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BRAIN RESEARCH

Research Report

Neuroprotective effects of emodin in rat cortical neurons against β-amyloid-induced neurotoxicity

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ABSTRACT

Accumulation of β-amyloid protein (Aβ) in the brain plays an important role in the pathogenesis of Alzheimer's disease (AD). In this study, the neuroprotective effect of emodin extracted from the traditional Chinese medicinal herb *Polygonum cuspidatum* Sieb. et Zucc against $A\beta_{25-35}$-induced cell death in cultured cortical neurons was investigated. We found that pre-treatment with emodin prevented the cultured cortical neurons from β-amyloid-induced toxicity. The preventive effect of emodin was blocked by pre-treatment with a phosphatidylinositol-3-kinase (PI3K) pathway inhibitor LY294002 or an estrogen receptor (ER) specific antagonist ICI182780, but not by pre-treatment with an extracellular signal-related kinases (ERK) inhibitor U0126. Furthermore, we found that emodin exposure induced the activation of the Akt serine/threonine kinase and increased the level of Bcl-2 expression. Moreover, the application of emodin for 24 h was able to induce the activation of $A\beta_{25-35}$-suppressed Akt and decrease the activation of the Jun-N-terminal kinases (JNK), but not of ERK. Interestingly, the up-regulation of Akt and Bcl-2 did not occur in the presence of LY294002 or ICI182780, suggesting that emodin-up-regulated Bcl-2 is mediated via the ER and PI3K/Akt pathway. Taken together, our results suggest that emodin is an effective neuroprotective drug and is a viable candidate for treating AD.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease characterized by the progressive neuron loss and dementia associated with deposits of β-amyloid in senile plaques and accumulation of abnormal tau filaments in neurofibrillary tangles (Selkoe, 1991; Mattson, 2004). Aggregates of β-amyloid protein have been a neurotoxic event that likely plays a critical role in the development and progress of AD brains (Grace et al., 2002). Evidence from in vivo and vitro experiments showed that Aβ peptides induced inflammatory response, oxidative stress, and neuronal apoptosis, resulting in neurodegeneration and cognitive dysfunctions (Behl et al., 1994; Robinson and Bishop, 2002). Several studies have shown that Aβ peptides are correlated with some intracellular

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Abbreviations: AD, Alzheimer's disease; Aβ, β-amyloid protein; PI3K, phosphatidylinositol-3 kinase; Akt, Akt serine-threonine kinase; ERK, extracellular signal-related kinases; JNK, Jun-N-terminal kinases; ER, estrogen receptor; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; PMSF, phenylmethanesulfonyl fluoride; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasolium bromide; LDH, lactate dehydrogenase

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signaling pathways including ERK, JNK and P38 mitogen-activated protein kinase (p38MAPK) (Morishima et al., 2001; Tamagno et al., 2003). However, the precise mechanism by which Aβ induces neuronal death remains unknown. Recent studies have proposed that the activation of PI3K pathway can prevent Aβ-induced neuronal death (Wei et al., 2002; Lee et al., 2008a). The PI3K/Akt signal transduction pathway is activated by several trophic factors in a variety of cell types (Wymann and Pirola, 1998), then phosphorylates the Akt serine/threonine kinase with principal roles related to the regulation of cell growth, proliferation, migration, glucose metabolism, transcription, protein synthesis, angiogenesis, and cell survival (Hill et al., 2001). It is well known that antiapoptotic protein Bcl-2 plays a central role in regulating programmed cell death (Desagher and Martinou, 2000). Recent reports showed that Akt suppressed apoptosis through the up-regulation of Bcl-2 (Numakawa et al., 2006; Ma, et al., 2009).

Currently, scientists are searching for a possible strategy to prevent AD. However, no valid therapeutic approaches have emerged. Now, much attention has been focused on the potential of using natural herbs as a neuroprotective agent. Some medicinal herbs were reported to protect neurons by stimulating the Akt survival pathway (Yu et al., 2004, 2007; Min, et al., 2006; Ho et al., 2007). Emodin (1,3,8-trihydroxy-6-methylanthraquinone) is an anthraquinone derivative from rhubarb (Fig. 1). It is regarded as a class of phytoestrogen due to a structure and function analogous to estrogen (Matsuda et al., 2001; Zhang et al., 2006). Studies have indicated that emodin inhibits the ras-dependent elevation in the level of tyrosine phosphorylated proteins (Chan et al., 1993) or activate the phosphoinositide 3-kinase signaling cascade (Lee et al., 2008b). Emodin is also proven to be an acetylcholinesterase inhibitor for an effective medicine curing AD (Chung et al., 1997) and prevents the rats from cycloheximide-induced amnesia (Lu et al., 2007). In the present study, we explore whether emodin can protect cultured neurons from β-amyloid-induced neurotoxicity and investigate what kind of mechanism is involved in the preventive effects of emodin.

2. Results

2.1. *Effects of Aβ and emodin on cell viability*

Cultured cortical neurons were treated with 0, 5, 10, 20, 30, 50 μM differently aggregated Aβ peptides for 24 h in serum-free neurobasal media containing B27 supplements at day 6. Then the cell viability was measured by colorimetric MTT assay. $A\beta_{1-42}$ and $A\beta_{25-35}$ caused up to 25–60% cell death at concentrations ranging from 10 to 50 μM, but $A\beta_{1-40}$ was not toxic at low concentrations. Only at 50 μM, $A\beta_{1-40}$ caused 30% cell death (Fig. 2A). To further confirm the cytotoxicity effect of Aβ, the LDH assay was performed (drug treatment was the same with MTT test). $A\beta_{1-42}$ and $A\beta_{25-35}$ caused the LDH release to increase to approximately 130–160% of control at concentrations ranging from 10 to 50 μM, but only at 50 μM did $A\beta_{1-40}$ cause a 130% LDH release of control (Fig. 2B).The above result showed that aggregated $A\beta_{25-35}$ induced similar toxic effects that were comparable to $A\beta_{1-42}$. Furthermore, $A\beta_{25-35}$ and full-length $A\beta_{1-42}$ have been found to induce neuronal apoptosis by similar mechanisms (Harkany et al., 2000). In this study, the survival rate of cortical neurons was about 53% when the cells were treated with 30 μM $A\beta_{25-35}$ for 24 h. Therefore, $A\beta_{25-35}$ (30 μM) was used in all subsequent experiments. To select a non-cytotoxic concentration of emodin in the study of the effect of emodin on $A\beta_{25-35}$ neurotoxicity, we evaluated the effect of various concentrations (1.25–80 μM) of emodin itself for 24 h on cell viability. The result showed that emodin exposure (80 μM) induced significant neuronal cell death compared with the control ($P<0.05$) (Fig. 2C and D).

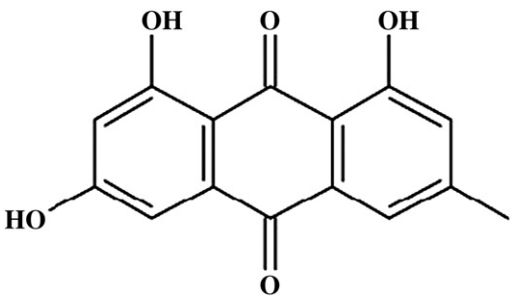

Fig. 1 – Chemical structure of emodin.

2.2. *Emodin protects neurons from $A\beta_{25-35}$ peptide-induced toxicity*

Cortical neurons were incubated with various concentrations of emodin (2.5–40 μM) for 24 h, and then treated by $A\beta_{25-35}$ for an additional 24 h. Viability of cortical neurons exposed to $A\beta_{25-35}$ was reduced to 46.1±3.1% compared to the control group. A pre-treatment of cortical neurons with emodin (5–40 μM) significantly reduced $A\beta_{25-35}$-induced cell death, with a maximal effect (92.8±9.3%) obtained at 20 μM (Fig. 3A). Next, we studied the action of emodin (at 20 μM) against $A\beta_{25-35}$-induced cell death at 1, 4, 8, 12, and 24 h (Fig. 3C). No survival effect was observed with 1 or 4 h emodin pre-treatment. The survival effect was significant 8 or 12 h after pre-treatment with emodin and reached a plateau after 24 h pre-treatment. The neuroprotective effect of emodin was also confirmed using the LDH assay. Exposure to $A\beta_{25-35}$ alone induced a significant increase in LDH release approximately 150% compared with control. Emodin significantly reduced LDH efflux. The maximal protection was observed at 20 μM (108.34% of control) (Fig. 3B) and pre-treatment with emodin for 8, 12 and 24 h significantly prevented the LDH release (75.68%, 77.73%, and 70.13%, respectively, reduction relative to $A\beta_{25-35}$-treated cells) (Fig. 3D).

Nuclear staining with Hoechst33258 was performed after various treatments. Control group (Fig. 4A) showed intact and relatively large nuclei whereas $A\beta_{25-35}$-treated neurons showed an increase in condensed nuclei (Fig. 4C). Pre-treatment of emodin significantly lowered the number of condensed nuclei induced by $A\beta_{25-35}$ (Fig. 4D). Emodin alone showed no effect (Fig. 4B). Apoptotic cortical cells were also detected by flow cytometry. The Annexin-V−/PI− population was regarded as normal healthy cells, while Annexin-V+/PI− cells were taken as a measure of early apoptosis and Annexin-V+/PI+ as necrosis/late apoptosis. The apoptotic rate was 48.72% in cortical cells cultured with 30 μM $A\beta_{25-35}$ for 24 h (Fig. 5C). When cells were pre-treated with 20 μM emodin for 24 h before being exposed to $A\beta_{25-35}$, the percentage of apoptotic cells was 19.52% (Fig. 5D).