Puerarin alleviates cognitive impairment and oxidative stress in APP/PS1 transgenic mice

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Abstract

Increasing evidence demonstrates that β-amyloid (Aβ) elicits oxidative stress, which contributes to the pathogenesis and disease progression of Alzheimer’s disease (AD). Thus, there is interest in developing antioxidant therapies for the prevention/treatment of cognitive decline during AD. We reported previously that puerarin has antioxidative properties in vitro. Therefore, the aim of the present study was to determine whether puerarin improves cognitive function and reduces oxidative stress in amyloid precursor protein/presenilin-1 (APP/PS1) mice, a well established AD mouse model, and explore its potential mechanism. Our results show that oral administration of puerarin significantly ameliorates cognitive impairment in APP/PS1 mice assessed by the Morris water maze (MWM) test. This was accompanied by a significant decrease in the levels of lipid peroxidation (LPO) through, at least in part, induction of nuclear factor erythroid 2-related factor 2 (Nrf2) target gene heme oxygenase 1 (HO-1) in the hippocampus of APP/PS1 transgenic mice at 9 months of age, but without altering brain Aβ burden. Furthermore, puerarin significantly activated Akt, reduced activation of glycogen synthase kinase 3β (GSK-3β), and induced nuclear translocation of Nrf2 in the hippocampus of APP/PS1 mice but did not alter ERK1/2 phosphorylation. Thus, puerarin may improve cognitive performance in APP/PS1 mice through activation of the Akt/GSK-3β signaling pathway. These findings suggest that puerarin might be an attractive agent for prevention and treatment of cognitive impairment and dementia.

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Key words: Alzheimer’s disease, APP/PS1 transgenic mice, cognitive impairment, glycogen synthase kinase 3β, puerarin.

Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder and causes significant dementia in elderly people, and it has no known cure (Lambracht-Washington and Rosenberg, 2013). The amyloid hypothesis of AD postulates that β-amyloid (Aβ) deposition and neurotoxicity play a causative role in AD (Coomaraswamy et al., 2010). There is abundant evidence that oxidative stress is not only an early event in AD, occurring prior to cytopathology, but also plays a role in nerve cell dysfunction and death in AD (Texel and Mattson, 2011; López et al., 2013). Although the mechanisms through which Aβ exerts its toxicity are numerous and have not yet been fully elucidated, it seems that oxidative injury is an important feature in AD pathogenesis, even before the appearance of Aβ deposits (Hamilton and Holscher, 2012). Recent evidence suggests that the neurotoxic properties of Aβ are mediated by oxidative stress (reviewed by Butterfield, 1997). The brain is particularly vulnerable to oxidative stress because of its high oxygen consumption coupled to a naturally lower antioxidant capability, resulting in an increment in lipid peroxidation (LPO) (Perluigi et al., 2009).

The heme oxygenase-1 (HO-1) is an inducible and redox-regulated enzyme that provides tissue-specific anti-antioxidant effects. HO-1 is considered a protective gene in many clinically relevant disease states, including AD (Barone et al., 2012). In redox signaling, nuclear factor erythroid 2-related factor 2 (Nrf2) plays a critical role in the transcription of a series of genes that contribute to phase II/III enzymes and the defense against oxidative stress. In the human AD brain, the amount of nuclear Nrf2 is reduced in the hippocampus (von Otter et al., 2010). Recent findings, including ours, have indicated that antioxidant induced Nrf2 to translocate from the cytoplasm into the nucleus to enhance the expression of HO-1 (Zou et al., 2013). Nrf2 over-expression in vitro protects against neurotoxicity of Aβ and is associated with increased expression of Nrf2 target genes and reduced oxidative stress (Kanninen et al., 2008).

Several lines of evidence directly link glycogen synthase kinase 3β (GSK-3β) to the neuropathology of
GSK-3β can be dephosphorylated and activated by Aβ in vitro, and its levels are increased in the AD brain (Hoshi et al., 2003; Rockenstein et al., 2007). Oxidative stress induces over-activation of GSK-3β in neuronal cells, while the inhibition of GSK-3β is involved in the control of oxidative stress in neuronal hippocampal cell lines (Schäfer et al., 2004). GSK-3β is tightly regulated by the survival pathway represented by phosphatidylinositol-3 kinase (PI3K) and its downstream effector the serine/threonine protein kinase (Akt) (Choi et al., 2012). It has been documented that translocation of Nrf2 into the nucleus might be induced by a decrease of GSK-3β phosphorylation (Ser9) (Rojo et al., 2008a,b).

Although it is unclear whether oxidative stress is initiated by the decrease in antioxidant capacity, treatment with antioxidants might offer theoretical benefits to improve cognition (Weinstein et al., 2009). Several synthetic antioxidants are available, but there is a growing trend towards the use of natural products as antioxidants (Fang et al., 2013). Epidemiological studies indicate that oestrogen replacement therapy lowers the risk of developing AD (Gutierrez-Zepeda et al., 2005). A large body of evidence indicates that oestrogens exert protective effects against Aβ toxicity and oxidative stress (Yao et al., 2007). Phytoestrogens have structural and functional similarities to mammalian oestrogens. Puerarin (for its structure, see Fig. 1), a phytoestrogen derived from the root of the wild leguminous creeper Pueraria lobata (Willd) Ohwi, is soluble in organic solvent (approximately 5 mg/ml in ethanol), and stable in rat plasma for at least 2 months when stored at −20 °C (Prasain et al., 2007). Work from our laboratory clearly shows that puerarin inhibits Aβ-induced oxidative stress in neuronal cultures from rat hippocampus (Zou et al., 2013), which is consistent with other studies (Zhang et al., 2008; Lin et al., 2012). No answer is yet available as to whether puerarin would ameliorate the cognitive deficits and oxidative stress in vivo in a genetic AD model. The aim of this study was to investigate the hypothesis that puerarin prevents cognitive impairment in amyloid precursor protein/presenilin-1 (APP/PS1) transgenic mice, and the cognitive enhancement effect of puerarin might mainly result from its antioxidant property by interrupting GSK-3β/Nrf2 signaling. A schematic diagram illustrating the hypothetical signaling pathway by which puerarin ameliorates cognitive impairment resulting from AD is presented in Fig. 2. Results in this report support our hypothesis and demonstrate that puerarin improves memory deficits in APP/PS1 mice through attenuating brain oxidative stress, suggesting a potential role of puerarin as a useful agent for the treatment of oxidative stress-mediated dementia disorders.

Methods

Animals

All studies were conducted under an approved protocol from the Animal Care and Use Committee of Qiqihar Medical University. Male APP/PS1 transgenic mice expressing mutant variants of human APP and PS1 [B6C3-Tg(APPswe, PSEN1dE9)85Dbo/J] aged 8 months, and age-matched nontransgenic littermate mice were purchased from the Animal Models Institution of Nanjing University (China). Mice were group-housed (four animals per cage) with a 12:12 h light/dark cycle and with ad libitum access to food and water.

Drug treatment

APP/PS1 transgenic mice and their wild-type (WT) controls were treated orally with puerarin (30 mg/kg/day, solubilized in 1,2-propanediol) (98% purity by HPLC; Sino-Herb Company, China) for 28 consecutive days. Control animals followed similar procedures but received vehicle instead of puerarin treatment. The doses and treatment schedules were selected based on previous reports in the literature (Xu and Zhao, 2002).
Morris water maze (MWM) test
The 6 d testing protocol used has been described in detail previously (Reed et al., 2010). Briefly, the mice underwent six consecutive days of testing with a submerged platform in a circular grey pool filled with water maintained at 24 °C. During the procedure, the platform location was kept constant, and the starting position varied between four constant locations at the pool rim. Mice had a maximum of 60 s to find the platform in trials of the acquisition phase (days 1–5). Probe trials were given on the sixth day, in which the platform was removed from the maze in the mouse’s absence to determine their search bias. Prior to the spatial learning training, all mice underwent non-spatial pre-training to assess swimming abilities and familiarize mice with the test. The time to find the platform (escape latency), the length of the swim path, and number of crossing over platform position were monitored and recorded semi-automatically by a tracking system using MWM software (Shanghai Jiliang Software Technology Co. Ltd, China). Swim speed was also assessed in the MWM to determine whether differences in performance could be attributed to non-cognitive factors.

Enzyme-linked immunosorbent assay (ELISA)
Mouse hemibrains were homogenized in T-PER® Tissue Protein Extraction Reagent (Pierce Chemical Co., USA) with a complete protease inhibitor cocktail (Roche Diagnostics GmbH, Germany) and centrifuged at 10000 r/min for 30 min at 4 °C. Supernatants and pellets were stored at −80 °C. Pellets were resuspended in the same volume of 70% formic acid, kept on ice for 30 min and centrifuged likewise. The supernatants were collected (SDS insoluble/FA soluble). Protein concentrations of all samples were measured using a BCA protein assay kit (Beyotime Biotechnology, China). ELISA was performed to quantify SDS soluble and SDS-insoluble/formic acid soluble Aβ using colorimetric β-amyloid ELISA kit (Invitrogen, USA) according to the manufacturer’s instructions, and was expressed as ng/mg of protein.

Measurement of LPO
Free malondialdehyde (MDA), a marker of LPO, was measured using a thiobarbituric acid-reactive substance (TBARS) assay kit (Jiancheng Bioengineering, China) according to the manufacturer’s instructions. Briefly, the brain tissues were homogenized in the presence of 5 mM butylated hydroxytoluene. Extracts were prepared by centrifugation at 10000 g for 10 min at 4 °C. The absorbance was measured by using a microplate reader (Safire2, Tecan Group Ltd, Switzerland) at 532 nm. MDA content was expressed as nmol/mg of protein.

Glutathione (GSH) assays
The levels of GSH in brain tissues were determined by using the components provided in a Glutathione Assay Kit obtained from Cayman Chemical Company (Ann Arbor, USA) according to their protocol. The concentration of total GSH was calculated as nmol/mg of protein according to the equation in the protocol.

Superoxide dismutase (SOD) activity measurement
The SOD activity was measured from the supernatant of the brain tissue homogenates using a SOD assay kit (Jiancheng Bioengineering, China) according to the manufacturer’s instructions. One unit of SOD activity was defined as the amount that reduced the absorbance at 550 nm by 50%. The enzyme activity was expressed as units/mg of protein.

Western blot
Cytosolic and nuclear fractions were isolated from the frozen hippocampal samples using a nuclear and cytoplasmic protein extraction kit (Beyotime Biotechnology, China), and protein content was determined using the BCA protein assay kit from Beyotime Biotechnology (China). Cytosolic or nuclear protein extracts were loaded into 10% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. After blocking, the membranes were treated with primary antibodies (HO-1, Nrf2, p-Akt, p-GSK-3β, p-ERK1/2, Akt1, GSK-3β, ERK1/2, GAPDH, or Lamin B), which were obtained from Santa Cruz Biotechnology (USA). Immunoreactive bands were then detected by incubating with conjugates of anti-rabbit IgG with horseradish peroxidase and enhanced chemiluminescence reagents (Amersham Biosciences UK, Ltd). To monitor potential artifacts in loading and transfer among samples in different lanes, the blots were reprobed with GAPDH antibody (USA). Band intensities were quantified by an AlphaImager™ 2200 using the SpotDenso function of AlphaEaseFC™ software version 3.1.2 (Witec, Switzerland).

Real-time polymerase chain reaction (PCR)
Total RNA was extracted from hippocampal tissues using a Trizol reagent (Invitrogen Corp., USA). First strand cDNA synthesis was performed using an ExScript™ RT kit (Takara Biotechnology, China). Gene expression levels were measured by real-time PCR on an ABI7300 machine (Applied Biosystems, USA) with iTaq™ SYBR® Green Supermix with ROX (Bio-Rad Laboratories, Hercules, USA) and the following primers: Nrf2: (F) 5′-GAT CCG CCA GCT ACT CCC AGG TTG-3′; (R) 5′-CAG GGC AAG CGA CTC ATG GTC ATC-3′; HO-1: (F) 5′-CAG CAT GTA CGT AGC CAT CC-3′; (R) 5′-TCA TTC GAG TCA-3′; β-actin: (F) 5′- GGTT GGA TTG GAG GGC TGG TTT-3′; (R) 5′-TCA CTC TCT TCT CAT TGT GAG-3′; Nrf2: (F) 5′-GGG TGT GGT GAC CTT GAT GAC-3′; (R) 5′-TCC CAA TTT TGT ACA TCT TTT ATG-3′; HO-1: (F) 5′-CTC TCA TTC GGC TTA AAG GAA-3′; (R) 5′-CTT TGC CAC TTT TCT TCA-3′; GAPDH: (F) 5′-CTT CTC TCT CTT TCA TAT TAC-3′; (R) 5′-CGA GCA ATT TAC CCA AAC TCA-3′.
GTG GTG GTG AA -3'. For quantification of the changes in gene expression, we used the comparative Ct method to calculate the relative fold changes normalized against the β-actin (Livak and Schmittgen, 2001).

**Statistical analysis**

Results are expressed as the mean±S.D. To determine significant main effects, three-way analysis of variance (ANOVA) was used to analyse the effects of genotype, treatment and time on the escape latency. Statistical differences among the experimental groups were tested by a repeated measures two-way ANOVA with puerarin-treated groups as between- and sessions as the within-subjects factors for behavioral tests when variable distributions were normal. Otherwise, the non-parametric Mann–Whitney U test was used. The biochemical estimations were separately analysed by ANOVA with the Student–Newman–Keuls test for post-hoc analysis. The value *p*<0.05 was considered significant. Owing to space limitation, only significant results are reported.

**Results**

**Puerarin treatment markedly attenuates cognitive deficits of APP/PS1 mice**

MWM analysis has been shown to be a reliable and noninvasive test to determine cognitive changes in the APP/PS1 mouse (Gallagher et al., 2013). Three-way ANOVA testing identified significant effects of the genotype (F$_{1,27}$=108.6, *p*<0.050, treatment (F$_{1,27}$=15.5, *p*<0.05) and time (F$_{4,27}$=411.4, *p*<0.05) on escape latency, with a significant interaction among the three effects (F$_{13,27}$=2.1, *p*=0.014). As shown in Fig. 3a, at 9 months of age, APP/PS1 mice took longer to locate the hidden platform than WT mice (ANOVA with repeated measures; F$_{3,27}$=75.52, *p*<0.01). Escape latencies did not significantly change in puerarin-treated WT mice compared with vehicle-treated WT mice (ANOVA with repeated measures; F$_{1,14}$=0.007, *p*=0.934). However, puerarin significantly improved spatial learning of APP/PS1 mice (ANOVA with repeated measures; F$_{1, 13}$=58.83, *p*<0.01). Because the swim speed of APP/PS1 and WT mice in MWM training conditions did not differ (data not shown), it is unlikely that the observed differences in escape latencies were caused by differences in mice locomotor abilities.

We further assess the retention of spatial memory in the APP/PS1 mice on the sixth day by a probe trial. Again, APP/PS1 mice show impaired spatial memory compared with WT mice, as expected. Similar to the results obtained from acquisition trials, the effect of puerarin was dependent on the genotype. Puerarin treatment significantly enhanced the number of crossing over platform position in APP/PS1 mice (Mann–Whitney U=5.5, *p*=0.008) (Fig. 3b), while no significant effect was observed in WT mice (Mann–Whitney U=30.00, *p*=0.832). One puerarin-treated APP/PS1 mouse that did not stay afloat was removed from the water and we were unable to collect data from that mouse in the MWM test.

**Puerarin treatment has no effect on brain Aβ burden in APP/PS1 mice**

Antioxidants have been reported to inhibit Aβ production (Avramovich-Tirosh et al., 2007). We analysed Aβ burden
Puerarin alleviates memory deficits

Puerarin treatment had no effect on soluble and insoluble Aβ levels in the brain of APP/PS1 mice. The content of Aβ1–42, both soluble and insoluble, in the brain were determined by ELISA and calculated based on the total protein content. Results are presented as means±S.D. of four animals per group. n.d.: none detected (below the ELISA detection limit).

Puerarin treatment attenuates oxidative stress in the brain of APP/PS1 mice

In the order to evaluate whether the antioxidant property of puerarin might play a critical role in the enhancement of spatial memory and learning, we assessed the effect of puerarin on the oxidative stress-related biomarkers. As shown in Fig. 5a, a significant increase in the level of MDA was observed in the brain of APP/PS1 mice as compared with that in WT mice. Treatment with puerarin significantly prevented the increase of MDA levels in the brain of APP/PS1 mice. In contrast, puerarin treatment prevented decrease of GSH in the brain of APP/PS1 mice, although decrease of GSH continued to be lower than that of WT mice (Fig. 5b). However, puerarin did not affect levels of MDA and GSH in the brain of WT mice. Puerarin had only a negligible effect on the SOD activity in the brain of APP/PS1 and WT mice (Fig. 5c).

Puerarin treatment upregulates the HO-1 expressions in the hippocampus of APP/PS1 mice

Previous studies have demonstrated that HO-1 plays important roles in protecting cells against oxidative stress as a cellular defence mechanism (Egea et al., 2007). We assumed that puerarin might induce HO-1 expression in the hippocampus of APP/PS1 mice, leading to the
increase in GSH level and decrease in LPO level. As shown in Fig. 6a, c, d, by Western blotting and real-time PCR analyses, hippocampus samples from APP/PS1 mice exhibited decreased HO-1 expression at both levels of protein and mRNA. Treatment with puerarin increased levels of protein and mRNA expression of HO-1 in the hippocampus of APP/PS1 mice, although HO-1 expression continued to be lower than that of WT mice. HO-1 expression also showed an upward trend in puerarin-treated WT mice than vehicle-treated WT mice, although, this did not reach statistical significance.

Puerarin treatment induces nuclear translocation of Nrf2 in the hippocampus of APP/PS1 mice

The nuclear translocation of Nrf2 and its target gene products, including HO-1, elicited an antioxidant response that may have therapeutic value for AD (Li et al., 2011). Therefore, we assessed the effects of puerarin on nuclear translocation of Nrf2 in the hippocampus of APP/PS1 mice. As demonstrated in Fig. 6a–c, a significant decrease in nuclear Nrf2 protein expression was observed in the hippocampus of APP/PS1 mice compared with
Puerarin treatment induces phosphorylations of Akt and GSK-3β in the hippocampus of APP/PS1 mice

Recent studies have suggested that the Akt/GSK-3β signaling pathway is involved in the nuclear translocation of Nrf2 (Rojo et al., 2008a,b). We assessed by Western blotting pSer9-GSK-3β (inactive form) and pSer473-Akt (active form) levels in the hippocampus from APP/PS1 and WT mice. The results showed that the amount of pAkt-Ser473, pSer9-GSK-3β, and p-ERK1/2 (Thr 202/Tyr 204) were significantly decreased in the hippocampus of APP/PS1 mice compared with WT mice. However, puerarin treatment significantly increased, as expected, pAkt-Ser473 (Fig. 6a, d) and pSer9-GSK-3β (Fig. 6a, d) in APP/PS1 mice but not in WT mice. The increased levels of pSer9-GSK-3β and pSer473-Akt were not attributable to an increase in total GSK-3β or total Akt, because there was no alteration of the total GSK-3β or total Akt expression by puerarin treatment. These results suggest, but do not prove, that puerarin-mediated enhancement of spatial memory appears to involve GSK-3β/Akt signaling pathways. Puerarin treatment did not significantly alter ERK1/2 phosphorylation in either WT or APP/PS1 mice (Fig. 6a, d).

Discussion

Oxidative stress is thought to be a key factor in the pathogenesis of AD and mild cognitive impairment (reviewed by Sultana and Butterfield, 2013). Reducing oxidative damage in the brain can be considered a promising strategy for therapeutic intervention in AD (Dumont et al., 2009). So far the possible effects of puerarin have not been studied in a genetic AD model, which mimics the amyloidosis and oxidative stress. In the present study, we found that puerarin significantly ameliorates the cognitive deficits accompanied by reduced LPO and increased HO-1 in APP/PS1 mice. Puerarin exerts beneficial therapeutic effects via regulating Akt/GSK-3β/Nrf2 signaling pathways.

The APP and PS1 knock-in mouse model utilized in the current study has been demonstrated to recapitulate some key features of AD pathology, including Aβ pathogenesis (Yu et al., 2009). Nine month old APP/PS1 mice have Aβ levels that are ~7-fold higher than WT mice (Danielyan et al., 2010). APP/PS1 mice at this age exhibit associative learning and memory impairments in the MWM test (Liu et al., 2011). This study shows that all of this is consistent with previous studies. We observed an amelioration of associative memory deficits after puerarin treatment for 28 d in APP/PS1 mice. The same treatment did not affect Aβ levels in the brain of these mice. We failed to observe a significant effect of puerarin treatment on cognitive performance in WT mice, as assessed in the MWM test. We cannot exclude that a larger dose of puerarin treatment might be needed to enhance cognitive performance in WT mice. Our results indicate memory deficits in APP/PS1 mice at this age are reversible. The underlying mechanisms by which puerarin alleviates cognitive impairment is independent of alteration of Aβ levels in APP/PS1 mice. These results did not support our initial assumption that puerarin might reduce Aβ levels in APP/PS1 mice. Given that male APP/PS1 transgenic mice were used in this study, we will investigate the anti-AD effects of puerarin in ovariectomized female mice in our future study. Although only one dose of puerarin (30 mg/kg) was used in this study, it may be that administration of higher doses would lead to even greater effects in APP/PS1 mice. Thus, the effect of several gradient doses of puerarin in anti-AD needs to be studied in detail in further studies. In addition, we chose 28 d as the duration of puerarin treatment on the basis of our preliminary studies that shorter durations showed less amelioration of cognitive deficits. Given the relatively short treatment duration of our study, however, longer-term treatment studies are warranted to determine whether or not long-term puerarin treatment can reverse elevated Aβ levels in APP/PS1 mice. Either way, the data presented here suggest that cognitive rescue can be achieved to some degree, even in APP/PS1 mice, by 30 mg/kg/day puerarin. Although much of the pathology associated with AD is driven by an increased load of Aβ in the brain of AD patients, a strategy for treating AD is to inhibit oxidative stress that results from a high Aβ environment (Block, 2008). Inhibition of oxidative stress may not only produce symptomatic relief, but may also slow the course of AD.

The brain may be particularly vulnerable to oxidative stress because of its high content of polyunsaturated fatty acids, high oxygen consumption and relatively low antioxidant levels (Moreira et al., 2010). Previous studies involving in vivo and in vitro experiments showed that Aβ increases oxidative damage (Wan et al., 2011). Our data in the present study show that 9 month-old APP/PS1 mice exhibit a significant basal increase in oxidative stress compared to WT mice, which has been likely caused by the APP/PS1 mutations. Our observations are consistent with other prior reports (Abdul et al., 2008). However, in this study, we used hemibrains, not the hippocampus, that mainly relates to learning and memory functions, for biochemical assay because the quantity of hippocampus was insufficient. Interestingly, in this work, we found that the enhancement of oxidative stress in the brain of APP/PS1 mice was significantly attenuated by puerarin, suggesting that antioxidant activity might play some role in the beneficial effects of puerarin in APP/PS1 mice. Although we cannot establish an
irrefutable cause–effect relationship between cognitive deficits and oxidative stress in APP/PS1 mice, our data support previous studies (Zou et al., 2013) and suggest that the neuroprotective effects of puerarin in AD may be mediated by a reduction of LPO production.

HO-1 is thought to be highly associated with AD pathology and expressed in the AD hippocampus (Hui et al., 2011). Upregulation of HO-1 has therapeutic potential for antioxidant function in AD (Kamalvand et al., 2003). Although HO-1 is known to correlate with the oxidative stress of AD, it is uncertain whether the increased HO-1 levels are associated with the improvement of cognitive functioning by antioxidant treatment. In this study, we found that increase of HO-1 expression by puerarin treatment is sufficient or necessary for the recovery of cognitive functions in APP/PS1 mice. Our results offer a remarkable parallel with previous in vitro studies showing that the HO-1 protein and mRNA levels increased ~2-fold in hippocampal neurons with puerarin treatment. Analysis of SOD activity in the brains of mice from this study revealed that there was no significant change by puerarin treatment. Schuessel et al. (2005) found that the activity of Cu/ZnSOD did not change in brains from male Thy1-APP/PS1L mice as compared to WT mice. In contrast, Sompol and colleagues demonstrated that developing APP/PS1 neurons have increased MnSOD protein and activity, and mature APP/PS1 neurons exhibited lower MnSOD levels compared to mature WT neurons (Sompol et al., 2008). Here, we show that SOD activity in brain showed a trend, but not a statistically-significant reduction in APP/PS1 mice vs. WT mice. In addition, we could not exclude the roles of any mechanisms and enzymes in the removal of LPO products, which requires additional studies using knockdown strategy, such as HO-1 null mutant mice.

A growing body of literature suggests that activation of Nrf2 provides neuroprotection in AD (Khodagholi et al., 2010). The Nrf2 antioxidant pathway was impaired in transgenic AD mice concomitantly with increased brain Aβ burden (Kanninen et al., 2009). Previous work by Choudry showed a 50% reduction of Nrf2 levels in transgenic AD mice (Choudry et al., 2012). Induction of the Nrf2 pathway by small-molecule compounds protects against neuronal oxidative stress and toxicity induced by Aβ in vitro (Eftekhazadeh et al., 2010). In this study, analysis of both the protein and mRNA levels of Nrf2 in hippocampus of APP/PS1 mouse revealed increased nuclear accumulation of Nrf2 protein after puerarin treatment, without affecting its transcription. The accumulation of Nrf2 in the nucleus results in the induction of cytoprotective gene expression. Although nuclear translocation of Nrf2 is responsible for induction of HO-1 expression, it is uncertain whether the puerarin-induced enhancement in HO-1 expression contributes to the improved cognitive functions in APP/PS1 mice.

GSK-3β exerts a negative form of regulation on Nrf2 by controlling its subcellular distribution (Rada et al., 2012). Prolonged oxidative stress, such as AD, causes inactivation of Akt, activation of GSK-3β and translocation of Nrf2 from the nucleus to the cytosol, thus limiting the antioxidant response of cells (Rojo et al., 2008a,b). In agreement with previous studies on AD patients and AD mouse models, this study shows that the inactive form of GSK-3 was decreased in the hippocampus of APP/PS1 mice, and puerarin treatment increased the active form of Akt and the inactive form of GSK-3β. Although the causal relationship remains unclear, it is conceivable that the improved spatial learning and memory can be attributed to the puerarin-induced activation of the Akt pathway. Why puerarin treatment has no effect on the above indices in WT mice is unclear. It is likely that several changes in cellular functions triggered by mutant PS1 and APP render the above indexes more responsive to puerarin. The overall effects of puerarin may be milder in WT brains, and the puerarin treatment, at the low doses used in the present study, may affect only disturbed cellular functions and cause relatively few side-effects. However, there is no experimental evidence for this hypothesis. Moreover, puerarin failed to modulate the phosphorylation of ERK1/2, demonstrating some specificity for its action on the Akt/GSK-3β pathway. Du et al. (2004) showed that oestrogen protects against Aβ-induced neurotoxicity by activation of the Akt cascade, although antioxidative action of oestrogen was not shown. Our results on Akt activation by phytooestrogen are consistent with this report. Similarly, curcumin, an antioxidants found in the turmeric root, protects human neuroblastoma SH-SY5Y cells against Aβ-induced mitochondrial dysfunction involving the inhibition of GSK-3β (Huang et al., 2012).

In summary, our results demonstrated that reversal of cognitive deficits by puerarin in APP/PS1 mice might mainly result from its antioxidant capability by increasing gene expression of HO-1, which is mediated by activation of Akt/GSK-3β/Nrf2 signaling. This unique mechanism explains, at least partially, its potent antioxidant capacity, which might allow puerarin to succeed where other ‘regular’ antioxidants have failed to inhibit AD. In addition, our results do not exclude possible involvement of any other mechanisms in the inhibition of oxidative stress by puerarin. Puerarin is well absorbed from the gastrointestinal tract, and is rapidly eliminated from the blood circulation after intragastric administration (t1/2 = 30 min), showing a very low toxicity, with an LD50 of 738 mg/kg in mice (Wu et al., 2009). Therefore, puerarin could be a potential candidate for further preclinical study aimed at the treatment of cognitive impairment and dementia.

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Statement of Interest

None.

References


