Oridonin Attenuates Aβ1–42-Induced Neuroinflammation and Inhibits NF-κB Pathway

Sulei Wang¹, Hui Yang¹, Linjie Yu², Jiali Jin², Lai Qian², Hui Zhao², Yun Xu¹,²*, Xiaolei Zhu²*

¹Department of Neurology, Nanjing Drum Tower Hospital Clinical College of Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine, Nanjing, P. R. China, ²Department of Neurology, Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, P. R. China

Abstract

Neuroinflammation induced by beta-amyloid (Aβ) plays a critical role in the pathogenesis of Alzheimer’s disease (AD), and inhibiting Aβ-induced neuroinflammation serves as a potential strategy for the treatment of AD. Oridonin (Ori), a compound of Rabdosia rubescens, has been shown to exert anti-inflammatory effects. In this study, we demonstrated that Ori inhibited gial activation and decreased the release of inflammatory cytokines in the hippocampus of Aβ1–42-induced AD mice. In addition, Ori inhibited the NF-κB pathway and Aβ1–42-induced apoptosis. Furthermore, Ori could attenuate memory deficits in Aβ1–42-induced AD mice. In conclusion, our study demonstrated that Ori inhibited the neuroinflammation and attenuated memory deficits induced by Aβ1–42, suggesting that Ori might be a promising candidate for AD treatment.

Introduction

Alzheimer’s disease (AD), as the major cause of dementia, is an irreversible neurodegenerative disorder with progressive cognitive dysfunction, memory impairment and behavioral damage. According to the World Alzheimer Report 2012, there are 36 million people suffering from dementia worldwide in 2010 [1]. The pathological features of AD compose of beta-amyloid (Aβ) plaques (accumulation of extracellular Aβ) and neurofibrillary tangles (NFTs, deposition of intracellular hyperphosphorylated tau protein) [2,3]. Although the exact mechanism of AD still remains unclear, evidence from experimental models and human brain studies indicates that Aβ-mediated neuroinflammation is associated with the development of AD [4,5]. The clinical trials also demonstrate that the levels of pro-inflammatory cytokines are significantly increased in the cerebrospinal fluid (CSF) of AD patients [6], and anti-inflammatory drugs including nonsteriodals therapy for AD.

Oridonin (Ori), a diterpenoid originated from Chinese herb of Rabdosia rubescens, exhibits a diverse of biological activities, such as anti-inflammatory, anti-tumor and anti-oxidation [16–18]. Recent studies have demonstrated that Ori inhibits the release of proinflammatory mediators through modulating the functions of microglia [19]. In addition, Ori inhibits the NF-κB activity in TNF-α-induced HepG2 cells [20]. In the current study, we investigated whether Ori could inhibit Aβ1–42-induced inflammation and attenuate memory deficits in Aβ1–42 induced AD mice.

Materials and Methods

Aβ1–42 induced AD mice model and Ori treatment

The Aβ1–42 (Millipore, CA, USA) was dissolved in 1% NH3·H2O at a concentration of 1 μg/μl and incuated at 37°C for 5 days to allow for fibril formation. Ori (Chengdu Must Bio-Technology Co., Ltd, Sichuan, China, purity more than 98% measured by reverse phase high-performance liquid chromatography) was dissolved in DMSO at a concentration of 20 mg/ml and diluted to the desired concentration in saline. Aβ1–42 (4 μg) was injected into the bilateral hippocampus of male C57BL/6 (B6) mice by infusion cannulae as described previously [21]. Mice were
divided into three groups: the control mice with saline, Aβ1–42-induced AD mice with saline, and Aβ1–42-induced AD mice with Ori (10 mg/kg/day, i.p. for 15 days). Our preliminary data has shown that Ori did not attenuate the memory impairment in Aβ1–42-induced AD mice at higher dose (20–50 mg/kg/day) (data not shown). After Ori treatment, the mice were trained and tested in Morris water maze for 6 days, and then sacrificed for the following experiments. All experimental procedures were approved by Animal Care Committee of Nanjing University.

Real-time PCR
As described previously [22], total RNA of the hippocampus was isolated using TriZol (Invitrogen, USA) and reverse transcribed to cDNA using a reverse transcriptase kit (Takara, Dalian, China). Quantitative PCR was performed using ABI 7500 system (Applied Biosystems, USA) by SYBR green kit (Takara, Dalian, China). The primers (Invitrogen, USA) are as follows:

- GAPDH: F: GCCAAGGCTGTGGGCAAGGT, R: TGAGGCC-CAGGCCCAACAGGT;
- IL-6: F: GCCTGTTGAACACCCCGGCT, R: AGCCCTCC-GACTTGTGAAGTTTG;
- TNF-α: F: CACGTGGGTGTCGTAACCTCT, R: TGAGTTAAGGACAGTCGCA;
- iNOS: F: CACGTGGGTGTCGTAACCTCT, R: CATTG-GAAAGTGCCCAAGTT;
- COX-2: F: GATGACTGCCCAACTCCC, R: ACCCA-CAGGTTCCTCGCTTTGA;
- MCP-1: F: CCAGCCACGGACACCAGCCAA, R: TGGATGCTCCAGCGCCGAC;
- GAPDH: F: GCCAAGGCTGTGGGCAAGGT, R: TCTCCAGCGCCGACGTCA.

Western blotting
Western blotting analysis was performed as previously [23]. Briefly, cytoplasmic and nuclear proteins of the hippocampus were collected using cytoplasmic and nuclear protein extraction kit (Thermo, USA) according to the manufacturer’s instruction. The proteins were separated by SDS-PAGE and electrophoretically transferred onto polyvinylidene fluoride membranes. Membranes were blocked with 5% skimmed milk for 1 h and incubated overnight at 4°C with anti-ionized calcium-binding adaptor molecule 1 (Iba1) (1:400, Abcam, USA) and anti-β-actin (1:500, Cell Signaling, USA), anti-p-IkBα (1:1000, Cell Signaling, USA), anti-p-IκBα (1:1000, Cell Signaling, USA), anti-β-actin (1:1000, Cell Signaling, USA), anti-cytokine c (1:500, Abcam, USA), anti-VDAC1 (1:500, BioWorld, USA), anti-Histone (1:1000, Epitomics, USA) or anti-β-actin (1:500, BioWorld, USA). β-actin was used as a loading control. Subsequently, the membranes were incubated with the corresponding secondary antibodies and the reaction was visualized with chemiluminescence reagents provided with an ECL kit (BioWorld) and exposed to a film. The intensity of the blots was quantified with densitometry.

Immunostaining
Mice were anesthetized and transcardially perfused with 0.9% saline, then perfused with paraformaldehyde. The brains were removed and cut into consecutive frozen sections and then incubated overnight with anti-ionized calcium-binding adaptor molecule 1 (Iba1) (1:400, Abcam, USA) and anti-glia fibrillary acidic protein (GFAP) (1:100, BD, USA) at 4°C. The secondary antibody (1:200, Invitrogen) was applied to the sections for 1 h at room temperature. The images were performed by a fluorescent microscope (Olympus, Japan). All images were analyzed by Image J for counting of automatically recognized cells. The means were calculated from 5 randomly selected fields in the hippocampus and 5 consecutive sections were analyzed for each brain. All counting procedures were conducted in a randomized and blinded manner.

Morris water maze test
The Morris water maze test was prepared as previously described [21]. Briefly, mice were trained to find a platform in an open circular pool 2 cm under the water surface in the middle of one quadrant. Four training trials per day were conducted for five consecutive days. In each trial, the latency to escape onto the platform was recorded up to 1 min. If a mouse could find the platform, it was allowed to remain on the platform for 5 s, and then returned to the home cage. If the mouse failed to find the platform within 1 min, it was picked up and placed on the platform for 10 s, and the latency was recorded for 1 min. On the 6th day, a probe trial was given for memory retention by removing the platform from the pool, and each mouse was allowed to swim freely for 1 min, the numbers of crossings of the platform were recorded. All data were collected using a computerized video system.

Statistical analysis
The results were expressed as means ± SEM. The data were subjected to statistical analysis using SPSS version 13.0 (SPSS, Chicago, IL, USA). Group differences in the escape latency and swimming distance during the MWM test were analyzed by two-way analysis of variance (ANOVA) with repeated measures followed by Bonferroni multiple comparison test with day and treatment as the sources of variation. All other data were analyzed with a one-way ANOVA followed by Bonferroni’s post hoc. P < 0.05 were considered statistically significant.

Results
Ori Inhibits the Release of Pro-inflammatory Factors in the Hippocampus of Aβ1–42-induced AD Mice
To examine the effects of Ori on Aβ1–42-induced inflammation in vivo, the relative mRNA levels of IL-1β, IL-6, IL-10, COX-2, iNOS, TNF-α, and MCP-1, and the relative protein levels of iNOS and COX-2 in the hippocampus of mice were tested. The results showed that Aβ1–42 could significantly increase the expression of inflammatory factors (IL-1β: P < 0.05, IL-6: P < 0.05, iNOS: P < 0.01, TNF-α: P < 0.05, MCP-1: P < 0.01 versus control group, Figure 1A–F). However, Ori could significantly decrease the levels of these pro-inflammatory cytokines induced by Aβ1–42 (IL-1β: P < 0.05, IL-6: P < 0.05, iNOS: P < 0.01, COX-2: P < 0.01, TNF-α: P < 0.05, MCP-1: P < 0.05 versus Aβ group). Meanwhile, the mRNA level of IL-10 was decreased in AD group, and it was upregulated by Ori treatment (Figure 1G). In addition, Ori attenuated the protein levels of iNOS and COX-2 in the hippocampus of AD mice (Figure 2).

Ori Ameliorates Microglia and Astrocytes Activation in the Hippocampus of AD Mice
To verify whether Ori could inhibit Aβ1–42 stimulated activation of microglia and astrocytes, Iba-1 and GFAP staining was performed. Iba-1 and GFAP were specific markers of activated microglia and astrocytes respectively. As shown in Figure 3, the expression of Iba-1 was significantly increased in the hippocampus of Aβ1–42-induced AD mice (P < 0.01), while treatment with Ori

PLOS ONE | www.plosone.org 2 August 2014 | Volume 9 | Issue 8 | e104745
significantly suppressed Iba-1 expression (P<0.05). Ori also reduced the GFAP expression in the hippocampus of AD mice (P<0.05).

**Ori Inhibits Aβ1–42-induced Activation of NF-κB p65 Signaling Pathway in vivo**

NF-κBp65 signaling pathway is one of the most important pathways in modulating inflammation in AD. Therefore, we explored the role of Ori in Aβ1–42-induced activation of NF-κB p65 signaling pathway in vivo. As shown in Figure 4, Aβ1–42 treatment significantly increased the phosphorylation of IκBα (P<0.01). However, Ori treatment could decrease the phosphorylation of IκBα in the hippocampus of AD mice (P<0.01). In addition, Ori inhibited the Aβ1–42-induced degradation of IκBα (P<0.05) and translocation of NF-κB p65 (P<0.01).

**Ori Decreases Mitochondrial Injury in the Hippocampus of AD Mice**

Since emerging evidence suggested that Aβ-induced inflammation may contribute to neuronal apoptosis in AD, we investigated the mitochondrial functions in the hippocampus of Ori-treated AD mice. As shown in Figure 5, Ori treatment inhibited the release of cytochrome c from the mitochondria to the cytoplasm (P<0.01). In addition, Aβ1–42 increased the expression of Bax (P<0.01) and decreased the expression of Bcl-2 (P<0.01). However, Ori treatment could decrease the level of Bax (P<0.05) and increased the level of Bcl-2 (P<0.01), which indicated that Ori could attenuate the mitochondrial dysfunction induced by Aβ1–42.

---

**Figure 1. Ori reduces the inflammatory factors in Aβ1–42 induced AD mice.** The mRNA levels of IL-1β (A), IL-6 (B), iNOS (C), COX-2 (D), TNF-α (E), MCP-1 (F) and IL-10 (G) were measured in each group by Real-time PCR. GAPDH was used as an internal control. n = 6 mice per group. *P<0.05, **P<0.01 vs. control group; #P<0.05, ##P<0.01 vs. Aβ1–42 induced AD mice. doi:10.1371/journal.pone.0104745.g001

**Figure 2. Effects of Ori on the protein levels of iNOS and COX-2 in Aβ1–42 induced AD mice.** (A) Representative images of western blotting showing Ori inhibited the expression of iNOS and COX-2 in Aβ1–42 induced AD mice. (B) Quantitative analysis of iNOS and COX-2 expression. n = 3 mice per group. *P<0.05, **P<0.01 vs. control group; #P<0.05, ##P<0.01 vs. Aβ1–42 induced AD mice. doi:10.1371/journal.pone.0104745.g002
Figure 3. Ori suppresses glial activation in Aβ1–42 induced AD mice. (A) Immunostaining for Iba1 and GFAP in the hippocampus of mice. (B) Quantitative analysis of Iba1 and GFAP staining. n = 6 mice per group. Scale bar = 50 μm. *P<0.05, **P<0.01 vs. control group; #P<0.05, ##P<0.01 vs. Aβ1–42 induced AD mice.
doi:10.1371/journal.pone.0104745.g003

Figure 4. Ori inhibits Aβ1–42 induced activation of NF-κB in Aβ1–42 induced AD mice. (A) Representative image of western blotting of p65, p-IκBα, IκBα in the cytosolic and p65 in nuclear. (B) Quantitative analysis of Figure 4A. n = 6 mice per group. *P<0.05, **P<0.01 vs. control group; #P<0.05, ##P<0.01 vs. Aβ1–42 induced AD mice.
doi:10.1371/journal.pone.0104745.g004
Ori Improves Cognitive Impairment in Aβ1–42-induced AD Mice

To explore whether Ori could improve cognitive impairment in Aβ1–42-induced AD mice, Morris water maze test was performed. As shown in Figure 6A, the mean escape latency of Aβ1–42 induced AD mice was significantly increased compared with control group (P < 0.01), while Ori-treated AD mice showed significant improvements compared with AD mice after the training periods (P < 0.01). The searching distance by the Ori-treated AD mice was significantly decreased compared with that of AD mice (P < 0.01, Figure 6B). Moreover, on the 6th day, the platform was removed and the probe trail was conducted. The number of platform crossings by the Ori-treated AD mice was significantly higher than that of AD mice (P < 0.01, Figure 6C). In addition, there were no differences for swimming speed among these three groups (Figure 6D).

Discussion

AD is characterized by neurodegeneration and is the most common type of dementia. The Aβ deposition and formation of plaques in the brain have been thought as key events during the progression of AD [24]. In the present study, we demonstrated that Ori attenuated memory impairment in Aβ1–42-induced AD mice. Furthermore, Ori could suppress the inflammatory response, and the underlying mechanism might be associated with the inhibition of NF-κB pathway.

Growing data from basic and clinical studies indicate that inflammation induced by Aβ is involved in neuronal degeneration in AD [25,26]. It has been reported that the levels of proinflammatory cytokines are significantly elevated in brains of AD patients, which suggests that inflammation might contribute to the pathogenesis of AD [27]. The deposition of Aβ can activate glial cells which will release a wide spectrum inflammatory cytokines, such as IL-6 and TNF-α [28]. Aβ-induced overproduction of cytokines could lead to neuronal dysfunction and eventually death in AD. Therefore, inhibiting the activation of glial cells and production of proinflammatory cytokines may contribute to neuroprotection. Previous study indicated that Iba-1 and GFAP was increased in the brain of AD patients [29,30]. In the present study, the levels of Iba-1 and GFAP were significantly decreased in the Ori-treated mice compared with AD mice, which suggested that Ori suppressed the activation of microglia and astrocytes. Consistently, the results indicated that Ori could inhibit the mRNA levels of IL-1β, IL-6, COX-2, iNOS, TNF-α, and MCP-1 induced by Aβ, and it also up-regulated the expression of IL-10. Collectively, the current findings suggested that Ori modulated inflammatory response by inhibiting the activation of glial cells, which ameliorated the cognitive impairment of Aβ1–42-induced AD mice.

NF-κB has been regarded as the key regulator of inflammatory processes, and many studies have showed that suppression of NF-κB pathway ameliorates the neuroinflammation [31,32]. NF-κB is activated in brains of patients with AD and activated NF-κB is also detected in Aβ surrounding areas [33,34]. In the resting cells, NF-κB family composed of five members, p65 (RelA), RelB, c-Rel, p50/p105 and p52 which bound to the inhibitory proteins IκB, thereby maintaining NF-κB in an inactive form in the cytoplasm. Upon stimuli, IκB is phosphorylated by IKK (IκB kinase), which then is ubiquitinated and subsequently degraded, leading to translocation of NF-κB to the nucleus and binding to specific target genes, and increased the expression of proinflammatory factors [35]. Hydrogen sulfide inhibits neuroinflammation via suppressing NF-κB pathway and attenuates neuronal death in the hippocampus of Aβ-induced AD rats [36]. Moreover, experimental studies show that suppressing NF-κB could decrease production of Aβ [37,38]. This study demonstrated that Ori significantly inhibited NF-κB p65 nuclear translocation by attenuating Aβ1–40-induced IκB phosphorylation and degradation in vitro.

In summary, the present study shows that Ori, a typical compound of Rabdosia rubescens, attenuates cognitive impairment and inhibits inflammatory response in Aβ1–42-induced AD mice. In addition, the anti-inflammatory effects of Ori might be
due to inhibiting the NF-κB pathway. These findings suggest that Ori might be a potential agent for AD treatment.

Author Contributions
Conceived and designed the experiments: YX XLZ SLW. Performed the experiments: SLW HY LJY XLZ. Analyzed the data: SLW LQ HZ. Contributed reagents/materials/analysis tools: SLW HY LJY. Contributed to the writing of the manuscript: SLW HY LJY XLZ.

References
1. Batsch N, Mittelman M (2012) World Alzheimer Report 2012: Overcoming the stigma of dementia. London: Alzheimer’s Disease International.
2. Kurt MA, Davies DG, Kidd M (1997) Paired helical filament morphology varies with intracellular location in Alzheimer’s disease brain. Neurosci Lett 239: 41–44.
3. Price DL, Sisodia SS, Gandy SE (1995) Amyloid beta amyloidosis in Alzheimer’s disease. Curr Opin Neurol 8: 268–274.
4. Zhang YY, Fan YC, Wang M, Wang D, Li XR (2013) Arotastatin attenuates the production of IL-1beta, IL-6, and TNF-alpha in the hippocampus of an amyloid beta1–42-induced rat model of Alzheimer’s disease. Clin Interv Aging 8: 103–109.
5. McGee PL, McGee EG (2013) The amyloid cascade-inflammatory hypothesis of Alzheimer disease: implications for therapy. Acta Neuropathol 126: 479–497.
6. Llane DA, Li J, Waring JP, Ellis T, Devanarayan V, et al. (2012) Cerebrospinal fluid cytokine dynamics differ between Alzheimer disease patients and elderly controls. Alzheimer Dis Assoc Disord 26: 322–328.
7. Jaturapatporn D, Isaac MG, McCleery J, Tabet N (2012) Aspirin, steroidal and non-steroidal anti-inflammatory drugs for the treatment of Alzheimer’s disease. Cochrane Database Syst Rev 2: CD006378.
8. Couturier J, Pacalain M, Morel M, Terro F, Milin S, et al. (2011) Prevention of the beta-amyloid peptide-induced inflammatory process by inhibition of double-stranded RNA-dependent protein kinase in primary murine co-cultures. J Neuroinflammation 8: 72.
9. Lyman M, Lloyd DG, Ji X, Yu Z, Yangchibi MP, Ma D (2014) Neuroinflammation: The role and consequences. Neurosci Res 79C: 1–12.
10. Wu J, Wang A, Min Z, Xiong Y, Yan Q, et al. (2011) Lipoxin A4 inhibits the production of proinflammatory cytokines induced by beta-amyloid in vitro and in vivo. J Biophi Res Phys Commun 408: 382-387.
11. Heneka MT, O’Banion MK, Terwel D, Kummer MP (2010) Neuroinflammatory processes in Alzheimer’s disease. J Neurol Traum 117: 919–947.
12. Lawrence T, Fong C (2010) The resolution of inflammation: anti-inflammatory roles for NF-kB. Int J Biochem Cell Biol 42: 519–523.
13. Boissiere F, Hunot S, Faucheux B, Duyckaerts C, Hauw JJ, et al. (1997) Nuclear translocation of NF-kappaB in cholinergic neurons of patients with Alzheimer’s disease. Neuroreport 8: 2494–2495.
14. Kaltschmidt B, Uhler M, Vol B, Bauerle PA, Kaltschmidt C (1997) Transcription factor NF-kB is activated in primary neurons by amyloid beta peptides and in neurons surrounding early plaques from patients with Alzheimer disease. Proc Natl Acad Sci U S A 94: 2642–2647.
15. Kim HG, Moon M, Choi JG, Park G, Kim AJ, et al. (2014) Donepezil inhibits the amyloid-beta oligomer-induced microglial activation in vitro and in vivo. Neurotoxicology 40: 25–32.
16. Xu CM, Lin JG (2013) Anti-inflammatory effects of 27 selected terpenoid compounds tested through modulating Th1/Th2 cytokine secretion profiles using murine primary splenocytes. Food Chem 141: 1104–1113.
17. Bu HQ, Liu DL, Wei WT, Chen L, Huang H, et al. (2014) Oridonin induces apoptosis in SW1990 pancreatic cancer cells via p38- and caspase-dependent induction of p38 MAPK. Oncol Rep 31: 973–982.
18. Bae S, Lee EJ, Lee JH, Park IC, Lee SJ, et al. (2014) Oridonin protects HaCaT keratinocytes against hydrogen peroxide-induced oxidative stress by altering microRNA expression. Int J Mol Med 33: 185–193.
19. Xu Y, Xue Y, Wang Y, Feng D, Lin S, et al. (2009) Multiple-modulation effects of Oridonin on the production of proinflammatory cytokines and neurotrophic factors in LPS-activated microglia. Int Immunopharmac 9: 360–365.
20. Leung CH, Grill SP, Lam W, Han QB, Sun HD, et al. (2005) Novel mechanism of inhibition of nuclear factor-kB DNA-binding activity by diterpenoids isolated from Isodon rubescens. Mol Pharmacol 68: 286–297.
21. Zhao H, Wang SL, Qian L, Jin JL, Li H, et al. (2013) Diammonium glycyrrhizinate attenuates Abeta(1–42)-induced neuroinflammation and regulates MAPK and NF-kB pathways in vitro and in vivo. CNS Neurosci Ther 19: 117–124.
22. Han L, Yin K, Zhang S, Wu Z, Wang C, et al. (2013) Dalesenol blocks inhibits lipopolysaccharide induced inflammation and suppresses NF-kB and p38/JNK activation in microglial cells. Neurochem Int 62: 913–921.
23. Zhu X, Chen C, Ye D, Guan D, Ye L, et al. (2012) Diammonium glycyrhrizinate upregulates PGC1alpha and protects against Abeta1-42-induced neurotoxicity. PLoS One 7: e35823.
24. Karran EL, Mercken M, De Strooper B (2011) The amyloid cascade hypothesis for Alzheimer’s disease: an appraisal for the development of therapeutics. Nat Rev Drug Discov 10: 698–712.
25. Rezvannuller AJ, Jansen C, Carrazo A, van Haastert ES, Houdini D, et al. (2012) Neuroinflammation and common mechanism in Alzheimer’s disease and prion amyloidosis: amyloid-associated proteins, neuroinflammation and neurofilibrillary degeneration. Neurodegener Dis 10: 301–304.
26. Meng QH, Lou FL, Hou WX, Liu M, Guo H, et al. (2013) Acetylpuerarin reduces inflammation and improves memory function in a rat model of Alzheimer’s disease induced by Abeta1–42. Pharmazie 68: 904–908.

27. Gubandru M, Margina D, Tsitsimikou C, Goutzourelas N, Tsarouhas K, et al. (2013) Alzheimer’s disease treated patients showed different patterns for oxidative stress and inflammation markers. Food Chem Toxicol 61: 209–214.

28. Capiralla H, Vingtdeux V, Zhao H, Sankowski R, Al-Ashed Y, et al. (2012) Resveratrol mitigates lipopolysaccharide- and Abeta-mediated microglial inflammation by inhibiting the TLR4/NF-κB/STAT signaling cascade. J Neurochem 120: 461–472.

29. Thangavel R, Stolmeier D, Yang X, Anantharam P, Zaheer A (2012) Expression of glia maturation factor in neuropathological lesions of Alzheimer’s disease. Neuropathol Appl Neurobiol 38: 572–581.

30. Overmyer M, Helisalmi S, Soininen H, Laakso M, Riekkinen P Sr, et al. (1999) Astroglisis and the ApoE genotype. an immunohistochemical study of postmortem human brain tissue. Dement Geriatr Cogn Disord 10: 252–257.

31. Wang CJ, Li J, Liu Q, Yang R, Zhang JH, et al. (2011) Hydrogen-rich saline reduces oxidative stress and inflammation by inhibit of JNK and NF-κB activation in a rat model of amyloid-beta-induced Alzheimer’s disease. Neurosci Lett 491: 127–132.

32. Zhang J, Zhen YF, Pu-Bu-Ci-Ren, Song LG, Kong WN, et al. (2013) Salidroside attenuates beta amyloid-induced cognitive deficits via modulating oxidative stress and inflammatory mediators in rat hippocampus. Behav Brain Res 244: 70–81.

33. Kaltschmidt B1, Uhrezek M, Volk B, Baueerle PA, Kaltschmidt C (1997) Transcription factor NF-kappaB is activated in primary neurons by amyloid beta peptides and in neurons surrounding early plaques from patients with Alzheimer disease. Proc Natl Acad Sci U S A 94: 2642–2647.

34. Huang Y1, Liu F, Grundke-Iqbal I, Iqbal K, Gong CX (2005) NF-kappaB precursor, p105, and NF-kappaB inhibitor, IkappaBgamma, are both elevated in Alzheimer disease brain. Neurosci Lett 373: 115–118.

35. Hayden MS, Ghosh S (2004) Signaling to NF-κB. Genes Dev 18: 2195–2224.

36. Fan H, Guo Y, Liang X, Yuan Y, Qi X, et al. (2013) Hydrogen sulfide protects against amyloid beta-peptide induced neuronal injury via attenuating inflammatory responses in a rat model. J Biomed Res 27: 296–304.

37. Paris D, Patel N, Quadros A, Linan M, Bakshi P, et al. (2007) Inhibition of Abeta production by NF-κB inhibitors. Neurosci Lett 415: 11–16.

38. Valerio A, Boroni F, Benarese M, Sarnico I, Ghisi V, et al. (2006) NF-κB pathway: a target for preventing beta-amyloid (Abeta)-induced neuronal damage and Abeta42 production. Eur J Neurosci 23: 1711–1720.