Neuroscience Letters 461 (2009) 172–176

Contents lists available at ScienceDirect

Neuroscience Letters

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

Hibifolin, a flavonol glycoside, prevents β-amyloid-induced neurotoxicity in cultured cortical neurons

Judy T.T. Zhu[a], Roy C.Y. Choi[a], Heidi Q. Xie[a], Ken Y.Z. Zheng[a], Ava J.Y. Guo[a], Cathy W.C. Bi[a], David T.W. Lau[a], Jun Li[a], Tina T.X. Dong[a], Brad W.C. Lau[b], Ji J. Chen[c], Karl W.K. Tsim[a,*]

[a] Department of Biology and Center for Chinese Medicine, The Hong Kong University of Science and Technology, Clear Water Bay Road, Kowloon, Hong Kong SAR, China
[b] Macau Institute for Applied Research in Medicine and Health, Macau University of Science and Technology, Avenida Wai Long, Taipa, Macao
[c] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, China

ARTICLE INFO

Article history:
Received 14 April 2009
Received in revised form 29 May 2009
Accepted 11 June 2009

Keywords:
Neuroprotection
Cortical neuron
Flavonoid
Hibifolin

ABSTRACT

The toxicity of aggregated β-amyloid (Aβ) has been implicated as a critical cause in the development of Alzheimer's disease (AD). Hibifolin, a flavonol glycoside derived from herbal plants, possessed a strong protective activity against cell death induced by aggregated Aβ. Application of hibifolin in primary cortical neurons prevented the Aβ-induced cell death in a dose-dependent manner. In cultured cortical neurons, the pre-treatment of hibifolin abolished Aβ-induced Ca^{2+} mobilization, and also reduced Aβ-induced caspase-3 and caspase-7 activation. Moreover, DNA fragmentation induced by Aβ could be suppressed by hibifolin. In addition to such protection mechanisms, hibifolin was able to induce Akt phosphorylation in cortical neurons, which could be another explanation for the neuroprotection activity. These results therefore provided the first evidence that hibifolin protected neurons against Aβ-induced apoptosis and stimulated Akt activation, which would be useful in developing potential drugs or food supplements for treating AD.

Alzheimer's disease (AD) is a multifaceted neurodegenerative disorder characterized by the progressive deterioration of cognition and memory and affects nearly 0.4% of the world population and expected to 1% in 2050. Pathologically, senile plaque and neurofibrillary tangles are the hallmarks of AD [21]. Peptide β-amyloid (Aβ) is the major protein component of senile plaques, and its excessive accumulation in the brain has been suggested as a possible cause of neurodegeneration [26]. The Aβ-related fragments are toxic to neurons *in vitro*, or *in vivo*, which might be attributed to Aβ production, aggregation and degradation [10]. The inhibition of Aβ-induced cell toxicity is one of the targets for AD treatment and drug discovery. However, the detailed mechanisms for Aβ-induced neuronal death remain controversial and unclear. In neurons, Aβ triggered the disturbance of calcium homeostasis [15]. In addition, cultured neurons treated with Aβ, or transgenic mice expressing Aβ, render neurons vulnerable to apoptosis [5].

A serine/threonine kinase Akt is proposed to have roles in the regulation of cell growth, proliferation, migration, glucose metabolism, transcription, protein synthesis, angiogenesis, cell survival and anti-apoptosis [2,18]. Akt is activated by phosphorylation at two critical residues: T308 in the kinase domain and S473 in the hydrophobic motif. The stimulation of Akt kinase therefore is another approach for AD therapy.

Among those neuroprotective compounds, estrogen and its chemically similar compounds are drawing lots of attention. The estrogen-treated patients could experience a reduced risk suffering from AD, and estrogen has been proposed to have neuroprotective roles in neurons [7]. Unfortunately, the increased estrogen level causes sever side effects in clinical evidence [8]. Polyphenolic compounds have been considered as substitutes for estrogen, and indeed some of these compounds have been proven to possess neuroprotective effects, e.g. baicalein, genistein and kaempferol [1,19]. Different flavonoids with neuroprotective effects have been identified as potential therapeutic agents against AD. Among these flavonoids, baicalein, scutellarin, hibifolin and quercetin-3′-glucoside possessed the strongest effects in neuroprotection; however, the neuroprotective activity did not directly correlate with the estrogenic activity of the flavonoids [29]. One of the potential candidates, hibifolin, having strong neuroprotective effects and no estrogenic effects, is a flavonol glycoside and isolated from herbal medicines, e.g. *Abelmoschus manihot* and *Melochia corchorifolia* [12,23,29]. Here, we showed that hibifolin could inhibit Aβ-induced toxicity in cultured neurons, and which possessed neuroprotective

Abbreviations: Aβ, β-amyloid protein; AD, Alzheimer's disease; A23187, Ca^{2+} ionophore; BAPTA-AM, 1,2-bis-(o-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetra-(acetoxymethyl) ester.

* Corresponding author. Tel.: +852 2358 7332; fax: +852 2358 1559.
E-mail address: botsim@ust.hk (K.W.K. Tsim).

0304-3940/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2009.06.010

activity by blocking Aβ-induced calcium mobilization, as well as by activating Akt signaling pathway.

17β-Estradiol, congo red, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), staurosporin, A23187, BAPTA-AM, tetrodotoxin and β-amyloid$_{25-35}$ (Aβ$_{25-35}$) were purchased from Sigma (St. Louis, MO); β-amyloid$_{1-40}$ (Aβ$_{1-40}$) was from American Peptide Company (Sunnyvale, CA); Cytotoxicity Detection Kit (LDH) was from Roche (Mannheim, Germany). Hibifolin was obtained from Kunming Institute of Botany (Kunming, China). The purity of hibifolin was over 98%, and dissolved in DMSO to give stock solutions at a concentration of 100 mM.

Cortical neurons were cultured as described previously with modifications [24]. In brief, cortex was dissected from embryonic day 18 rats and undergone trypsin digestion. The dissociated cortical neurons were grown in neural basal medium with B27 and 0.5 mM GlutaMax in a humidified incubator with 5% CO_2 at 37 °C. The cultures were treated with 2.5 μM cytosine arabinoside on the third day of culture as to eliminate glial cells. The primary cortical neurons were cultured for 2 weeks before the treatments. Reagents for cell cultures were purchased from Invitrogen Technologies (Carlsbad, CA). Un-aggregated Aβ (Aβ$_{1-40}$) was dissolved in water and incubated for 4 days at 37 °C. The aged Aβ together with the active fragment of Aβ (Aβ$_{25-35}$) were used for the toxicity tests. Cultured cortical neurons in 96-well plate were treated with 10 μM Aβ (either aged Aβ or Aβ$_{25-35}$) for 24 h. Followed by addition of 3-(4,5-dimethylthiazol-2)-2,5 diphenyltetrazolium bromide (MTT; 0.5 mg/ml) in PBS for 2 h, the medium was aspirated, and the cultures were re-suspended by DMSO to determine the cell viability by measuring the absorbance at 570 nm. To measure lactate dehydrogenase (LDH), cultures were treated as in the viability assay. After the treatment, the conditioned medium was collected and centrifuged for 14,000 rpm at 25 °C for 5 min. The supernatant was added into Cytotoxicity Detection Kit and incubated for 20 min at room temperature before taking the absorbance at 490 nm. In the blocking experiment, cultures were pre-treated with DMSO (control, 1/2000 dilution) or hibifolin (0.5, 5 and 50 μM) for 24 h or 1,2-bis-(o-aminophenoxy) ethane-N,N,N′,N′-tetra-acetic acid tetra-(acetoxymethyl) ester (BAPTA-AM; 50 μM) for 3 h before the addition of Aβ.

Analysis of DNA fragmentation was performed according to the previous report [6]. In brief, the cultured cortical neurons were pre-treated with or without hibifolin (0.5, 5, 50 μM) for 24 h before the addition of Aβ for 24 h. The cultures were lysed by 10 mM Tris–HCl pH 7.5, 10 mM EDTA and 1% Triton X-100, and centrifuged for 14,000 rpm at 4 °C for 10 min. The supernatant treated with proteinase K (0.1 mg/ml) at 55 °C for 1 h, RNase A (0.2 mg/ml) 37 °C for 30 min, and then extracted with phenol:chloroform:isoamyl alcohol (25:24:1) and then precipitated by isopropanol in the presence of 300 mM sodium acetate. The fragmented DNA was visualized by agarose gel electrophoresis.

For the detection of caspases, 2-week-old cultured cortical neurons in 12-well plates were pre-treated for 24 h with or without hibifolin (50 μM) before the addition of Aβ for 24 and 48 h. Staurosporin (1 μM) was used as a control to induce apoptosis. For the detection of Akt, 2-week-old cortical neurons in 12-well plates were starved by neurobasal medium or DMEM for 3 h with tetrodotoxin (100 nM) and then treated with hibifolin (50 μM) for 0, 5, 15 min. Cultures were then collected immediately by lysis buffer containing 0.125 M Tris–HCl, pH 6.8, 4% SDS, 20% glycerol, 2% 2-mercaptoethanol, and the cell lysates were analyzed by Western blot analysis. The antibodies were specific for cleaved caspase-3, total caspase-3, cleaved caspase-7, total caspase-7, phospho-Akt S473, phospho-Akt T308 and total Akt (1:2000; Cell Signaling Technology, Danvers, MA). The detection was performed according to the ECL (Amersham Biosciences, Piscataway, NJ). The intensities of the bands in the control and drug-stimulated samples, run on the same gel and under strictly standardized ECL conditions, were compared on an image analyzer, using in each case a calibration plot constructed from a parallel gel with serial dilutions of one of those samples.

Cultured cortical neurons in 96-well clear-bottom black plates were labeled by 2 μM Fluo-4-AM (Invitrogen) in HEPES buffer saline (Invitrogen) for 1 h at 37 °C, and then the change of intracellular Ca^{2+} mobilization was determined by FlexStation II (Molecular Devices, Sunnyvale, CA). For the blocking experiment, 50 μM hibifolin was included during the labeling process. The Ca^{2+} mobilization was monitored for 2 min after the addition of Aβ. Data were analyzed by SoftMax Pro 4.7 software.

Protein concentrations were measured with a kit from Bio-Rad Laboratories (Hercules, CA). Statistical tests were done by using

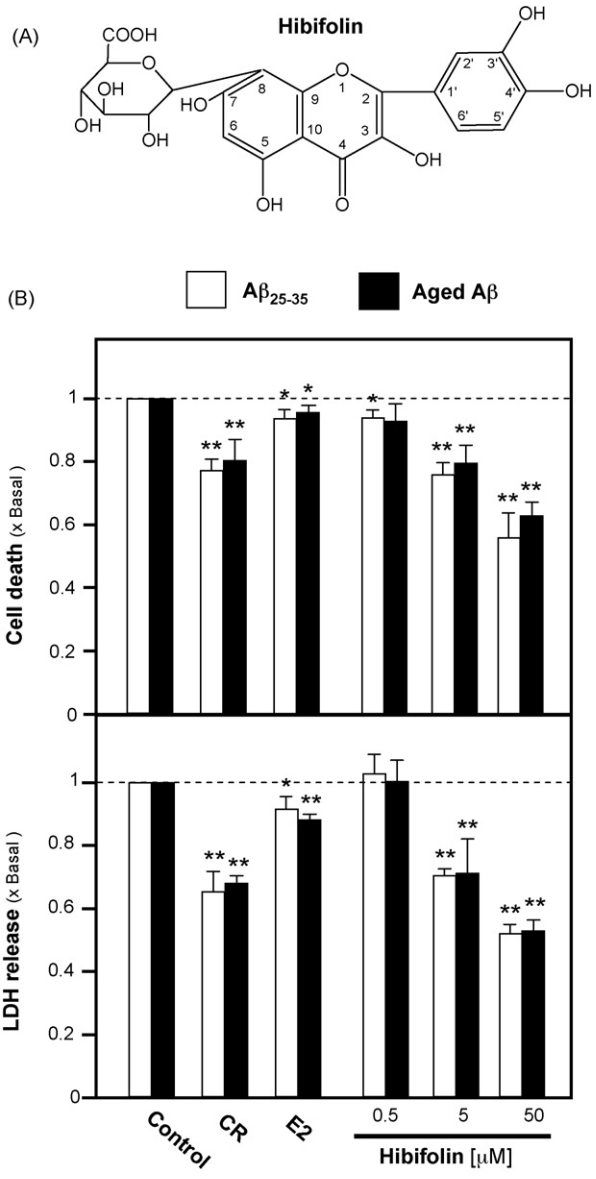

Fig. 1. Hibifolin prevents Aβ-induced cell toxicity. (A) The chemical structure of hibifolin. (B) Cultured cortical neurons were pre-treated with hibifolin for 24 h, and then were washed away. Aged Aβ (10 μM; 37 °C for 4 days of aging), or Aβ$_{25-35}$ (10 μM), was applied onto cultured neurons for 24 h before the MTT cell viability assay and LDH assay. 17β-Estradiol (E2, 10 nM) and congo red (CR, 1 μM) were used as positive controls. The results are normalized and expressed as a ratio to the value obtained at control (with Aβ but no drug): that is arbitrarily set to 1. Data are expressed as mean ± SEM, where $n = 5$, each with triplicate samples. $^{*}p < 0.05$ and $^{**}p < 0.01$ as compared to the control group (with Aβ but no drug).

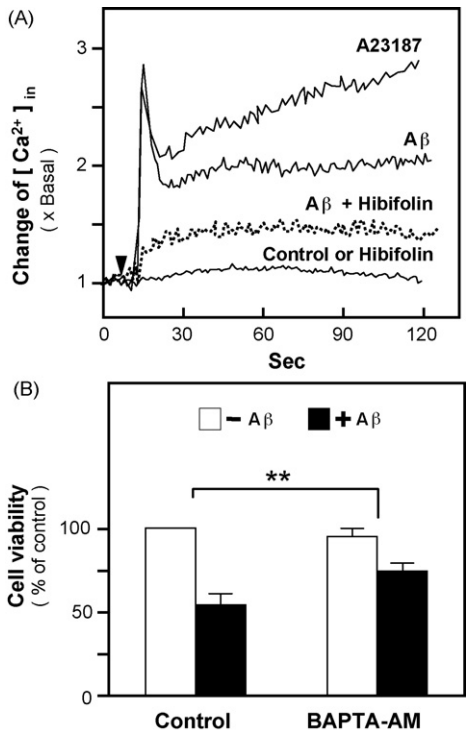

Fig. 2. Pre-treatment of hibifolin prevents intracellular Ca^{2+} mobilization induced by Aβ. (A) Cultured cortical neurons were labeled with Ca^{2+} indicator Fluo-4-AM (2 μM), and challenged by DMSO (control), Aβ (100 μM), Aβ (100 μM) + hibifolin (50 μM) and calcium ionophore A23187 (10 μM). Arrow-head indicated the addition of Aβ or drug. Value at 0 min was set as the basal level 1. (B) Cultured cortical neurons were pre-treated with DMSO (0.1%), or Ca^{2+} chelator BAPTA-AM (50 μM), for 3 h before the addition of Aβ (10 μM) for 24 h treatment, and which was subjected to cell viability assay. Value of DMSO-treated culture without Aβ was considered as a control. **Indicated the significant value with $p < 0.01$.

one-way ANOVA. Statistically significant changes were classed as significant [*] where $p < 0.05$ and highly significant [**] where $p < 0.01$.

The role of hibifolin (Fig. 1A) in protecting against cell death induced by aged Aβ, or $Aβ_{25–35}$, in cultured cortical neurons were elucidated. By using MTT assay for cell viability, application of Aβ (either aged Aβ or $Aβ_{25–35}$) for 24 h caused 40–50% death of the cultured cells (supplementary material Fig. 1S). Similarly the release of LDH, a cytosolic marker for cell death, into medium was markedly increased to ~2-fold after Aβ (either aged Aβ or $Aβ_{25–35}$) application (supplementary material Fig. 1S). The protective effect of hibifolin was quantified by MTT and LDH assays. The pre-treatment of hibifolin prevented Aβ-induced cell death in a dose-dependent manner (Fig. 1B, upper panel). In parallel, the release of LDH induced by Aβ was reduced by hibifolin in cortical neurons (Fig. 1B, lower panel). Congo red and estrogen served as positive controls [7,14,25]. The morphological change of the drug-treated cortical neurons also indicated the toxicity of Aβ and neuroprotection of hibifolin (supplementary material Fig. 2S). Due to the similar results of aged Aβ and $Aβ_{25–35}$ shown in the cell death assays, $Aβ_{25–35}$ was used routinely in the subsequent experiments.

The role of hibifolin in calcium mobilization was determined. In cultured neurons, about ~2-fold increase of intracellular Ca^{2+} level was revealed after application of Aβ and sustained for few minutes. Calcium ionophore A23187 served as a control for Ca^{2+} elevation (Fig. 2A). To reveal the correlation between Aβ-induced Ca^{2+} influx and neuroprotection mechanisms of hibifolin, the cultured neurons were pre-treated with the flavonol for 24 h before the measurement of intracellular Ca^{2+}. Interestingly, hibifolin pre-treatment reduced the amount of Ca^{2+}, induced by Aβ by over 70% (Fig. 2A). Moreover, hibifolin itself did not trigger any Ca^{2+} mobilization upon treatment. These results indicated that Aβ-induced Ca^{2+} elevation was one of the signaling pathways in mediating cell death, and hibifolin could partially block this action. Serving as a control, the pre-treatment of a cell permeable Ca^{2+} chelator BAPTA-AM for 3 h reduced the Aβ-induced cell death (Fig. 2B), which suggested that the intracellular Ca^{2+} indeed was one of downstream mediators for Aβ-induced cell death.

In cultured cortical neurons, the application of Aβ caused an increase of cleaved caspase-3 and caspase-7, both at ~16 kDa, by ~2-fold (Fig. 3A); however, the pre-treatment of hibifolin fully prevented this cleavage (Fig. 3A and B). Similarly, the pre-treatment of hibifolin was shown to block the DNA fragmentation triggered by Aβ (Fig. 3C). The general apoptotic inducer, staurosporin, served as a control for both the assays. These results suggested that hibifolin exerted its neuroprotection by blocking Aβ-induced apoptosis.

In addition to the above protection mechanism, Akt pathway has been demonstrated to mediate neurotrophic and anti-apoptotic effects [18]. Therefore, we targeted the role of Akt signaling in the neuroprotection of hibifolin. The phosphorylation of Akt was determined by using antibodies against T308 and S473 positions. Results revealed that the application of hibifolin induced the phosphorylation of Akt to ~2-fold at T308 and S473 (~60 kDa) in a time-dependent manner (Fig. 4). The total amount of Akt (~60 kDa)

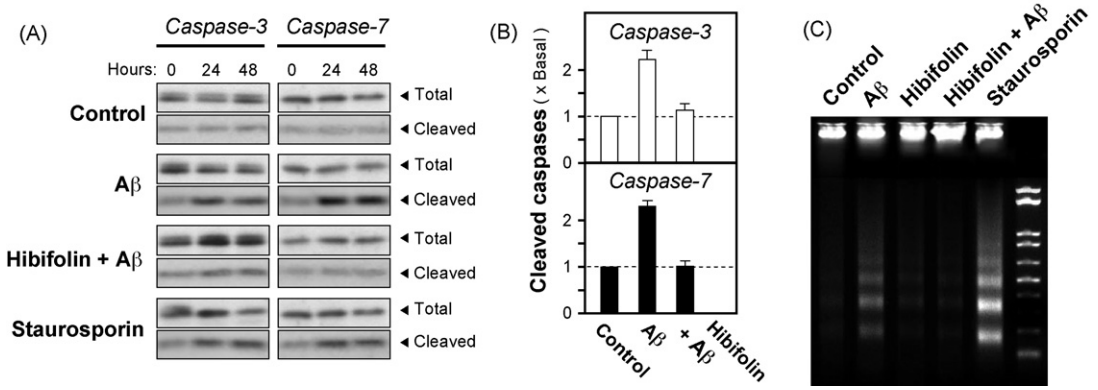

Fig. 3. Hibifolin protects neurons from Aβ-induced apoptosis. (A) Cultured cortical neurons were pre-treated with hibifolin (50 μM), or DMSO, for 24 h before the addition of 10 μM Aβ for 0–48 h. Cultures were collected at different time points to determine the amount of caspase-3 (16 kDa) and caspase-7 (20 kDa) by Western blot analysis. Application of staurosporin (10 μM) for 2 h served as a control. (B) Quantification of band intensity from (A) was performed. The values are expressed as the ratio of treated culture to background level (untreated culture), and in all cases values are mean ± SEM for five independent experiments, each with triplicate samples. (C) Cultured neurons were pre-treated with hibifolin (50 μM), or water control, for 24 h before the addition of 10 μM Aβ for 24 h. Application of staurosporin (10 μM) served as a control. The last lane on the right shows the DNA markers from 100 to 2000 bp. A representative gel photo was shown.

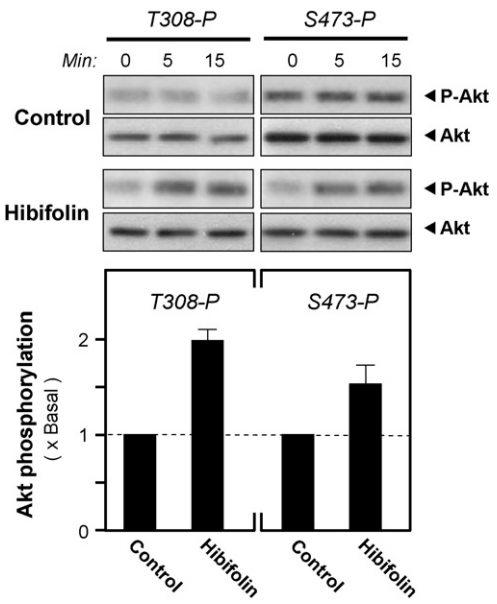

Fig. 4. Activation of Akt signaling pathway by hibifolin in cortical neurons. Cultured cortical neurons were serum starved for 3 h before the addition of hibifolin (50 μM) for different times. Total Akt and phosphorylated Akt at T308 or S473 (~60 kDa) were revealed by using specific antibodies (upper panel). The phosphorylations were quantified from the blots by calibrated densitometry (lower panel). Data are normalized and expressed as the ratio to the basal activity where time 0 (untreated) equals to 1. Data are expressed as mean ± SEM, where $n = 5$, each with triplicate samples.

was unchanged. Thus, the activity of hibifolin on Akt phosphorylation could be another explanation for its protective effect against Aβ-induced cell death.

Estrogen has been proven to improve cognitive impairments in animal model or in AD patients [7]. Being the alternative drugs of estrogen, flavonoids were firstly selected to study the mechanism for its neuroprotection: this study could pave an avenue in developing new drugs for AD [29]. Here, our results indicated that hibifolin blocked calcium mobilization and apoptosis induced by Aβ, and simultaneously which stimulated Akt phosphorylation in cultured neurons. Flavonoids are polyphenolic compounds with multiple biological functions and widely existed in herbal medicines, fruits and vegetables. Hibifolin belongs to flavanol subclass with 3-hydroxyflavone backbone (3-hydroxy-2-phenylchromen-4-one (IUPAC). In this subclass, some flavonols such as kaempferol, quercetin and myricetin have been studied to exert neuroprotection for the substitution of hydroxyl group at C-3 position and double bond between C-2 and C-3 positions [19,22]. Stronger neuroprotective activity of hibifolin, as compared to other flavonols, illuminates that the sugar residues might increase the neuroprotective effects of flavonols which is similar to the case of ginsenoside [16].

In the present study, we have shown the neuroprotective role of hibifolin against Aβ-induced toxicity. The calcium mobilization and apoptosis induced by Aβ have been proposed to be one of the causes of AD [4]. Hibifolin mediated its neuroprotection by blocking the upstream calcium elevation and apoptosis induced by Aβ in cultured neurons. Alternatively, the activation of Akt kinase has been demonstrated to mediate neurotrophic and anti-apoptotic effects, which could be used as another target for drug development against AD [2,18]. Such speculation was further supported by the fact that estrogen could activate PI3K/AKT pathway to exert its neuroprotection [9]. In addition, the activation of Akt by a flavanol quercetin has been revealed to downregulate the pro-apoptotic proteins [20]. The involvement of Akt in Aβ-induced toxicity therefore supported the role of this kinase in neuroprotective activity [17]. However, the mechanism of Aβ-induced neuronal apoptosis remains incompletely defined. Other routes related to Aβ-induced apoptosis, e.g. Bcl-2 family protein, c-Jun N-terminal kinase, mitochondrial malfunctioning metal ions, reactive oxygen species (ROS) release, and apoptogenic acetylcholinesterase variants should also be considered in revealing the neuroprotective mechanisms of hibifolin [13,27,28]. Complete elucidation of the signaling mechanism promises the acceleration of developing neuroprotective interventions for AD treatments.

The generation of free radicals and oxidative stress has been proposed to be another cause of AD. Indeed, many natural products particular flavonoids are the well-known anti-oxidants or free radical scavengers. The relationship between flavonoid structure and anti-oxidant activity has been studied [11]. Possibly, the anti-oxidant effects of flavonoids may be one of the mechanisms for its neuroprotection, and the estrogenic activity could be correlated with the neuroprotection activity [14]. In opposite to this notion, most of the flavonoids having strong estrogenic activity do not exert strong neuroprotective effects, e.g. hibifolin [29]. Under this scenario, the neuroprotective effect of flavonoids could not be fully accounted by estrogenic effect, or which is not the only factor involved in neuroprotection [3,14]. Nevertheless, the association of estrogenic activity and neuroprotective effect is required to be further explored.

Acknowledgments

This research was supported by grants from RGC of Hong Kong (HKUST 6419/06M, N_HKUST629/07, 662608), Croucher Foundation (CAS-CF07/08.SC03), and Areas of Excellence Scheme established under the UGC of Hong Kong (AoE/B-15/01) to KWKT.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neulet.2009.06.010.

References

[1] O.Y. Bang, H.S. Hong, D.H. Kim, H. Kim, J.H. Boo, K. Huh, I. Mook-Jung, Neuroprotective effect of genistein against beta amyloid-induced neurotoxicity, Neurobiol. Dis. 16 (2004) 21–28.
[2] D.P. Brazil, B.A. Hemmings, Ten years of protein kinase B signalling: a hard Akt to follow, Trends Biochem. Sci. 26 (2001) 657–664.
[3] D.N. Bryant, M.A. Bosch, O.K. Rønnekleiv, D.M. Dorsa, 17-Beta estradiol rapidly enhances extracellular signal-regulated kinase 2 phosphorylation in the rat brain, Neuroscience 133 (2005) 343–352.
[4] C. Cerella, C. Mearelli, S. Coppola, M. D'Alessio, M. De Nicola, M. Diederich, L. Ghibelli, Sequential phases of Ca^{2+} alterations in pre-apoptotic cells, Apoptosis 12 (2007) 2207–2219.
[5] S. Estus, H.M. Tucker, C. van Rooyen, S. Wright, E.F. Brigham, M. Wogulis, R.E. Rydel, Aggregated amyloid-beta protein induces cortical neuronal apoptosis and concomitant "apoptotic" pattern of gene induction, J. Neurosci. 17 (1997) 7736–7745.
[6] H.J. Fu, Q.S. Hu, Z.N. Lin, T.L. Ren, H. Song, C.K. Cai, S.Z. Dong, Aluminum-induced apoptosis in cultured cortical neurons and its effect on SAPK/JNK signal transduction pathway, Brain Res. 980 (2003) 11–23.
[7] A.R. Genazzani, N. Pluchino, S. Luisi, M. Luisi, Estrogen, cognition and female ageing, Hum. Reprod. Update 3 (2007) 175–187.
[8] M.G. Glazier, M.A. Bowman, A review of the evidence for the use of phytoestrogens as a replacement for traditional estrogen replacement therapy, Arch. Intern. Med. 161 (2001) 1161–1172.
[9] K. Honda, H. Sawada, T. Kihara, M. Urushitani, T. Nakamizo, A. Akaike, S. Shimohama, Phosphatidylinositol 3-kinase mediates neuroprotection by estrogen in cultured cortical neurons, J. Neurosci. Res. 60 (2000) 321–327.
[10] K. Irie, K. Murakami, Y. Masuda, A. Morimoto, H. Ohigashi, R. Ohashi, K. Takegoshi, M. Nagao, T. Shimizu, T. Shirasawa, Structure of beta-amyloid fibrils and its relevance to their neurotoxicity: implications for the pathogenesis of Alzheimer's disease, J. Biosci. Bioeng. 99 (2005) 437–447.
[11] J.M. Jeong, C.H. Choi, S.K. Kang, I.H. Lee, J.Y. Lee, H. Jung, Antioxidant and chemosensitizing effects of flavonoids with hydroxy and/or methoxy groups and structure–activity relationship, J. Pharm. Pharm. Sci. 10 (2007) 537–546.
[12] H.P. Kim, K.H. Son, H.W. Chang, S.S. Kang, Anti-inflammatory plant flavonoids and cellular action mechanisms, J. Pharmacol. Sci. 96 (2004) 229–245.

[13] J. León, D. Acuña-Castroviejo, G. Escames, D.X. Tan, R.J. Reiter, Melatonin mitigates mitochondrial malfunction, J. Pineal Res. 38 (2005) 1–9.
[14] N. Linford, C. Wade, D. Dorsa, The rapid effects of estrogen are implicated in estrogen-mediated neuroprotection, J. Neurocytol. 29 (2000) 367–374.
[15] A. MacManus, M. Ramsden, M. Murray, Z. Henderson, H.A. Pearson, V.A. Campbell, Enhancement of (45) $Ca^{(2+)}$ influx and voltage-dependent $Ca^{(2+)}$ channel activity by beta-amyloid-$_{(1–40)}$ in rat cortical synaptosomes and cultured cortical neurons. Modulation by the proinflammatory cytokine interleukin-1beta, J. Biol. Chem. 275 (2000) 4713–4718.
[16] S.Y. Nah, D.H. Kim, H. Rhim, Ginsenosides: are any of them candidates for drugs acting on the central nervous system? CNS Drug Rev. 13 (2007) 381–404.
[17] M. Nassif, J. Hoppe, K. Santin, R. Frozza, L.L. Zamin, F. Simão, A.P. Horn, C. Salbego, Beta-amyloid peptide toxicity in organotypic hippocampal slice culture involves Akt/PKB, GSK-3beta, and PTEN, Neurochem. Int. 50 (2007) 229–235.
[18] V. Ries, C. Henchcliffe, T. Kareva, M. Rzhetskaya, R. Bland, M.J. During, N. Kholodilov, R.E. Burke, Oncoprotein Akt/PKB induces trophic effects in murine models of Parkinson's disease, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 18757–18762.
[19] A. Roth, W. Schaffner, C. Hertel, Phytoestrogen kaempferol (3,4′,5,7-tetrahydroxyflavone) protects PC12 and T47D cells from beta-amyloid-induced toxicity, J. Neurosci. Res. 57 (1999) 399–404.
[20] J.P. Spencer, C. Rice-Evans, R.J. Williams, Modulation of pro-survival Akt/protein kinase B and ERK1/2 signaling cascades by quercetin and its in vivo metabolites underlie their action on neuronal viability, J. Biol. Chem. 278 (2003) 34783–34793.
[21] R.E. Tanzi, L. Bertram, Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective, Cell 120 (2005) 545–555.
[22] C.N. Wang, C.W. Chi, Y.L. Lin, C.F. Chen, Y.J. Shiao, The neuroprotective effects of phytoestrogens on amyloid beta protein-induced toxicity are mediated by abrogating the activation of caspase cascade in rat cortical neurons, J. Biol. Chem. 276 (2001) 5287–5295.
[23] L.L. Wu, X.B. Yang, Z.M. Huang, H.Z. Liu, G.X. Wu, *In vivo* and *in vitro* antiviral activity of hyperoside extracted from *Abelmoschus manihot* (L) Medik, Acta Pharmacol. Sin. 28 (2007) 404–409.
[24] H.Q. Xie, R.C. Choi, W.K. Leung, V.P. Chen, G.K. Chu, K.W. Tsim, Transcriptional regulation of proline-rich membrane anchor (PRiMA) of globular form acetylcholinesterase in neuron: an inductive effect of neuron differentiation, Brain Res. 1265 (2009) 13–23.
[25] F. Yang, G.P. Lim, A.N. Begum, O.J. Ubeda, M.R. Simmons, S.S. Ambegaokar, P.P. Chen, R. Kayed, C.G. Glabe, S.A. Frautschy, G.M. Cole, Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid *in vivo*, J. Biol. Chem. 280 (2005) 5892–5901.
[26] B.A. Yankner, Mechanisms of neuronal degeneration in Alzheimer's disease, Neuron 16 (1996) 921–932.
[27] M. Yao, T.V. Nguyen, C.J. Pike, Beta-amyloid-induced neuronal apoptosis involves c-Jun N-terminal kinase-dependent downregulation of Bcl-w, J. Neurosci. 25 (2005) 1149–1158.
[28] X.J. Zhang, L. Yang, Q. Zhao, J.P. Caen, H.Y. He, Q.H. Jin, L.H. Guo, M. Alemany, L.Y. Zhang, Y.F. Shi, Induction of acetylcholinesterase expression during apoptosis in various cell types, Cell. Death Differ. 9 (2002) 790–800.
[29] J.T. Zhu, R.C. Choi, G.K. Chu, A.W. Cheung, Q.T. Gao, J. Li, Z.Y. Jiang, T.T. Dong, K.W. Tsim, Flavonoids possess neuroprotective effects on cultured pheochromocytoma PC12 cells: a comparison of different flavonoids in activating estrogenic effect and in preventing β-amyloid-induced cell death, J. Agric. Food Chem. 55 (2007) 2438–2445.