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Soybean isoflavone alleviates β-amyloid 1-42 induced inflammatory response to improve learning and memory ability by down regulation of Toll-like receptor 4 expression and nuclear factor-κB activity in rats

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ABSTRACT

β-amyloid 1-42 (Aβ1-42)-induced learning and memory impairment in rats is believed to be associated with inflammation. Cytokine production is a key pathologic event in the progression of inflammatory processes.

In this rat study, soybean isoflavones (SIF) was used to investigate it's protective effects on inflammation caused by β-amyloid 1-42 (Aβ1-42), which is associated with learning and memory impairment in Alzheimer disease. We characterized the learning and memory ability. cytokine profiles of circulating interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) in the serum and the expression of Toll like receptor4 (TLR4) and nuclear factor-κB p65 (NF-κB p65) mRNA and protein in the brain tissue following intracerebroventricular administration of Aβ1-42 by miniosmotic pump for 14 days. The results showed that functional deficits of learning and memory in SIF treatment groups were significantly improved compared to the control group without SIF treatment in water maze test. The serum IL-1β and TNF-α level were significantly increased, and the expressions of TLR4 and NF-κB p65 mRNA and protein in the brain were up-regulated, indicating inflammation response was initiated following administration of Aβ1-42. After intragastric pre-treatment with SIF, inflammatory cytokines was significantly reduced and also SIF reversed the Aβ1-42 induced up-regulation of TLR4 and NF-κB p65 mRNA and protein expression in the brain and expression of NF-κB p65 in nuclei. These results suggested that SIF reduced the cytokine cascade and inflammatory response induced by Aβ1-42 which could result in the improvement of spatial learning and memory ability impairment in the rats.

1. Introduction

β-Amyloid peptides (Aβ), a peptide of 39–43 amino acids, was considered to induce inflammation and oxidative stress in the brain. These factors have been postulated to play very important roles in the pathogenesis of Alzheimer's disease (AD) with property of learning and memory ability impairment. Researchers have found that the senile plaques were infiltrated by reactive microglias and astrocytes in brain of (AD) patients (Schwab and McGeer, 2008), suggesting inflammation contributes to the pathogenesis of AD. Despite of two decades of work, many of the questions about the inflammatory response in AD remain unanswered. The recent in vitro study indicates that Aβ might function as a stimulus for glial cell activation (Kim et al., 2009). Overactivation of glial cell could result in neuro-inflammation related neurodegenerative disorders by producing reactive oxygen species and inflammatory mediators such as tumor necrosis factor (TNF). When such inflammation affects hippocampus function even without apparent neuron death, learning and memory impairment could occur (McGeer et al., 2006; Tanaka et al., 2006).

Toll-like receptors (TLRs) are evolutionary conserved, type I integral membrane glycoprotein, mainly expressed in glial cells (microglia, astrocytes and oligodendrocytes) (Walter et al., 2007) and neurons (Kigerl et al., 2007) in brain. As cell surface receptors, TLR2 and TLR4 can recognize danger-associated host-derived molecules, such as lipid, carbohydrate, peptide and nucleic-acid structures expressed in microorganisms (Trinchieri and Sher, 2007), and released heat-shock protein (Miyake, 2007), as well as Aβ and oligomers. Research has proved that CD14 and Toll-like receptors 2 and 4 were required for fibrillar Aβ-stimulated microglial activation (Reed-Geaghan et al., 2009). When TLR2 and TLR4 receive signals from stimulus factors, several protein kinase cas-

Abbreviations: Bw, body weight; ELISA, enzyme linked immunosorbent assay; D, day; RT-PCR, reverse transcription-polymerase chain reaction; IL-1, interleukin-1; TLR4, Toll like receptor4; TNF, tumor necrosis factor; NF-κB, nuclear factor-κB; RNA, ribonucleic acid.

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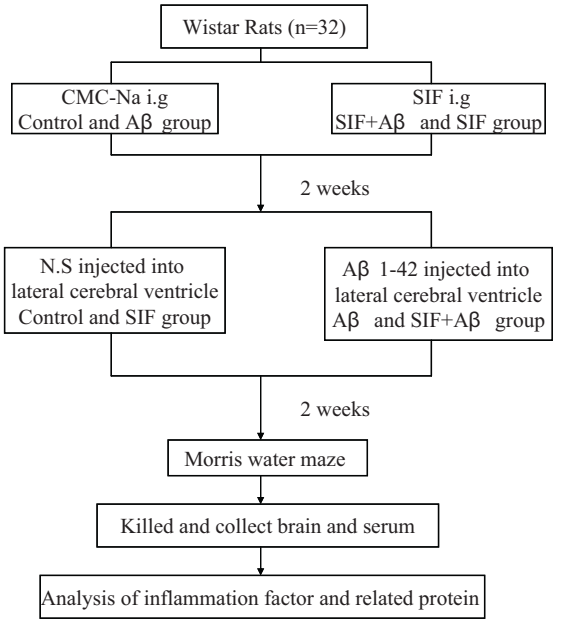

Fig. 1. Experimental schedule and groups.

cades will be activated, which subsequently modulate the gene expression through specific adaptor proteins (Barton, 2007; Lee and Kim, 2007). One of these important protein kinase cascades activated by TLRs is nuclear factor-κB (NF-κB), a transcription factor ubiquitously expressed in human tissue including brain (Doyle and O'Neill, 2006; Hashimoto et al., 2009; Snihur et al., 2008). Once NF-κB is activated in cytoplasm, it will shift to nuclei and binding with DNA consensus sequence to induce inflammation-related transcription process (Walter et al., 2007).

Soybean isoflavone (SIF), a kind of soy phytochemicals, has many beneficial effects such as antiosteoporosis effect (Taku et al., 2010), anti-inflammatory (Velasquez and Bhathena, 2001), antiproliferative (Barnes et al., 1996), and estrogenic effects antioxidant effect, and lowing total cholesterol in plasma (Taku et al., 2007). Hsu suggested that SIF may inhibit hormone-induced proinflammatory NF-κB signals associated with prostate cancer development (Hsu et al., 2010). However, the bioactivity and mechanism of SIF in the mammal central nervous system are still in the darkness. Our previous study showed that SIF could improve the learning and memory ability of rats damaged by Aβ treatment characterized by decrease in the number of Aβ-positive neurons and increase in antioxidant activity (Ma et al., 2009). Isoflavone modulates the inflammatory response by inhibiting the production of NO and PGE2 in activated macrophages (Blay et al., 2010). It is hypothesized that SIF may have an impact on inflammation induced by Aβ. In this article, we characterized the learning and memory ability, cytokine profiles of circulating interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) in the serum and the expression of Toll like receptor4 (TLR4) and nuclear factor-κB p65 (NF-κB p65) mRNA and protein in the brain tissue and tried to understand the potential mechanisms of neuroprotective effects of SIF

2. Materials and methods

2.1. Experimental animals

Thirty two adult male Wistar rats (SPF class, weighing 250–300 g) were provided by the Chinese Academy of Sciences. The animal experiments were conducted to follow the guidelines established by the Chinese Committee on Experimental Animal Supervision. All rats were randomly divided into four groups (control, Aβ1-42, SIF + Aβ1-42 and SIF group) according to body weight. The experimental schedule is shown in Fig. 1. The rats in SIF + Aβ and SIF group were treated by intra gastric of SIF (80 mg/kg body weight per day) daily for 14 days before Aβ injection, while the rats in control group and Aβ group were treated by intra gastric of 0.5% CMC-Na (sodium carboxyl methyl cellulose).

2.2. Preparation of SIF, Aβ and surgery

SIF was purchase from North China Pharmaceutical Company (Shi Jiazhuang, China). Total isoflavones content of SIF in study was 91.96%, and its chemical composition mainly included genistin13.82%, daidzin55.49% and glycitin18.63%. SIF was dissolved in 0.5% CMC-Na by 50 mg/ml. Synthetic Aβ42 (1-42) was dissolved in phosphate-buffered saline (PBS) at 0.1 mg/ml and incubated at 37 °C for 3 days to form Aβ1-42 aggregation (Durairajan et al., 2008). Surgery was performed according to the publication by Hashimoto (Hashimoto et al., 2009) and our before study (Ma et al., 2009). Briefly, the skull of the anaesthetized rats were opened and drilled with one holes (anteroposterior 1.2 mm from Bregma, mediolateral 2.0 mm, dorsoventral 4.0 mm) using a stereotaxic frame (Narishige, Tokyo, Japan). A miniosmotic pump (Alzet 2002; Durect Co., Cupertino, CA, USA) containing either Aβ1-42 solution (20 μg/200 μl) or vehicle alone was quickly implanted into the neck of the rats. The outlet of the pump was inserted 3.5 mm into the lateral cerebral ventricle and fixed at screws with dental cement. Aβ1-42 solution was spontaneously infused to lateral cerebral ventricle by miniosmotic pump for 2 weeks.

2.3. Morris water maze-learning and memory ability

Two weeks later, the learning and memory ability of rats was evaluated by the Morris water maze (Morris, 1984; Gong et al., 2005). Briefly, these animals were released from four randomly assigned start positions respectively. Each rat was trained for four consecutive days to find the hidden platform. The full acquisition time course was recorded. The final readout included the escape latency, the distance to arrive the hidden platform, and the frequency of the rat spanning the place where the platform laid.

2.4. Measurement of cytokines in serum by ELISA

Rat blood was collected from arteria cruralis of rats. Briefly, the rat was fixed, and localized anesthesia. Skin in inguen was cut and the arteria cruralis was exposed by blunt dissection. Injector was used to take blood. Blood was centrifuged at 1500 rpm for 5 min. Upper light yellow liquid was serum. Serum levels of circulating IL-1 and TNF-α were measured in duplicate by immunoassay using a commercially available enzyme-like immunosorbent assay (ELISA) kit (Groundwork Biotechnology Diagnosticate Ltd., USA). The procedure was strictly according to the manufacturers' instructions. The resulting color reaction was measured at 450 nm with an ELISA reader (Infinite M200, TECAN, Switzerland). Values were expressed as ng/g protein.

2.5. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was purified by using Trizol (Invitrogen, Carlsbad, CA, USA) and reverse transcription was performed by A reverse transcriptase kit purchased from Applied Promega, Madison, WI, USA. Briefly, double-stranded DNA was synthesized from 2 μg of total RNA, and the cDNA obtained was used as a template for PCR. The mRNA expression of TLR4, p65 subunit of NF-κB, and β-actin in brain was measured. The forward and reverse primer sequence for TLR4 was: 5′- GAATGAGGACTGGGTGAGAAA-3′ and 5′- TCTGCTAAGAAGG CGATACAA-3′, respectively; for p65 subunit of NF-κB, forward and reverse prime sequence was 5′- CACAGATACCACTAAGACGCAC C-3′ and 5′- GACCGCATTC AAGTCATA GTCC-3′ respectively; for β-actin, forward and reverse prime sequence was 5′- TGGAATCCTGTGGCATCCATGAAAC-3′ and 5′- TAAAACGCAGCTCAGTA ACAGTCCG-3′ respectively. The annealing temperature for TLR4, p65 subunit of NF-κB, and β-actin was 53 °C, 57 °C and 58 °C, respectively. After 35 cycles, amplification products were electrophoresed on a 2.0% agarose gel. Then FluorChem FC2 software (Alpha Innotech, America) was used to photo and analyze results.

2.6. Western blot analysis

Brain tissues (about 0.05 g) were harvested and washed with phosphate buffered saline, and grounded in lysis RIPA buffer containing 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris–HCl (pH 7.4), 1 mM DTT, and protease inhibitors. The homogenate was kept at 4 °C for 40 min, and then centrifuged at 15,000 rpm for 15 min. Supernatant was separated and collected for protein analysis. Nucleoprotein was extracted by kit (Novagen, Germany). The protein concentration was determined by using the BCA (bicinchoninic acid) protein assay kit (Pierce Biotechnology, USA). 50 μg of protein was loaded and separated by a 10% or 12% SDS-acrylamide gel electrophoresis and transferred to polyvinylidene fluoride blots at the voltage of 60 V for 2 h. The transferred membrane was blocked by fresh blocking buffer (Tris-buffered saline, containing 5% nonfat dry milk) at room temperature for 1 h. The different primary antibodies for anti-TLR4, and anti-p65 were added to the membrane and incubated for 12 h at 4 °C. After completing the primary antibody reaction and washed with appropriate buffer, the secondary antibodies were added and incubated for 1 h (1:5000 in a 1% non-fat milk solution). The membrane was finally washed with TBST. ProtoBlot®II kit (Promege Corporation, USA) was used