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Effects of Fucoidan on Neuronal Cell Proliferation: Association with NO Production through the iNOS Pathway

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Abstract

Fucoidan, that is high-molecular-weight sulfated polysaccharides extracted from brown seaweeds has been shown to elicit various biological activities. Here, we investigated the effects of fucoidan on cell proliferation and nitric oxide (NO) production in neuronal blastoma cell (SH-SY5Y). In the present study, we demonstrated that fucoidan treatment resulted in increase of cell proliferation and NO production. When cells were treated with amyloid-β (Aβ) in the absence or presence of fucoidan, fucoidan recovered the cell viability decreased by Aβ peptides. To further determine whether nitric oxide synthase (NOS) is involved in proliferative effect of fucoidan, cells were treated with NOS inhibitors in the absence or presence of fucoidan. Selective constitutive nitric oxide synthase (cNOS) inhibitor, diphenylene iodonium chloride (DPI), caused a decrease of cell viability, whereas cell viability was increased by specific inducible nitric oxide synthase (iNOS) inhibitor, S-methylisothiourea (SMT), in the fucoidan-untreated cells. Treatment with fucoidan inhibited the cell viability decreased in DPI-exposed cells. In contrast, fucoidan had no effect on cell growth in SMT-treated cells, indicating that cNOS may not play a role in the proliferation of fucoidan-treated cells. The present data suggest that fucoidan has proliferative and neuroprotective effects and these effects may be associated with iNOS.

Key words: fucoidan, amyloid-beta, proliferation, iNOS, cNOS

INTRODUCTION

The brown seaweeds are the most important economic seaweeds cultured in China. They are also widely contributed in Japan and Korea. For many years, they have been consumed as food and relish to provide a dietary fibre source. Cell walls from brown seaweeds contain abundant sulfated polysaccharides and alginate. Fucoidan is the collective name for algal sulfated polysaccharides extracted from the edible brown seaweeds. Fucoidan was first isolated by Kylin almost one century ago, containing substantial percentages of L-fucose and sulfate ester groups (1). In recent years, fucoidan has been extensively studied due to its numerous biological activities including anticoagulant, antithrombotic, antitumor, antiviral, anti-complement and anti-inflammatory activities (2-6).

The most prevalent form of dementia, Alzheimer's disease is proposed to be a result of the accumulation of amyloid-beta (Aβ) peptides together with oxidative stress mechanisms and inflammation in nervous system. Nitric oxide (NO) plays many different roles in the nervous system. Under various neurological conditions the levels of NO produced by the enzymes inducible NO synthase (iNOS) or constitutive NOS (cNOS) are considered to be important regulator to neurons and glial cells. The comparatively small amount of NO produced by cNOS in neuronal cells is important for cellular signaling events such as blood pressure regulation and neurotransmission (7). In contrast, the much larger amount of NO generated by iNOS functions as both a regulator and effector during infection and neuroinflammation (8). O'Leary et al. (3) reported that fucoidan modulates the effect of transforming growth factor (TGF)-β on fibroblast proliferation and might be beneficial in the treatment of wound healing. They have also shown that the anti-proliferative effects of TGF-β on dermal fibroblasts were abrogated by fucoidan. However, the effect of fucoidan on cell proliferation and NO production in neuronal cells remains unclear. In the present study, we determined the cell proliferative effect of fucoidan in neuronal cells induced by Aβ aggregation form.

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MATERIALS AND METHODS

Materials

Unless otherwise indicated, all the chemicals were purchased from Sigma Chemical Co. (St Louis, MO). The Dulbecco's modified Eagle's medium (DMEM), penicillin, streptomycin, trysin-EDTA and fetal bovine serum (FBS) were purchased from Gibco Co. (Grand Island, NY).

Cell culture

The neuroblastoma cell line SH-SY5Y was purchased from the American Type Culture Collection (Manassas, VA). The cells were cultured in DMEM medium containing 10% inactivated FBS and antibiotics (100 IU/mL penicillin and 100 μg/mL streptomycin) and incubated at 37°C in a humidified atmosphere containing 5% CO_2.

Prepartaion of amyloid-β aggregation

Amyloid-β peptide 25-35 (Aβ 25-35) in lyophilized form was purchased from Sigma. For aging Aβ under acidic conditions, a 1 mM peptide stock solution in 10 mM HCl was prepared, and then diluted 1:10 into phosphate buffered saline (PBS). This 0.1 mM peptide solution was allowed to aggregate at room temperature for 2 days, and then diluted to the appropriate concentrations in DMEM medium for cell treatment.

MTT assay for cell viability

Cell viability was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay adapted from Mosmann for measuring cellular proliferation (cell growth) (9). The MTT assay is an alternative to the thymidine incorporation test which measures cell proliferation by determining the amounts of incorporated tritiated thymidine into freshly synthesized DNA. The proliferative profiles of cells as determined by the colorimetric assay, which essentially measures energy metabolism, or radioisotope assay usually do not show large differences (10,11). Briefly, the cells were seeded in 96-well plates at density of 5×10^4 cells per well to which 200 μL of DMEM medium supplemented with various concentrations of fucoidan and other reagents. After 24 hr of incubation, cell were washed once with PBS and 200 μL of MTT solution (25 μg/mL in media) was added to each well. After incubation at 37°C under 5% CO_2 for 4 hr, the MTT solution was carefully discarded and the formazan blue crystals formed by the reduction of MTT was dissolved in 150 μL DMSO. The amount of formazan was determined by absorbance at 540 nm using a microplate reader (Menlo Park, CA).

Nitrite determination

The cells were seeded in 96-well plates at density of 5×10^4 cells per well and cultured for 24 hr. The accumulation of nitrite in culture supernatant was measured using the assay system described by Ding et al. (12) Briefly, 100 μL aliquots of culture supernatant were mixed with an equal volume of Griess reagent (0.1% naphthylethylenediamine dihydrochloride, 1% sulphanilamide, and 2.5% phosphoric acid) and incubated at room temperature with mild shaking for 10 min. The absorbance at 540 nm was measured using a microplate reader (Menlo Park, CA). Nitrite concentration was calculated from a $NaNO_2$ standard curve.

Statistical analysis

The data is represented as a mean±SEM. The statistical difference between the groups was determined using a one-way analysis of variance (ANOVA) with a Dunnett's *t*-test. A p value <0.05 was considered significant.

RESULTS AND DISCUSSIONS

Effect of fucoidan on proliferation and NO production in SH-SY5Y cell

It has been shown that fucoidan has an effect on cell proliferation. Giraux et al. (13) reported that fucoidan enhanced endothelial progenitor cells (EPC). They showed that fucoidan induced cell proliferation in a concentration-dependent manner, whereas it had no effect on human umbilical vein endothelial cells (HUVEC) at concentration below 100 μg/mL. At higher concentration of fucoidan, EPC was proliferated significantly more rapidly than HUVEC. In contrast, Religa et al. showed the inhibitory effect of fucoidan on the proliferation of rat aortic smooth muscle cell (SMC) at 100 μg/mL (14). These results suggest that effect of fucoidan on cell proliferation is different depending on cell type.

In this study our data showed that fucoidan enhanced SH-SY5Y cell proliferation (Fig. 1A). We also examined the effect of fucoidan on NO production. Treatment of the cells with fucoidan (1, 10, 100 μg/mL) caused an increase in the production of nitrite. They increased gradually and reached the highest value at 100 μg/mL. Although large amount of NO damages the nervous system, the small amount of NO production has a beneficial effect for neuroendocrine activities, modulation of autonomic functions, neuroprotective effects and improvement on cognitive tasks (15,16). Thus, a lightly increase of NO production by fucoidan in SH-SY5Y cell may indicate another evidence for the beneficial effect of fucoidan.

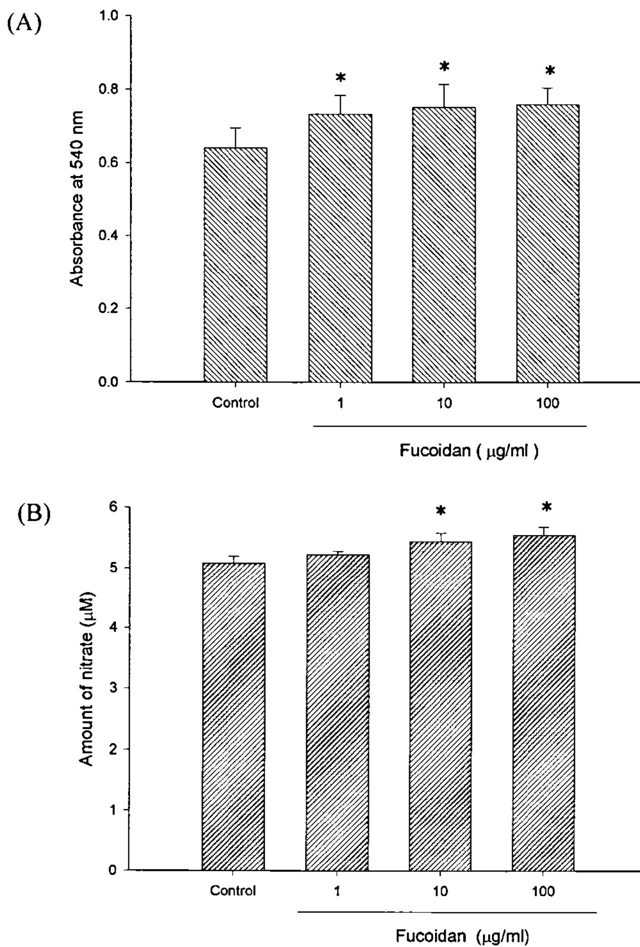

Fig. 1. The effect of fucoidan on proliferation (A) and production of nitrite (B) in SH-SY5Y cells. Cells (5×10^4 cells/well) were treated with various concentrations of fucoidan for 24 hr. Cell proliferation was determined by MTT assay. Optical density was measured at 540 nm. For NO determination, culture supernatants were collected and the levels of nitrite were measured as described in materials and method. The data represents the mean±SEM of quadruplicate experiments. *$p<0.05$; significantly different from the control (no treatment).

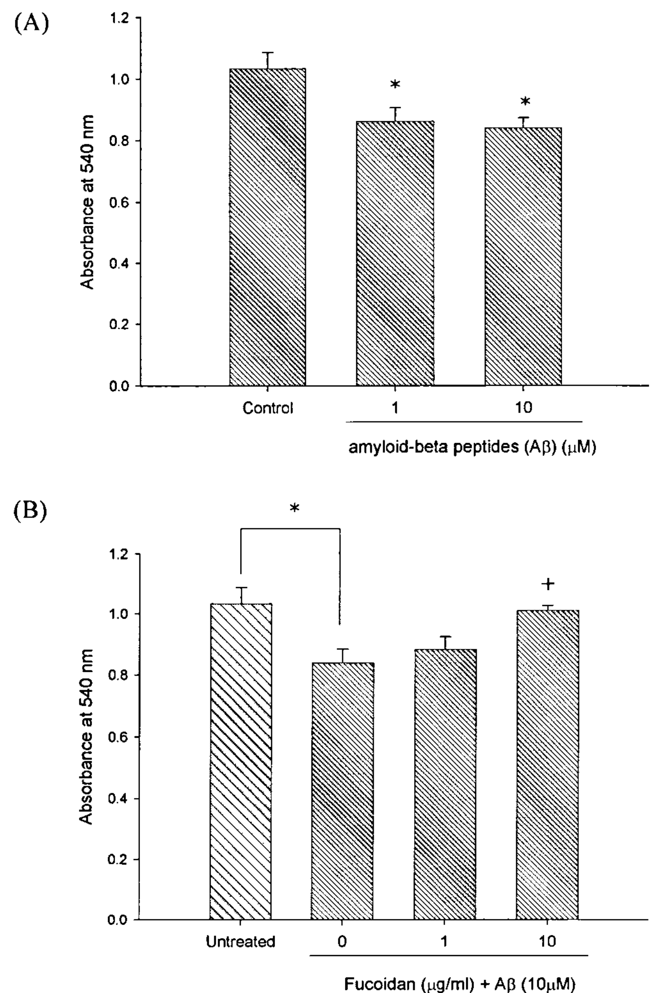

Fig. 2. Fucoidan protects against Aβ 25-35 cytotoxicity in SH-SY5Y cells. Cells (5×10^4 cells/well) were treated with acetic acid (vehicle) and Aβ 25-35 (1 and 10 μM) in the absence or presence of fucoidan (1 and 10 μg/mL) for 24 hr, respectively. Cell viability was determined by MTT assay. Optical density was measured at 540 nm. The data represents the mean±SEM of quadruplicate experiments. *$p<0.05$; significantly different from the control (no treatment). $^+p<0.05$; significantly different from fucoidan-untreated.

Fucoidan attenuates Aβ 25-35 cytotoxicity in a concentration-dependent manner in SH-SY5Y cell

Progressive memory impairment, Aβ plaques associated with local inflammation, neurofibrillary tangles, and loss of neurons in selective brain areas are hallmarks of Alzheimer's disease (AD) (17). Our data demonstrate that aggregated form of Aβ 25-35 induced SH-SY5Y cell death at concentration 1 and 10 μM, indicating a neurotoxicity effect of Aβ 25-35 (Fig. 2A). However, fucoidan attenuated this cytotoxicity of Aβ 25-35 (Fig. 2B). This result suggests the neuroprotective effect of fucoidan. Since AD is a progressive neurodegenerative characterized by memory impairment and cognitive dysfunction that is linked to the loss of cholinergic neurons in basal forebrain, the enhancement of SH-SY5Y cell proliferation by fucoidan may give another evidence about the beneficial effect of fucoidan for AD.

Effect of cNOS and iNOS inhibitors on fucoidan-induced cell growth in SH-SY5Y cell

NO, a molecule synthesized by NOS, has multiple functions in brain, neuroprotection and neurotoxicity, and behavior (18-20). NO production is determined by both constitutive and inducible NOS activities. The constitutive NOS activity (cNOS) is consists of neuronal NOS (nNOS) and endothelial NOS (eNOS) isoforms that are constitutively expressed in the brain. The inducible NOS activity is controlled by the expression of the inducible NOS (iNOS), induced after infection or injury (21).

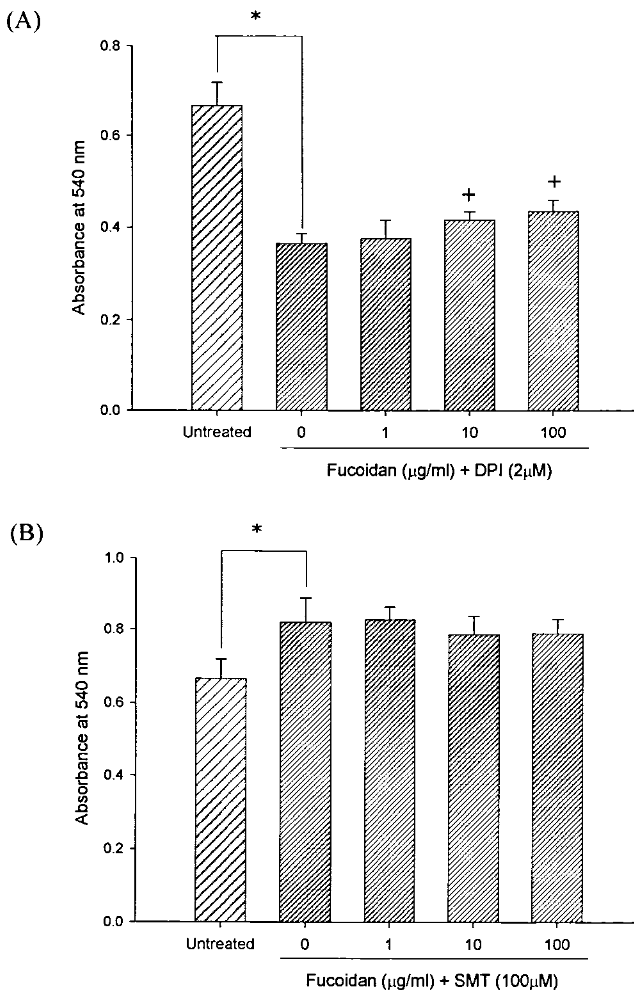

Fig. 3. Effect of iNOS and cNOS inhibitors on fucoidan-induced cell growth in SH-SY5Y cells. Cells (5×10^4cells/well) were treated with DPI (2 μM) (A) or SMT (100 μM) (B) in the absence or presence of fucoidan for 24 hr. Cell viability was determined by MTT assay. Optical density was measured at 540 nm. The data represents the mean±SEM of quadruplicate experiments. *$p<0.05$; significantly different from the control (no treatment). $^{+}p<0.05$; significantly different from fucoidan-untreated.

A deficit in long-term potentiation and the occurrence of abnormal sexual/aggressive behaviors have been reported in animals mutant for nNOS (nNOS-/-) or eNOS (eNOS-/-) or treated with NOS inhibitors (22,23). A deficiency in cNOS activity or NO production could compromise NO-related neuroprotection and render patients with schizophrenia and depression more susceptible to infectious agents (24). In addition, the decreased cNOS enzyme activity could increase the risk for apoptotic cell damage with many reports of decreased cell number in these illnesses and the consistent findings of decreased Bcl-2 protein in all the three diseases (25).

To examine whether effect of fucoidan on SH-SY5Y proliferation is mediated by NO and which NOS is involved in this mediation, the cells were treated with DPI (inhibitor of cNOS) and SMT (inhibitor of iNOS) in the absence or presence of various doses of fucoidan, respectively. Our data showed that DPI 2 μM strongly suppressed the proliferation of SH-SY5Y cells, whereas treatment with fucoidan inhibited the decrease of cell viability in DPI-exposed cells (Fig. 3A). In the other hand, fucoidan had no effect on cell growth in SMT-treated cells (Fig. 3B). Therefore, although cNOS is needed for cell proliferation (or cell viabilty), cNOS may not play a role in the proliferation of fucoidan-treated cells. Thus, these data suggest that fucoidan-induced cell proliferation may be mediated by iNOS.

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