ABSTRACT

Title Neuroprotective effects of cultivar extracts derived from *Chrysanthemum Morifolium* flowers on the cerebral nerve cells

Doctor Course in Mechanical and Biochemical Engineering Doctor of Engineering

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［Abstract］（1,000words, 10points, single space）

Chrysanthemum flowers have been used for the treatment of inflammation, fever alleviation and detoxification. It is used for food which displayed a healthy function for disease prevention and health promotion actively. Phytochemicals in vegetables and fruits have been reported to exhibit various significant pharmacological effect. Systematic reviews to show clear causal relationships between the active ingredient constitution and the pharmacological effect for variety of fresh food are critical. Meanwhile, psychiatric disorders have rapidly increased and become a major social problem, but many of pathogenic mechanisms are unknown. The degeneration and injury of neurons due to oxidative stress and chronic inflammation, and they are thought to be an essential common cause of neurological disorders from dementia to depression. The protection from oxidative stress, restoration of neural plasticity, control of neuroinflammatory response of microglial cells are being studied as new therapeutic targets. In this study, chemical profile analysis (chapter 3), neurotrophic-like effects (chapter 4), neuroprotective effects (chapter 5) and anti-inflammatory effects (chapter 6) of two typical *Chrysanthemum* cultivar flowers were compared to examine the correlation between chemical profile and bioactivity.

We investigated the phenolic compounds contents of the extracts from Aboukyu and Enmeiraku flower, which are well known *Chrysanthemum spp.* cultivars in Japan (chapter 3). About 80% of soluble extracts ingredient was in methanol fraction. The level of hexane extract of Aboukyu was higher than those of Enmeiraku (5.53 % vs. 1.78 %). Aboukyu contained more phenolic compounds compared to Enmeiraku. Contents of luteolin and apigenin were assayed by quantitative HPLC. Enmeiraku contained more luteolin and less apigenin compared to Aboukyu. Two cultivar petal extracts were subjected to HPLC-DAD to characterize the phenolic components. The compounds corresponding to twelve specific peaks are common components of Aboukyu and Enmeiraku flowers, and six peaks were estimated as luteolin, apigenin, and their glycosides. The phenolic compounds differed greatly between two cultivars in terms of the content and composition of luteolin, apigenin and their glycosides. GC-MS analysis also showed that the composition of triterpenoids also differed between two cultivars.

Neurotrophic factors such as BDNF play an important role in regulating differentiation, survival and functional maintenance of neurons, and the microtubule-dependent extension. Reorganization of lost neuronal networks in the injured brain is necessary for restoration of normal physiological functions. Because of these properties, neurogenesis substances are anticipated as promising therapeutic tools for neuronal injuries. In chapter 4, we evaluated the neurogenesis capacity of the petal extracts of two cultivars. Individually, Aboukyu and Enmeiraku extracts only slightly induced neurite outgrowth (Aboukyu; 25.3 ± 2.5%, Enmeiraku; 26.3 ± 4.0%). However, in combination with NGF, the extracts acted synergistically to induce full neurite outgrowth in PC12 cells (Aboukyu; 69.0 ± 6.2%). These result suggests the role of chrysanthemum extract in enhancing the neurite outgrowth activity of NGF. The MAPK family include ERK and p38MAPK, is known to be involved in various cellular events such as survival/death, differentiation and migration. To evaluate the signal transmission pathway for

neurite outgrowth during chrysanthemum extract stimulation, we compared the effects of two cultivar extracts on phosphorylated ERK and p38MAPK levels in PC12 cells by immunoblot analysis. The ratio of pERK was higher in the cells treated with Aboukyu extract compared to Enmeiraku. However, there were no significant differences in neurite outgrowth activity between cells treated with two extracts. Phosphorylation of ERK, as an upstream signal, is necessary for neurite outgrowth, however, there was no correlation between strength of ERK activity and the length of neurite outgrowth.

Nerve cells are extremely sensitive to oxidative stress. The injury of glial cells and neurons due to toxicity of free radicals, leading to disorders. The polyphenolics have positive effects on the treatment and prevention of a wide range of oxidative stress injuries. In chapter 5, we compared the bioactive characteristics of two cultivar extracts by analyzing the composition of phenolic components and assessing the neuroprotective. Extracts of Enmeiraku protected neuroblastoma SH-SY5Y cell viability against oxidative stress-induced injury in a concentration-dependent manner. By contrast, the neuroprotective effect of Aboukyu extracts was biphasic and was weakened at concentrations > 25 μg/mL. The differences in the neuroprotective effects of Aboukyu and Enmeiraku extracts might be attributable to the anti-apoptotic capacity of their phenolic compounds as well as compositional differences. The levels of flavonoids such as apigenin that easily induce apoptosis were higher in Aboukyu extract than Enmeiraku extract. Thus, the characteristics of the neuroprotective capacity of chrysanthemum cultivars differed markedly according to the flavonoid composition. The Nrf2-ARE system, a body's antioxidant system, induces expression of antioxidant enzymes in response to reactive oxygen species. Certain phenolic compounds activate the Nrf2-ARE pathway, and activate these defense mechanisms in advance and put the cells in an alert state. Treatment of neuronal cells with chrysanthemum flower extract prior to oxidative stress alleviate the oxidative damage to neuronal cells. In addition, the expression levels of Nrf2 and the antioxidant enzyme HO-1 were increased in neuronal cells treated with chrysanthemum flower extract. These effects were higher with extract of Enmeiraku characterized by a high content of luteolin. These data suggest that chrysanthemum flower extract may enhance the ability of body's defense mechanisms through activation of Nrf2-ARE system, and may help reduce oxidative stress to neurons.

Although pathogenic mechanisms remain unknown for the majority of neurodegenerative diseases, activation of microglia and persistence of chronic inflammation are generally thought to be common triggers of the pathogenesis of neurodegenerative diseases. In chapter 6, the suppressing effect of chrysanthemum flower extract on neuroinflammation was examined. Extracts of solvent fraction from chrysanthemum flowers were assayed for inhibition of NO production by LPS-stimulated macrophages, and the hexane extract fraction showed the most potent inhibitory effect. Triterpenoids and fatty acid esters thereof in the hexane extract fraction were suggested to have a strong inhibitory activity. The effects of the Enmeiraku hexane extract demonstrated on glial cells, which play a central role in immune regulation in the brain, were markedly decreased production levels of NO and TNF-α. These compounds are known to cross the blood-brain barrier and reach the inside of brain, and suggested to suppress glial cell activation and exert the anti-inflammatory effect.

Although chrysanthemum flowers have been shown to have excellent pharmacological effects such as anti-inflammation and detoxification, the present study showed that chrysanthemum flower extract exerted the protective effect against oxidative damage and the anti-inflammatory effect in neurons and glial cells. This study also showed that its action characteristics varied greatly depending on cultivar, and it was shown that it depends on the species and composition ratio of phytochemical depending on the cultivar.

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