Abstract

Lipopolysaccharide (LPS)-induced neuroinflammation is associated with the pathogenesis of Alzheimer’s disease (AD), which is a key factor underlying the generation of amyloid beta. Requirements for therapies of neurodegenerative diseases such as AD are not only consistent efficacy for long periods, but also the elimination of potential risks for hepatotoxicity. Thus, this study aimed to evaluate the effect of Frunus mume water extract (FME) on LPS-induced hippocampal damage associated with inflammatory signals. ICR male mice (n=30) were divided into three groups. The normal group remained untreated, LPS group (positive control) was stimulated to express LPS-induced hippocampus damage, and FME group (treatment) was stimulated to express LPS-induced hippocampal damage and administered FME at 40 mg/kg/day. Mouse brain tissues were tested by hematoxylin and eosin staining and immunohistochemistry following inflammation markers such as tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2). FME administered at 40 mg/kg/day for seven days in saline solution significantly reduced brain inflammation in the hippocampi of LPS-stimulated mice. Moreover, FME regulated the expression of inflammatory proteins such as TNF-α, iNOS and COX-2. Our data exhibits that FME attenuates neuroinflammation and damage in brains of LPS-stimulated mice. These findings suggest that FME is a potential therapeutic agent in treating neuroinflammation and recommend further testing on the matter.

[Keywords] Frunus Mume, Crude Extract, Migroglia, Neuroinflammation, Anti-Inflammation

1. Introduction

Until now, the major cause of degenerative brain diseases such as Parkinson’s disease, Huntington’s disease, and Alzheimer’s disease (AD), have been reported to be potentially incurable reasons such as the death of neuronal cells, synaptic failures, and abnormal neuronal transmission [1]. More specifically, AD is characterized by the accumulation of β-amyloid (Aβ) peptides in the brain, which play an important role in the pathogenic changes of the central nervous system (CNS) during Aβ-induced neurotoxicity [2].

Microglia in the CNS exists to prevent the brain from damage against infection and inflammation. Despite the essential processes microglial responses play for host defense, the excess production of its intermediated signal pathway by interleukin (IL)-1β and tumor necrosis factor-α (TNF-α),—known as pro-inflammatory cytokines—can lead to the development of the chronic neurodegenerative diseases [3]. This lipopolysaccharide stimulation as a pathogenic infection leads to hyper-stimulated microglia [4]. Aβ accumulates through continuous or immoderate inflammatory responses of microglia. Therefore,
neuro-inflammation is one of the major contributors to the initial pathogenesis of AD[5][6].

Owing to this, the development of drugs for AD has been difficult because the initial neurodegeneration-related etiology is unknown. Many phytochemicals from herbs and fruits have been studied as potential medication for neuropathological disorders and have been considered traditional medicine[7]. Some studies have reported abundant phytochemical interventions, which have a minimal amount of side effects during long-term treatment[8]. Therefore, the phytochemistry studies should provide various opportunities for the development of drugs against neurodegenerative disease.

Fructus mume is a dry material from the unripe fruit of Prunus mume, which is traditionally used to treat digestive problems. Recently, Fructus mume has been reported to have an effect on the underlying mechanisms regarding inflammation of Lipopolysaccharide(LPS)-stimulated macrophages, the colitis model, and neurodegenerative models[9][10][11]. However, the effect of Fructus mume on the pathogenesis of neurodegeneration through the LPS-stimulated AD model has still not been investigated. Thus, in the present study, we explored the biological activity of Fructus mume in LPS-induced neuroinflammation in mouse brains.

2. Methods

2.1. Chemicals

Vectastain ABC Kits were purchased from Vector Laboratories(Burlingame, CA, USA). The following chemicals and antibodies were also used in this study: anti-TNF-α, anti-inducible nitric oxide synthase(iNOS), and anti-cyclooxygenase-2(COX-2) antibodies were purchased from Santa Cruz(Dallas, TX, USA), and LPS and all other reagents were purchased from Sigma(Saint Louis, MO, USA).

2.2. Design of experiments

We performed the histopathological assay to determine the neuroprotective effect of FME.

2.3. Procedure

2.3.1. Preparation of aqueous fructus mume extracts(FME)

One-hundred grams of Fructus mume were blended, and the crude powder was precipitated with 1000 mL of sterile deionized water at 100°C for 3 h. The aqueous extracts were concentrated by evaporation at 60°C in vacuum. The extract was dissolved in 50 mL of sterile deionized water. The aqueous extract was lyophilized at -60°C.

2.3.2. Animal care and in vivo test

Six-week-old male ICR mice were obtained from Orient Bio. Inc., Seoul, Korea. Prior to the experiments, all animals were allowed to acclimate to their new environment for one week at room temperature(24 ± 2°C; humidity, 50 ± 15%; 12:12-h light/dark cycle). All experiments and animal care were conducted in conformity with the institutional guidelines of the Konkuk University. The mice were housed individually in ventilated cages with ad libitum access to water and food under specific pathogen-free conditions. To test the anti-neuroinflammatory effect of FME, the animals were randomly divided into three different groups(n = 10 mice/group): normal (normal mice, untreated), LPS-treated(Positive control), and FME treated groups. FME (40 mg/kg/day in normal saline) was administered orally every day for 7 days at a regular schedule. Brain damage was induced by LPS (diluted in normal saline) for 7 days. LPS was administered by a single intraperitoneal injection at a dose of 250 μg/kg, 1 h after the last FME treatment.

2.3.3. Tissue preparation and histochemical staining

Histochemical staining was performed as previously reported[12]. Briefly, the mice were anesthetized with sodium pentobarbital solution and killed 24 h after acetaminophen injection. Their livers were dissected out and fixed in 10% formalin for 24 h. The livers were embedded in paraffin and 6-mm-thick tissue sections were stained with hematoxylin-eosin.
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2.3.4. Immunohistochemical staining

Immunohistochemical staining was performed as previously reported[12]. Some of the prepared sections were used for immunostaining assays. Samples were treated with 3% H2O2 for 5 min to inactivate endogenous peroxidase, then blocked with 10% normal serum for 1 h at 25°C and incubated overnight at 4°C with primary antibodies against TNF-α, COX-2, and iNOS. The following day, the sections were washed and incubated with the corresponding secondary antibodies for 1 h at room temperature. The Vectastain ABC Kit was used to carry out the avidin-biotin complex interaction, in accordance with the manufacturer’s instructions. Signal development was carried out in a substrate solution of 0.05% DAB, and the slides were counterstained with hematoxylin. The sample sections were examined using a light microscope (Olympus BX50, Japan) at 200× magnification and proteins were visualized and analyzed using the Image J Software.

2.3.5. Statistical analysis

The results are expressed as the mean ± standard error(SE) of at least three independent experiments(n ≥ 3). The data of the differences were determined by using the t-test between the experimental two groups and one-way analysis of variance(ANOVA). Tukey’s test was used for multiple comparisons(GraphPad Prism ver. 4.00 for Windows; San Diego, CA), and P-values < 0.05 were considered statistically significant.

3. Results

3.1. The protective effect of FME on LPS-induced brain damage in mice

First, to examine the protective effect of FME against the LPS-induced brain damage, we histological analysis using H&E staining was performed. As shown in <Figure 1>A, tissue samples treated with LPS(i.p.; 250 μg/kg) exhibited tissue atrophy with a dark violet color of the Cornu Ammonis area-1(CA1) and the dentate gyrus(DG) regions in the hippocampus. In contrast, the FME(oral administration; 40 mg/kg) group showed a morphology similar to a normal hippocampus when compared to the untreated(normal) group. Next, a histological analysis using cresyl violet staining was performed. As shown in <Figure 1>B, FME significantly reduced the aggregate cell morphology on the CA1 and DG regions in the hippocampus of LPS-induced brain damage.

Figure 1. The effect of FME in LPS-induced brain injury in mice The untreated group of animals was provided water only(Normal), one group of animals was treated with LPS(i.p. 250 μg/kg)(Positive control), the other group was treated with LPS and 40 mg/kg of FME for 7 days(Treatment). The photomicrographs at a magnification of ×400. These brain sections are stained with H&E(A) and cresyl violet(B). Upper panels indicate the Cornu Ammonis area-1(CA1) region and bottom panels indicate the dentate gyrus(DG) regions region in mice brain.

3.2. FME reduced the expression of TNF-α, iNOS and COX-2 in LPS-stimulated brain tissue in mice

Expression of inflammation-related signaling molecules such as TNF-α, iNOS and COX-2 in mice was studied as well. As shown in <Figure 2>, the expression levels of TNF-α, iNOS and COX-2 significantly increased in the injured tissue (positive control panels). An expression level of TNF-α, iNOS and COX-2 to
100% in the positive control group was observed. FME group decreased the each expression of 66% ± 4.98% of TNF-α, 56.4% ± 3.96% of iNOS, and 50.41% ± 1.36% of COX-2, respectively when compared with positive control group.

**Figure 2.** Expression of iNOS, COX2, and TNF-α in the Cornu Ammonis area-1(CA1) region of LPS-induced brain damage Immunohistochemical staining of iNOS, COX2, and TNF-α in liver tissue performed with cross-sections obtained from three groups. (A)The samples are photomicrographed at a magnification ×400 (B)The expression levels of iNOS, COX-2, and TNF-α. Expression changes are relative to the LPS alone-treated group.

4. Discussion and Conclusion

Despite the noble efforts of the past decade, the medicine to cure or control diseases progression against the neurodegenerative disorders underlying AD has not progressed. It is a disease classified as “incurable,” its cause having not been fully elucidated. The inability to control AD may be due to the majority of medications being dependent only on the cholinergic hypothesis. For example, the reversible acetylcholinesterase inhibitor-dependent prescription makes up the majority of AD therapies[1].

However, the prescriptions of neurodegeneration treatment are required to be effective and safe for long periods. Western medicines are known to have a potentially undislosed side effect. The results of the former study suggest that the natural product of Fructus mume should be one of the candidates for AD prescription in the foundation of alternative medicine, reinforced by Kim et al.[13], who found the effectiveness of Fructus mume on a chronic cerebral hypoperfusion model. Moreover, Lee et al.[11]. showed the effectiveness of Fructus mume on the chronic cerebral hypoperfusion-induced white matter and hippocampal damage. The regulatory effect of FME has not yet been evaluated in Aβ accumulated model.

The former study clearly demonstrates the anti-neuroinflammatory effect of FME in vivo. Particularly, Lee et al. reported that 250 mg kg of LPS induced brain damage by accumulating Aβ[4]. The study also showed that FME has an inhibitory effect on brain injury and neuroinflammation in an LPS-stimulated AD mouse model. FME can significantly regulate the TNF-α-related inflammation, and the administration of FME significantly diminished the LPS-induced the overexpressed COX-2 and iNOS. The results of the study suggest that FME may have regulated the accumulation of Aβ through anti-neuroinflammation. Hence, it can be reasonably suggested that Fructus mume should be one of the candidates of AD prescription.

5. References

5.1. Journal articles


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