open-label, compassionate-use study of glatiramer acetate in relapsing-remitting multiple sclerosis, Mult. Scler. 14 (2008) 494–499.
[42] S.M. Barton, V.A. Janve, R. McClure, A. Anderson, J.A. Matsubara, J.C. Gore, W. Pham, Lipopolysaccharide induced opening of the blood brain barrier on aging 5XFAD mouse model, J. Alzheimers Dis. 67 (2019) 503–513.
[43] P.M. Ardestani, A.K. Evans, B. Yi, T. Nguyen, L. Coutellier, M. Shamloo, Modulation of neuroinflammation and pathology in the 5XFAD mouse model of Alzheimer's disease using a biased and selective beta-1 adrenergic receptor partial agonist, Neuropharmacology 116 (2017) 371–386.
[44] S.Y. Kook, K.M. Lee, Y. Kim, M.Y. Cha, S. Kang, S.H. Baik, H. Lee, R. Park, I. Mook-Jung, High-dose of vitamin C supplementation reduces amyloid plaque burden and ameliorates pathological changes in the brain of 5XFAD mice, Cell Death Dis. 5 (2014), e1083.
[45] S.B. Rangasamy, G.T. Corbett, A. Roy, K.K. Modi, D.A. Bennett, E.J. Mufson, S. Ghosh, K. Pahan, Intranasal delivery of NEMO-binding domain peptide prevents memory loss in a mouse model of Alzheimer's disease, J. Alzheimers Dis. 47 (2015) 385–402.
[46] C. Arcuri, C. Mecca, R. Bianchi, I. Giambanco, R. Donato, The pathophysiological role of microglia in dynamic surveillance, phagocytosis and structural remodeling of the developing CNS, Front. Mol. Neurosci. 10 (2017) 191.
[47] M. Jana, C.A. Palencia, K. Pahan, Fibrillar amyloid-beta peptides activate microglia via TLR2: implications for Alzheimer's disease, J. Immunol. 181 (2008) 7254–7262.
[48] M.V. Guillot-Sestier, T. Town, Let's make microglia great again in neurodegenerative disorders, J. Neural Transm. 125 (2018) 751–770.
[49] E.G. Reed-Geaghan, J.C. Savage, A.G. Hise, G.E. Landreth, CD14 and toll-like receptors 2 and 4 are required for fibrillar A{beta}-stimulated microglial activation, J. Neurosci. 29 (2009) 11982–11992.
[50] P. Zhou, R. Weng, Z. Chen, R. Wang, J. Zou, X. Liu, J. Liao, Y. Wang, Y. Xia, Q. Wang, TLR4 signaling in MPP(+)-induced activation of BV-2 cells, Neural Plast. 2016 (2016) 5076740–5076749.
[51] W. Lin, M. Ding, J. Xue, W. Leng, The role of TLR2/JNK/NF-kappaB pathway in amyloid beta peptide-induced inflammatory response in mouse NG108-15 neural cells, Int. Immunopharmacol. 17 (2013) 880–884.
[52] B.L. Fiebich, C.R.A. Batista, S.W. Saliba, N.M. Yousif, A.C.P. de Oliveira, Role of microglia TLRs in neurodegeneration, Front. Cell Neurosci. 12 (2018) 329.
[53] K. Fassbender, S. Walter, S. Kuhl, R. Landmann, K. Ishii, T. Bertsch, A.K. Stalder, F. Muehlhauser, Y. Liu, A.J. Ulmer, S. Rivest, A. Lentschat, E. Gulbins, M. Jucker, M. Staufenbiel, K. Brechtel, J. Walter, G. Multhaup, B. Penke, Y. Adachi, T. Hartmann, K. Beyreuther, The LPS receptor (CD14) links innate immunity with Alzheimer's disease, FASEB J. 18 (2004) 203–205.
[54] M.L. Udan, D. Ajit, N.R. Crouse, M.R. Nichols, Toll-like receptors 2 and 4 mediate Abeta(1-42) activation of the innate immune response in a human monocytic cell line, J. Neurochem. 104 (2008) 524–533.
[55] S. Walter, M. Letiembre, Y. Liu, H. Heine, B. Penke, W. Hao, B. Bode, N. Manietta, J. Walter, W. Schulz-Schuffer, K. Fassbender, Role of the toll-like receptor 4 in neuroinflammation in Alzheimer's disease, Cell. Physiol. Biochem. 20 (2007) 947–956.
[56] T.D. Gilmore, M. Herscovitch, Inhibitors of NF-kappaB signaling: 785 and counting, Oncogene 25 (2006) 6887–6899.
[57] S. Ouk, M.L. Liou, H.C. Liou, Direct Rel/NF-kappaB inhibitors: structural basis for mechanism of action, Future Med. Chem. 1 (2009) 1683–1707.
[58] N.S. El-Sayed, Y. Bayan, Possible role of resveratrol targeting estradiol and neprilysin pathways in lipopolysaccharide model of Alzheimer disease, Adv. Exp. Med. Biol. 822 (2015) 107–118.
[59] J. Hoppstadter, N. Hachenthal, J.V. Valbuena-Perez, S. Lampe, K. Astanina, M. M. Kunze, S. Bruscoli, C. Riccardi, T. Schmid, B. Diesel, A.K. Kiemer, Induction of glucocorticoid-induced leucine zipper (GILZ) contributes to anti-inflammatory effects of the natural product curcumin in macrophages, J. Biol. Chem. 291 (2016) 22949–22960.
[60] Y.X. Sun, D.K. Dai, R. Liu, T. Wang, C.L. Luo, H.J. Bao, R. Yang, X.Y. Feng, Z. H. Qin, X.P. Chen, L.Y. Tao, Therapeutic effect of SN50, an inhibitor of nuclear factor-kappaB, in treatment of TBI in mice, Neurol. Sci. 34 (2013) 345–355.
[61] C. Adessi, M.J. Frossard, C. Boissard, S. Fraga, S. Bieler, T. Ruckle, F. Vilbois, S. M. Robinson, M. Mutter, W.A. Banks, C. Soto, Pharmacological profiles of peptide drug candidates for the treatment of Alzheimer's disease, J. Biol. Chem. 278 (2003) 13905–13911.
[62] N.N. Dewji, S.J. Singer, E. Masliah, E. Rockenstein, M. Kim, M. Harber, T. Horwood, Peptides of presenilin-1 bind the amyloid precursor protein ectodomain and offer a novel and specific therapeutic approach to reduce ss-amyloid in Alzheimer's disease, PLoS One 10 (2015), e0122451.
[63] A.J. Kastin, C. Nissen, D.H. Coy, Permeability of blood-brain barrier to DSIP peptides, Pharm. Biochem. Behav. 15 (1981) 955–959.
[64] W.M. Nolte, D.M. Tagore, W.S. Lane, A. Saghatelian, Peptidomics of prolyl endopeptidase in the central nervous system, Biochemistry 48 (2009) 11971–11981.
[65] K. Sakamoto, T. Shinohara, Y. Adachi, T. Asami, T. Ohtaki, A novel LRP1-binding peptide L57 that crosses the blood brain barrier, Biochem. Biophys. Rep. 12 (2017) 135–139.
[66] L.J. Ball, R. Kuhne, J. Schneider-Mergener, H. Oschkinat, Recognition of proline-rich motifs by protein-protein-interaction domains, Angew. Chem. Int. Ed. Engl. 44 (2005) 2852–2869.
[67] Y. Takada, S. Singh, B.B. Aggarwal, Identification of a p65 peptide that selectively inhibits NF-kappa B activation induced by various inflammatory stimuli and its role in down-regulation of NF-kappaB-mediated gene expression and up-regulation of apoptosis, J. Biol. Chem. 279 (2004) 15096–15104.