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Review Article

Compounds for dementia from *Hericium erinaceum*

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Abstract

Our group has been conducting a search for compounds for dementia derived from medicinal mushrooms since 1991. A series of benzyl alcohol derivatives (named hericenones C to H), as well as a series of diterpenoid derivatives (named erinacines A to I) were isolated from the mushroom *Hericium erinaceum*. These compounds significantly induced the synthesis of nerve growth factor (NGF) *in vitro* and *in vivo*. In a recent study, dilinoleoyl-phosphatidylethanolamine (DLPE) was isolated from the mushroom and was found to protect against neuronal cell death caused by β-amyloid peptide (Aβ) toxicity, endoplasmic reticulum (ER) stress and oxidative stress. Furthermore, the results of preliminary clinical trials showed that the mushroom was effective in patients with dementia in improving the Functional Independence Measure (FIM) score or retarding disease progression.

Introduction

Hericium erinaceum is an edible and medicinal mushroom, known in Japan as 'Yamabushitake', in China as 'Hou Tou Gu' and in Europe and the United States as 'Lion's Mane'. It has been demonstrated that *H. erinaceum* exerts important bioactivities, including: 1) the induction of nerve growth factor (NGF) synthesis (1-7); 2) inhibition of the cytotoxicity of β-amyloid peptide (Aβ) and protection against neuronal cell death caused by oxidative or endoplasmic reticulum (ER) stress (8-10); 3) antitumor activity (11); 4) anti-HIV activity (12); 5) immune enhancement (13-15); 6) hemagglutinating activity (16, 17); 7) cytotoxicity against cancer cells (18-20); 8) antimicrobial activity (21-23); 9) hypoglycemic effects (24); and 10) hypolipidemic effects (25).

Alzheimer's disease (AD) is the most common form of dementia, causing memory loss, language deterioration, impaired ability to manipulate visual information mentally, poor judgement, confusion, restlessness and mood swings due to progressive neurodegeneration. It eventually leads to the loss of cognition, personality and function. It has been reported that the susceptibility to AD is closely related to a number of factors, including age, genes, lack of NGF and excessive accumulation of Aβ. Conventional treatments for AD only address the symptoms, but there is presently no cure. For this reason, hericenones, erinacines and dilinoleoyl-phosphatidylethanolamine (DLPE), isolated from *H. erinaceum* and showing significant activities in inducing the synthesis of NGF or protecting against neuronal cell death caused by Aβ, ER stress or oxidative stress, are attracting great attention and may be developed into medicinal products or dietary supplements used for preventing and improving dementia in general and AD in particular. In this review, we discuss the isolation and bioactivities of these compounds, and the possible clinical application of the fungus.

NGF and AD

NGF, one of a family of neurotrophins that induce the survival and proliferation of neurons, plays an important role in the repair, regeneration and protection of neurons. It has been suggested that NGF may be used to treat AD (26). One report described how a woman with AD experienced improvement in symptoms (including impaired mental ability) after the administration of NGF directly to the brain using a catheter (27). However, since NGF is a protein that cannot pass through the blood-brain barrier (BBB) and needs to be injected directly into the brain to be effective, it is a high-risk treatment. A safer therapy for this disease would be a compound that could be administered orally, pass through the BBB and so induce NGF

synthesis inside the brain. Even if this compound could not pass through the BBB, it might still be beneficial for disorders of the peripheral nervous system, since NGF has a similar effect on neurons in that system.

Based on this concept, a search for natural inducers of NGF synthesis has been conducted worldwide, and several compounds with a lower molecular weight were found to have such bioactivity. Among those bioactive compounds, hericenones and erinacines from *H. erinaceum* were the first natural compounds and were found to possess remarkable activities.

Hericenones

The fruiting bodies of *H. erinaceum* were extracted with acetone at room temperature. The acetone extract was concentrated under reduced pressure, and then fractionated by solvent partition between chloroform and water. The chloroform-soluble layer was further fractionated and purified by various chromatographies, and six compounds, named hericenones C to H, were obtained (1, 2). As shown in Figure 1, hericenones C to H (**1**-**6**) are benzyl alcohol derivatives having simple fatty acids.

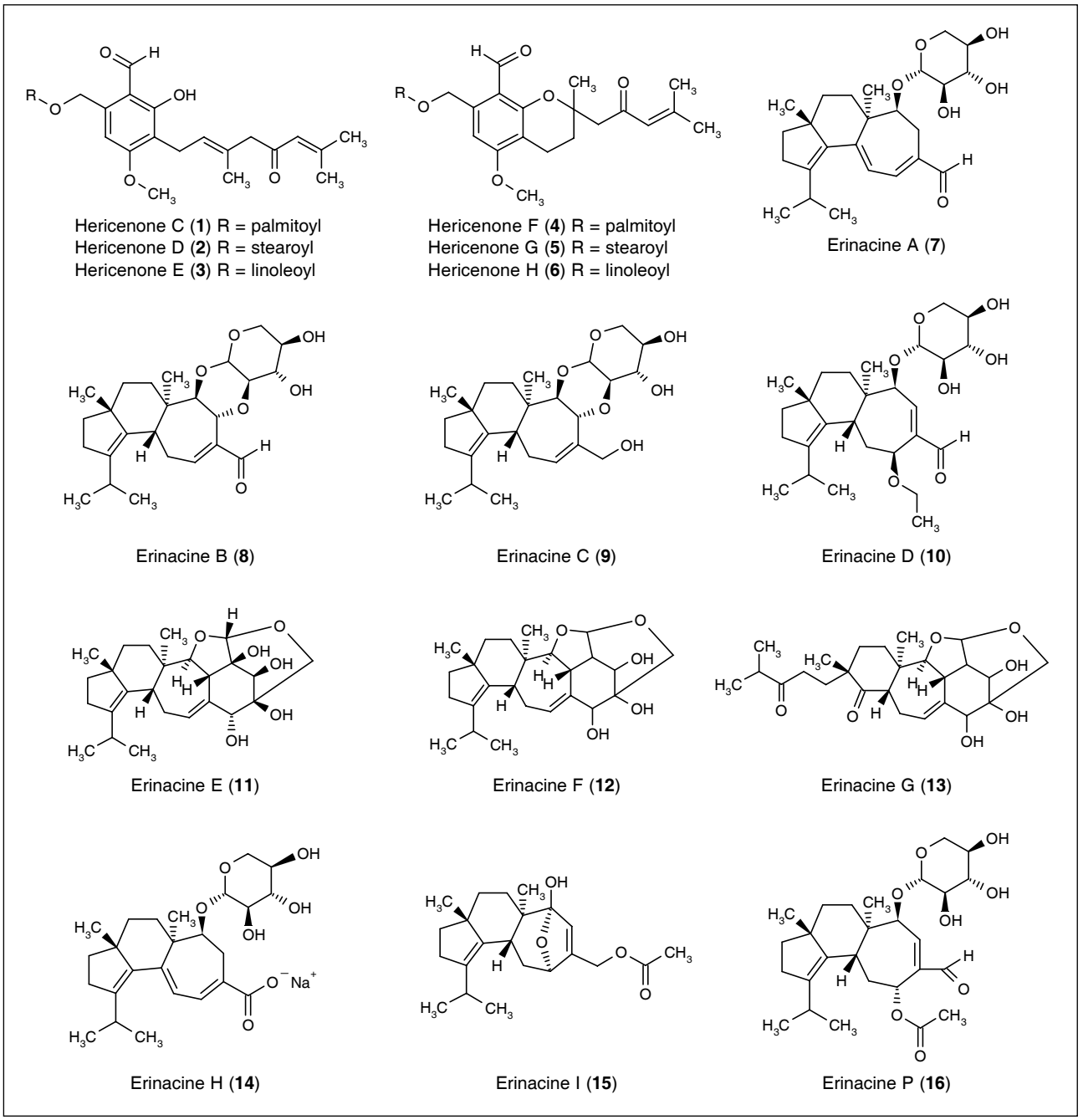

Fig. 1. Hericenones and erinacines, inducers of NGF synthesis isolated from *Hericium erinaceum.*

Among these compounds, hericenones F to H (**4**-**6**) are probably formed by cyclization between the phenolic hydroxyl and the side-chain of hericenones C to E (**1**-**3**), and exist in racemic forms.

Erinacines

The mycelia of *H. erinaceum* were cultivated in a tank for 4 weeks, and the mycelia and the culture media were separated by centrifugation. The mycelia obtained were extracted with 85% ethanol at room temperature. The ethanol extract was concentrated under reduced pressure, and then fractionated by solvent partition between ethyl acetate and water. The ethyl acetate-soluble layer was further fractionated and purified with various chromatographies, and nine compounds, named erinacines A to I (**7**-**15**), were obtained (Fig. 1) (3-6). Erinacines A to I are diterpenoid derivatives with different chemical structures from those of the hericenones. In addition, it has been reported that another compound, named erinacine P (**16**), was isolated from the mycelia of *H. erinaceum* (28). Erinacine P is considered to be a precursor of erinacines A (**7**) and B (**8**), and can be successfully converted to these compounds by a biomimetic reaction (Fig. 2).

Bioactivities of hericenones and erinacines

Within the brain, the neuron and astroglia are responsible for NGF production. It has been reported that the neuron controls NGF synthesis in order to maintain function in the mature brain, while the astroglia play the same role when the brain is growing or damaged. Therefore, primary astroglia derived from rat cerebral cortex were used in screening for bioactive compounds that induce the synthesis of NGF. The above compounds were added to these cells, maintained in a 96-well microplate, at various concentrations for 24 h, after which NGF secreted into the culture media was measured by an enzyme immunoassay (29-32). Hericenones C to E (**1**-**3**) induced the synthesis of NGF *in vitro*. In the presence of 33 μg/ml of hericenones D (**2**), E (**3**) and C (**1**), the mouse astroglial cells secreted 23.5 ± 1.0, 13.9 ± 2.1 and 10.8 ± 0.8 pg/ml of NGF, respectively. Hericenone D was as effective as epinephrine (a potent inducer used as a positive control) (1). It is interesting to note that the difference in activity among these compounds was dependent on the chain length and the double bond of the fatty acid part. As shown in Figure 3, erinacines A to G (**7**-**13**) were more potent inducers of NGF synthesis

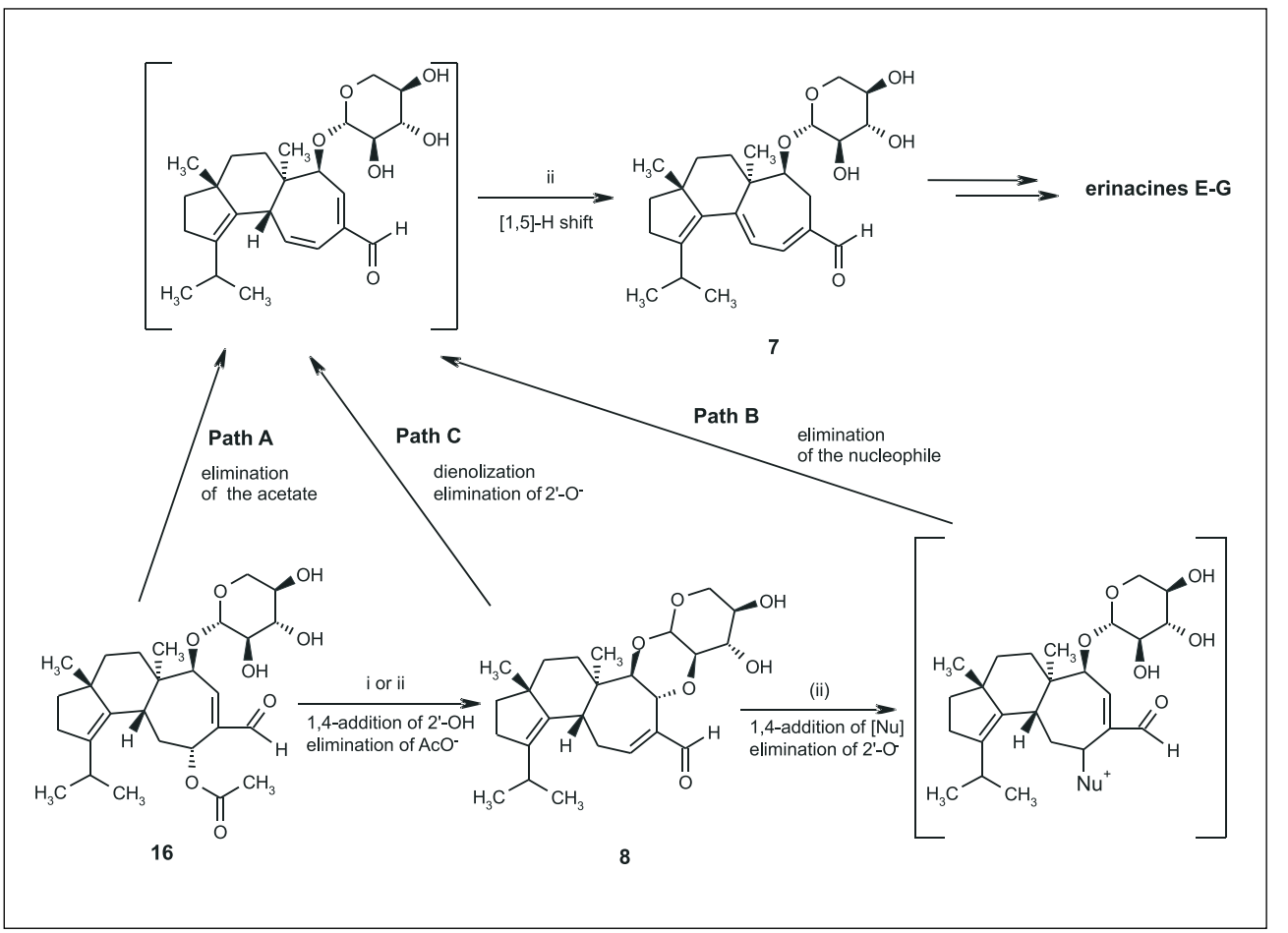

Fig. 2. Biogenesis for the erinacine family.

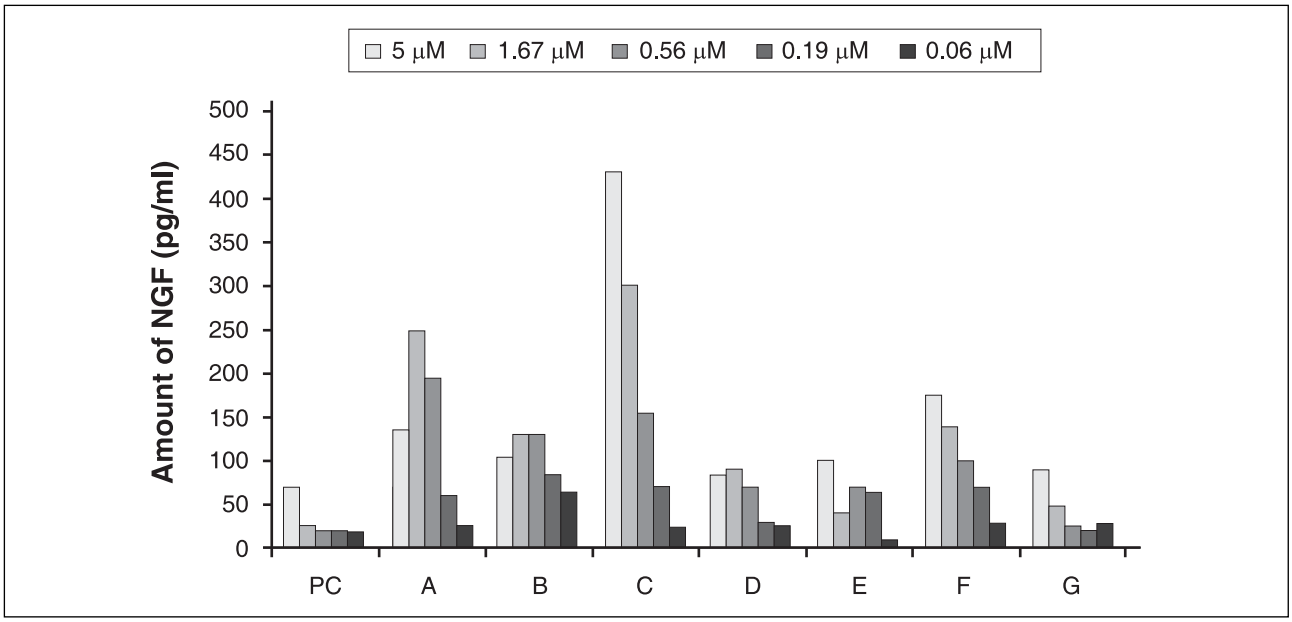

Fig. 3. Effects of erinacines A to G on NGF synthesis. PC (positive control): epinephrine.

than epinephrine, and erinacine C (**9**) showed the strongest activity (3-5, 33).

A further study was conducted *in vivo* in rats to examine the effects of erinacine A (**7**) on the production of catecholamines and NGF in the various regions of the central nervous system (7). Twenty newborn rats were divided equally into two groups. The control group was given 5% ethanol in phosphate-buffered saline (PBS) (10 ml/kg p.o.), and the treatment group was given 5% ethanol in buffer with erinacine A (8 mg/kg p.o.) for 4 weeks. After the last administration, the rats were decapitated under anesthesia and the catecholamine and NGF content was measured in the following brain regions: olfactory bulb, locus coeruleus, hippocampus and cerebral cortex. The results are shown in Figure 4. In the locus coeruleus, dopamine levels were unchanged in both groups, but the levels of its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were significantly higher in the erinacine A-treated group than in the control group. Levels of noradrenaline (NA), which is mainly produced in the locus coeruleus, significantly differed between control rats and those treated with erinacine A. There was no significant difference in the contents of 5-hydroxyindoleacetic acid (5-HIAA), 5-hydroxytryptamine (5-HT) and 5-HT + 5-HIAA (5-HIs). The effects of erinacine A on NGF synthesis in various brain regions are shown in Figure 5. In the locus coeruleus and hippocampus, the NGF content of the erinacine A-treated group was much higher than that of the control group. There was no significant difference between the groups in the NGF content of the olfactory bulb and cerebral cortex. The above findings suggest that erinacine A may enhance the synthesis of NGF by increasing the secretion of noradrenaline and catecholamines (7).

Another *in vivo* study was performed to investigate the effects of hericenone C and erinacine A in rats with ibotenic acid-induced dementia and rats with artificially induced cerebrovascular dementia. The results suggest that these compounds were beneficial in maintaining memory and improving learning skills in these models (manuscript in preparation).

Aβ and AD

Neuronal cell death is an essential feature of neurodegenerative diseases, including AD, Parkinson's disease and the prion diseases. It has been reported that many types of neuronal cell death are related to Aβ (34), glutamate (35) and nitric oxide (36).

Aβ, a major component of senile plaques, is considered to cause the inflammation and degradation of neurons due to ER and oxidative stress and lead to AD following its deposition in the brain. AD has a long symptom-free incubation period but is basically irreversible when diagnosed in advanced stages. Therefore, an effective way to reduce the risk of AD may be the daily intake of foods or dietary supplements that can inhibit the toxicity of Aβ (37, 38).

DLPE

The fruiting bodies of *H. erinaceum* were extracted with 85% ethanol and then acetone. The extracts were combined and concentrated under reduced pressure. The concentrated extract was partitioned between chloroform and water. The chloroform-soluble layer was concentrated and then fractionated by chromatography on a silica gel column to obtain 14 fractions. Fraction 10, which showed the highest activity in the screening test, was further purified by various chromatographies and DLPE was obtained (10).

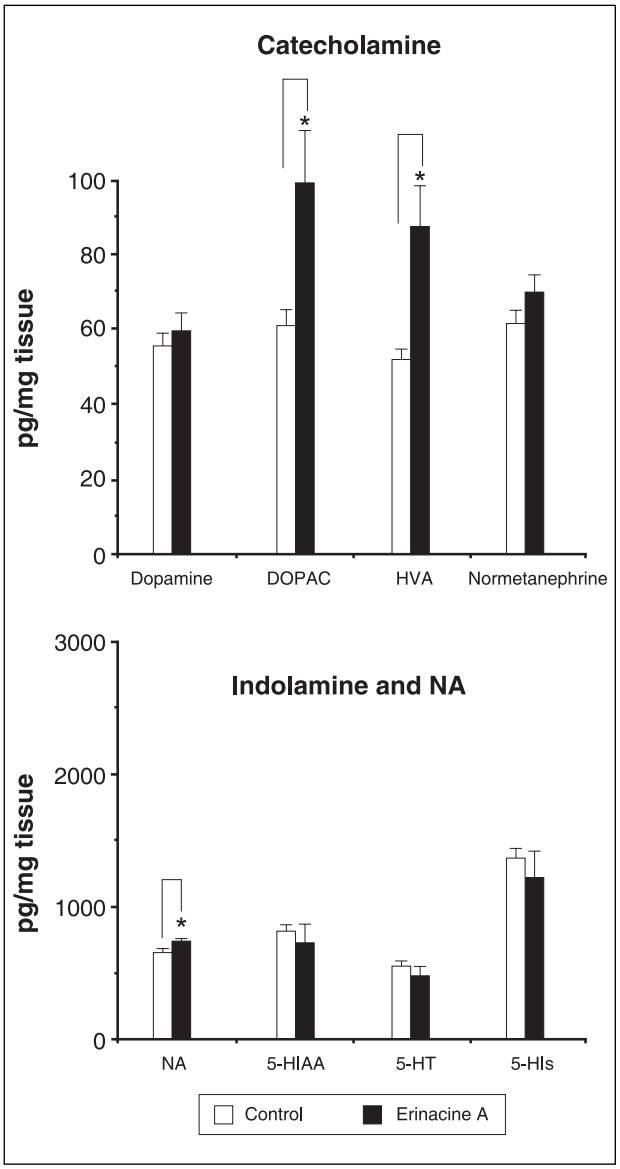

Fig. 4. Monoamine content in the locus coeruleus of rats fed erinacine A. *$p < 0.05$.

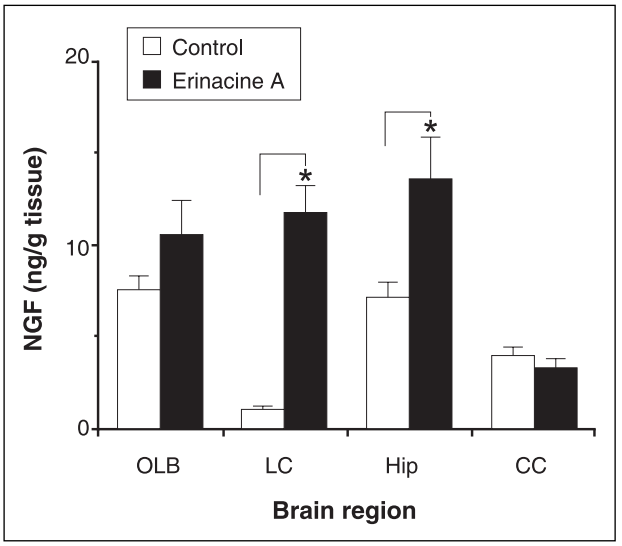

Fig. 5. Effects of erinacine A on NGF synthesis in the brain of rats. OLB, olfactory bulb; LC, locus coeruleus; Hip, hippocampus; CC, cerebral cortex. *$p < 0.05$.

Bioactivities of DLPE

The protective effect of DLPE against ER stress-induced neuronal cell death was investigated. Neuro2a cells were cultured in a 96-well plate at a cell density of 5,000 cells/well. After 1 day of culture, the cells were cultured in Dulbecco's modified Eagles medium (DMEM) without PBS, and 0.5 μg/ml of tunicamycin (an inducer of ER stress) and varying concentrations of DLPE or HE extract (chloroform-soluble fraction of *H. erinaceum*) were applied to the medium. The cells were incubated for 24 h and the cell viability was measured by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay. The results showed that the cell viability in cultures exposed to 10 and 100 ng/ml of HE extracts were 64.8 ± 10.56% and 76.9 ± 5.65%, respectively, compared to 8.88 ± 4.64% for control. In particular, the cell viability increased markedly to 67.0 ± 2.32% and 92.4 ± 1.63% upon the addition of 50 and 150 μg/ml of DLPE (Fig. 6). In the caspase-12 activation (a marker of the ER stress signal) assay, tunicamycin clearly reduced the amount of procaspase-12, whereas DLPE and HE extract inhibited the decrease in procaspase-12 or caspase-12 activation (10). Also, HE extract was shown to protect neuronal cells from Aβ- or oxidative stress-induced cell death (8, 9).

Preliminary clinical trials

A clinical trial was conducted to investigate the effects of *H. erinaceum* on dementia in a rehabilitative hospital in Japan, with 50 patients in the treatment group (average age: 75.0 years) and 50 patients used as controls (average age: 77.2 years) (39). All patients were suffering from cerebrovascular disease, degenerative orthopedic disease, Parkinson's disease, spinocerebellar degeneration, diabetic neuropathy, spinal cord injury or disuse syndrome. Seven of the patients in the treatment group suffered from AD or cerebrovascular dementia. The patients in this group received 5 g/day of the hot air-dried mushroom in their soup for a 6-month period. All patients were evaluated before and after the treatment period for their Functional Independence Measure (FIM), an international evaluation standard of independence in physical capabilities (eating, dressing, evacuating, walking, bathing/showering, etc.) and perceptive capabilities (understanding, expression, communication, problem solving, memory). After 6 months of taking the mushroom, 6 of 7 dementia patients showed improvement in their perceptual capacities, and all 7 had improvement in their overall FIM score (Fig. 7). In particular, 3 bedridden patients were able to get up for meals after the administration (39).

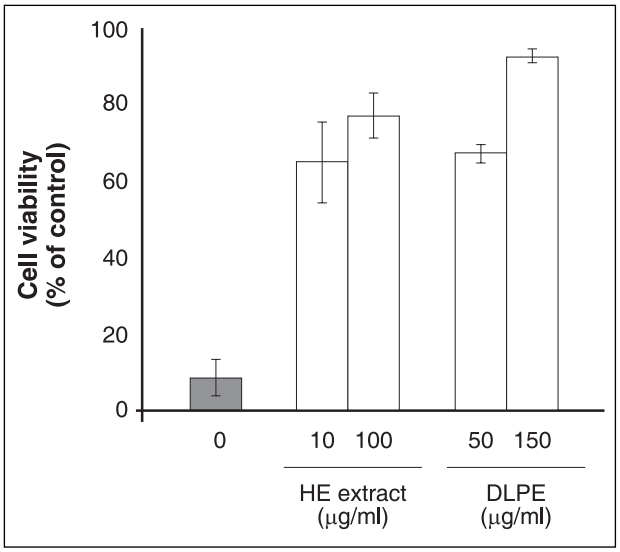

Fig. 6. Protective effects of HE extract and DLPE on ER stress-induced Neuro2a cell death.

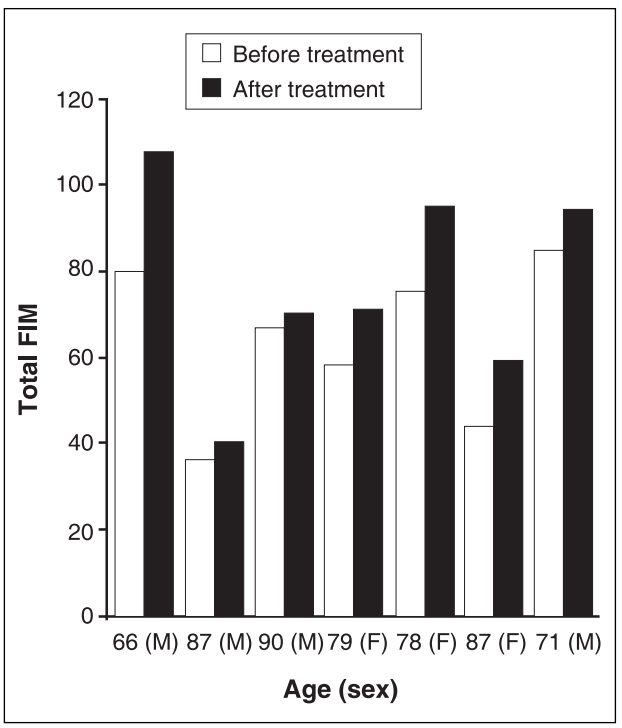

Fig. 7. Effects of *Hericium erinaceum* treatment on total FIM score.

To date, two other preliminary clinical trials in patients with senile dementia and AD have been conducted in Japan. The results showed that *H. erinaceum* retarded the progression of the disease or improved cognitive abilities (40, 41).

Conclusions

Several compounds (hericenones, erinacines and DLPE) isolated from *H. erinaceum* have shown significant activities in the induction of NGF synthesis or the protection of neuronal cells against Aβ-, ER stress- or oxidative stress-induced cell death *in vitro* and *in vivo*. The results of preliminary clinical trials indicate that *H. erinaceum* appears to be effective for senile dementia, especially AD.

Bioactivities observed in animal studies *in vitro* or *in vivo* are not always found when applied to humans. However, in the case of *H. erinaceum*, the positive results obtained in the laboratory were confirmed by analogous results in preliminary clinical trials.

Overall, it appears that *H. erinaceum* may have great potential as a medicine or dietary supplement for dementia, especially AD. However, further studies on its mechanism of action and more extensive clinical trials are clearly needed to substantiate the positive results seen so far.

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