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Prophylactic effects of sulforaphane on depression-like behavior and dendritic changes in mice after inflammation

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Abstract

Inflammation plays a role in the pathophysiology of depression. Sulforaphane (SFN), an isothiocyanate compound derived from broccoli, is a potent activator of the NF-E2-related factor-2 (Nrf2), which plays a role in inflammation. In this study, we examined whether the prevention effects of SFN in lipopolysaccharide (LPS) induced depression-like behavior in mice. Pretreatment with SFN significantly blocked an increase in the serum tumor necrosis factor-α (TNF-α) level and an increase in microglial activation of brain regions after a single administration of LPS (0.5 mg/kg). Furthermore, SFN significantly potentiated increased serum levels of IL-10 after LPS administration. In the tail-suspension test and forced swimming test, SFN significantly attenuated an increase of the immobility time after LPS administration. In addition, SFN significantly recovered to control levels for LPS-induced alterations in the proteins such as brain-derived neurotrophic factor, postsynaptic density protein 95 and AMPA receptor 1 (GluA1) and dendritic spine density in the brain regions. Finally, dietary intake of 0.1% glucoraphanin (a glucosinolate precursor of SFN) food during the juvenile and adolescence could prevent the onset of LPS-induced depression-like behaviors and dendritic spine changes in the brain regions at adulthood. In conclusion, these findings suggest that dietary intake of SFN-rich broccoli sprout has prophylactic effects on inflammation-related depressive symptoms. Therefore, supplementation of SFN-rich broccoli sprout could be prophylactic vegetable to prevent or minimize the relapse by inflammation in the remission state of depressed patients.

Keywords: Brain-derived neurotrophic factor; Depression; Inflammation; Nrf2; Prevention; Sulforaphane; Synaptogenesis

1. Introduction

Depression is one of the most common psychiatric disorders in the world. Although the precise mechanism underlying the pathophysiology of depression is unknown, multiple lines of evidence suggest that inflammation plays a key role in the pathophysiology of depression [1–6]. Meta-analyses showed higher blood levels of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin-6 (IL-6), in drug-free depressed patients, compared with healthy controls [7,8]. A study using postmortem brain samples showed elevated gene expression of proinflammatory cytokines in the frontal cortex of participants with a history of depression [9]. When the bacterial endotoxin lipopolysaccharide (LPS) is administered to rodents, depression-like behaviors are observed 24 h after inflammation [1,6,10]. The current antidepressants, including selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs), are able to prevent depression-like behavior and alterations in serum proinflammatory cytokines, such as TNF-α, induced by LPS administration [10–13]. These all findings suggest that inflammation is associated with depressive symptoms and that antiinflammatory drugs could ameliorate depressive symptoms in patients with depression.

Accumulating evidence suggests that nutrition could influence the development of numerous psychiatric disorders, including depression [14–18]. A recent meta-analysis including 21 studies demonstrated that high intake of fruit, vegetables, fish and whole grains are associated with a reduced risk of depression [19]. Taken together, it seems that nutrition such as vegetable plays an important role in mental health.

The potent antioxidant and antiinflammatory compound sulforaphane (SFN: 1-isothiocyanato-4-methylsulfinylbutane) is an organosulfur compound found in cruciferous vegetables, such as broccoli Brussels sprouts and cabbage [20–25]. The protection afforded by SFN is thought to be mediated *via* the activation of the NF-E2-related factor-2 (Nrf2) pathway and subsequent up-regulation of Phase II detoxification enzymes and antioxidant proteins through an enhancer sequence referred to as the electrophilic-responsive element or the antioxidant-responsive element (ARE) [25–28]. We previously reported that SFN could prevent behavioral abnormalities in mice after administration of methamphetamine [29] or phencyclidine (PCP) [30]. Subsequently, we also reported that dietary intake of SFN-rich broccoli sprout extracts during juvenile and adolescence can prevent PCP-induced cognitive deficits and loss of parvalbumin (PV) immunoreactivity in the prefrontal

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cortex (PFC) at adulthood [30,31]. These finding suggest that SFN could be a potential prophylactic natural compound for neuropsychiatric diseases [29–31].

We previously reported that SFN showed antiinflammatory and antioxidative effects in methamphetamine (or PCP)-induced behavioral abnormalities in mice thorough antiinflammatory and antioxidant activities [29–31]. Therefore, the present study was undertaken to examine whether SFN or dietary intake of glucoraphanin (GF) (a glucosinolate precursor of SFN) food can prevent the onset of depression-like behaviors in mice after inflammation. Alterations in the brain-derive neurotrophic factor (BDNF), synaptogenesis proteins [*e.g.*, postsynaptic density protein 95 (PSD-95) and AMPA receptor 1 (GluA1)] and dendritic spine density are involved in the pathophysiology of depression [32–42]. Therefore, we also examined the effects of SFN or dietary intake of GF on alterations in BDNF, PSD-95, GluA1 and dendritic spine density in the selected brain regions, including PFC, nucleus accumbens (NAc) and hippocampus [CA1, CA3, dentate gyrus (DG)].

2. Methods and materials

2.1. Animals

Male adult C57BL/6 mice, aged 8 weeks (body weight 20-25 g, Japan SLC, Inc., Hamamatsu, Japan) were used in experiments. Animals were housed under controlled temperature and 12-h light/dark cycles (lights on between 07:00–19:00), with *ad libitum* food and water. All experiments were carried out in accordance with the Guide for Animal Experimentation of Chiba University. The protocol was approved by the Chiba University Institutional Animal Care and Use Committee.

2.2. Drug administration

On the day of injection, fresh solutions were prepared by dissolving compounds in sterile endotoxin-free isotonic saline. Lipopolysaccharide (LPS, 0.5 mg/kg; L-4130, serotype 0111:B4, Sigma-Aldrich, St Louis, MO, USA) was dissolved in physiological saline. Sulforaphane (SFN, 3.0, 10 and 30 mg/kg; LKT Laboratories, Inc., St Paul, MN, USA) was dissolved in distilled water including 10% corn oil. LPS and SFN were administered intraperitoneally (ip) into mice. The doses of LPS (0.5 mg/kg) and SFN were selected by previous reports [10–13,29–31].

2.3. Preparation of 0.1% glucoraphanin (GF)

Food pellets (CE-2; Japan CLEA, Ltd., Tokyo, Japan) containing 0.1% glucoraphanin (GF) were prepared as follows. Broccoli sprout extract powder containing SFN precursor GF was industrially produced by KAGOME CO., LTD. In brief, broccoli sprout was grown from specially selected seeds (Caudill Seed Co., Inc. Louisville, KY, USA) for 1 day after the germination. The 1-day broccoli sprout was plunged into boiling water and maintained at 95°C for 30 min, and the sprout residues were removed by filtration. The boiling water extract was mixed with a waxy corn starch dextrin and then spray dried to yield the broccoli sprout extract powder containing 135 mg (approx. 0.31 mmol) of GF per gram. For preparing the animal diet containing 0.1% GF (approx. 2.3 mmol GF per 1 kg-diet), the extract powder was mixed with a basal diet CE-2 (CLEA Japan Inc., Tokyo, Japan) and then pelletized at a processing facility (Oriental Yeast Co., ltd., Tokyo, Japan). The GF content in the diet was determined by high-performance liquid chromatography as previously described [31].

2.4. Serum or brain levels of TNF-α and IL-10

The mice were anesthetized with pentobarbital, and blood or brain (PFC) was collected. Blood was centrifuged at 2000 *g* for 20 min to generate serum samples. Brain samples were homogenized in 5× volume of 1X PBS. Homogenates were centrifuged at 14,000×*g* for 30 min, and supernatant was retained. Proteins were measured using the DC protein assay kit (Bio-Rad, Hercules, *CA*, USA). The serum samples or brain samples were diluted 10-fold with ELISA diluent solution (eBioscience, San Diego, CA, USA). The tumor necrosis factor (TNF)-α and interlukin-10 (IL-10) concentrations were measured using a Ready-SET-Go ELISA kit (eBioscience) according to the manufacturer's instructions.

2.5. Immunohistochemical detection of MAC1 in mouse brains MAC1

Immunohistochemistry was performed, as reported previously [29,31,43]. Twenty four hours after the administration of LPS (0.5 mg/kg), mice were deeply anesthetized with sodium pentobarbital and perfused transcardially with 10 ml of isotonic saline, followed by 40 ml of ice-cold 4% paraformaldehyde in 0.1-M phosphate buffer (pH 7.4). Brains were removed from the skulls and postfixed overnight at 4°C. For immunohistochemical analysis, 50-μm thick serial coronal sections of brain tissue were cut in ice-cold, 0.01-M phosphate-buffered saline (pH 7.5), using a vibrating blade microtome (VT1000S, Leica Microsystems AG, Wetzlar, Germany). Brain sections were identified according to stereotaxic coordinates in Franklin and Paxinos' Mouse Brain [44]. Free-floating sections were treated with 0.3% H_2O_2 in 0.05 M Tris–HCl saline (TBS) for 30 min and blocked in TBS containing 0.2% Triton X-100 (TBST) and 1.5% normal serum for 1 h, at room temperature. Samples were then incubated for 36 h at 4°C, with rat anti-MAC1 (CD11b) antibody (1:1000, cat. no. MCA74G, Serotec Ltd., Oxford, UK). The sections were washed twice in TBST and processed according to the avidin–biotin–peroxidase method (Vectastain Elite ABC, Vector Laboratories, Inc., Burlingame, CA, USA). Sections were then incubated for 5 min in a solution of 0.15-mg/ml diaminobenzidine, containing 0.01% H_2O_2. The sections were mounted on gelatinized slides, dehydrated, cleared and coverslipped under Permount® (Fisher Scientific, Fair Lawn, NJ, USA). Next, sections were imaged, and MAC1 immunoreactivity (activated microglia) was quantified in the anterior regions (0.018 mm^2) of the each brain regions in a blinded manner.

2.6. Western blot analysis and Golgi staining

2.6.1. Western blot analysis

The mice brain sample of CA1, CA3 and DG of the hippocampus, prefrontal cortex (PFC) and nucleus accumbens (NAc) were prepared as previously reported [43,45,46]. Tissue samples were homogenized in Laemmli lysis buffer. Aliquots (10 μg for brain sample) of protein were measured using the DC protein assay kit (Bio-Rad, Hercules, CA, USA) and incubated for 5 min at 95°C, with an equal volume of 125-mM Tris/HCl, pH 6.8, 20% glycerol, 0.1% bromophenol blue, 10% β-mercaptoethanol and 4% sodium dodecyl sulfate and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis, using 10% mini-gels (Mini-PROTEAN® TGX™ Precast Gel; Bio-Rad). Proteins were transferred onto polyvinylidenedifluoride (PVDF) membranes using a Trans Blot Mini Cell (Bio-Rad). For immunodetection, the blots were blocked with 2% BSA plus 5% nonfat dry milk in TBST (TBS+0.1% Tween-20) for 1 h at room temperature (RT) and kept with primary antibodies overnight at 4°C. The following primary antibody was used: Brain derived neurotrophic factor (BDNF) (1: 1000, Santa Cruz Biotechnology, Inc., CA, USA), postsynaptic density protein 95 (PSD-95) (1 μg/ml, Invitrogen, Carlsbad, CA, USA) and glutamate receptor 1 (GluA1) (1 μg/ml, Abcam, Cambridge, MA, USA). The next day, blot were washed three times in TBST and incubated with horseradish peroxidase conjugated antirabbit antibody (1:5000 for BDNF, PSD-95 and GluA-1) 1 h, at RT. After final three washes with TBST, bands were detected using enhanced chemiluminescence (ECL) plus the Western Blotting Detection system (GE Healthcare Bioscience). The blots then were incubated in the stripping buffer (2% SDS, 100-mM β-mercaptoethanol, 62.5-mM Tris/HCL PH 6.8) for 30 min at 60°C followed by three time washed with TBST. The stripped blots were kept blocking solution for 1 h and incubated with the primary antibody directed against β-Actin. Images were captured with a Fuji LAS3000-mini imaging system (Fujifilm, Tokyo, Japan), and immunoreactive bands were quantified.

2.6.2. Golgi staining

Golgi staining was performed using the FD Rapid GolgiStain™ Kit (FD Neuro Technologies, Inc., Columbia, MD, USA), following the manufacturer's instructions. Twenty four hours after ip administration of LPS (0.5 mg/kg) or saline (10 ml/kg), animals were deeply anesthetized with sodium pentobarbital, and brains were removed from the skull and rinsed in double distilled water. Brains were immersed in the impregnation solution, made by mixing equal volumes of Solution A and B, overnight and then stored in fresh solution, for 2 weeks in the dark. Brains were transferred into Solution C overnight and then stored in fresh solution at 4°C for 1 week, in the dark. Coronal brain sections (100-μm thickness) were cut on a cryostat (3050S, Leica Microsystems AG, Wetzlar, Germany), with the chamber temperature set at −20°C. Each section was mounted in Solution C, on saline-coated microscope slides. After absorption of excess solution, sections were dried naturally, at room temperature. Dried sections were processed following the manufacturer's instructions. Briefly, images of dendrites within CA1, CA3 and DG of the hippocampus, PFC and NAc were captured using a 100× objective with a Keyence BZ-9000 GenerationIImicroscope (Osaka, Japan). Spines were counted along CA1, CA3, DG, PFC and NAc dendrites starting from their point of origin from the primary dendrite, as previously reported [43,45–47]. For spine density measurements, all clearly evaluable areas containing 50–100 μm of secondary dendrites from each imaged neuron were used. To determine relative spine density, spines on multiple dendritic branches from a single neuron were counted to obtain an average spine number per 10 μm. For spine number measurements, only spines that emerged perpendicular to the dendritic shaft were counted. Three neurons per section, three sections per animal and six animals were analyzed. The average value for each region in each individual was obtained. These individual averages were then combined to yield a grand average for each region.

2.7. Statistical analysis

The data are shown as the mean ± standard error of the mean (S.E.M.). Analysis was performed using PASW Statistics 20 (formerly SPSS statistics; SPSS, Tokyo, Japan). All data, including TNF-α, IL-10, locomotion, TST, FST, BDNF, PSD-95 GluA-1 and Golgi staining, were analyzed using one-way ANOVA, followed by *post-hoc* LSD test. *P* values of less than 0.05 were considered statistically significant.