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Effects of Non-saponin Red Ginseng Components on the Function of Brain Cells

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Abstract : Non-saponin gingseng fraction components (NSRG) have been known to have a variety of biological activity. However, the effects of these components on the function of brain cell have not been characterized in detail. In this study, we investigated the preventive effect of non-saponin red ginseng components on acrylamide (ACR)-induced suppression of neural cell adhesion molecule (NCAM), which is highly expressed in neuronal cells. The data showed that NSRG blocked the suppression of NCAM expression by ACR in neuroblastoma cells (SK-N-SH). In addition, NSRG significantly increased NCAM expression in ACR-nontreated neuroblastoma cells. NSRG treatment resulted in the increase of cell proliferation in a concentration-dependent manner. We also examined whether NSRG could modulate the NO production of astrocytes. When glioma cells (C6) were treated with various concentrations of NSRG (100-300 ug/ml) in the presence or absence of IFN-γ for 24 hours, NO production was suppressed in IFN-γ-stimulated C6 cells. Taken together, these results demonstrate that treatment of brain cells with NSRG results in the enhancement of proliferation, the suppression of NO production and the protective effect on NCAM expression impaired by ACR. Thus, the present data suggest that NSRG has proliferative and neuroprotective effects and these effects could be useful in neuronal diseases.

Key words : non-saponin red ginseng, NCAM, SK-N-SH, Acrylamide

INTRODUCTION

Red ginseng (Ginseng Radix Rubra) is produced by steaming the root of White ginseng (Ginseng Radix Alba) followed by drying. During the heat processing for preparing red ginseng, it has been found to exhibit changes in ginsenosides composition[1]. Hiromichi *et al*. suggests that pharmacological actions of ginseng roots may be derived from its non-saponin fractions as well as from ginsenosides[2]. Several publications documented that acidic polysaccharides from non-saponin red ginseng components (NSRGs) are known to prevent obesity or hyperlipemia by enhancing immunity against cancer, inhibiting lipolysis and lowering intestinal absorption of cholesterol or fat[3,4]. Larina *et al.* also showed that saponin-free fraction from Ginseng significantly improves an oxygen-dependent anti-microbial function of neutrophils[5]. Thus, several studies have been carried out to determine the biological activities of NSRG[2]. However, there is still large potential for further industrial screening and research on ginseng products and their therapeutic potential remains pharmaceutically unexploited.

The brain is made up of two principal cell types: neurons and glial cells. About 90% of the cells within the brain is not neurons but glial cells. Since neurons are much larger than glial cells, the mass of nervous tissue is divided about equally between neurons and glia. The four major types of glial cells in the brain are astrocyte, oligodendrocytes, microglia and ependymal cells. Among them, astrocyte is the most abundant glial cells[6]. Neural cell adhesion molecule (NCAM) is cell surface macromolecules that belong to the immunoglobulin (Ig) superfamily, which is expressed on the surface of neurons, glia, skeletal muscle and natural killer cells. NCAM has been implicated as having a role in cell-cell adhesion, neurite outgrowth, synaptic plasticity, and learning and memory[7].

The aim of this study was to investigate the biological effects of NSRG on the expression of NCAM in neuron cells impaired by acrylamide (ACR) and IFN-γ-induced

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NO production in glial cells.

MATERIALS AND METHODS

NSRG preparations

Six-year Korean red ginseng was obtained from the Korean Society of Ginseng. 100 g of 6-year red ginseng was extracted three times with 1000 ml of MeOH for 4 h under reflux. The resulting extract 13.5 g, was subjected to filtration and concentration. 10 g of this was dissolved in 100 ml of water and was extracted 3 times with 100 ml ether. The supernatant was dehydrated by Na_2SO_4, and was decompressed for concentration within 40°C yielding 830 mg of the powder (Fig. 1). The water saturated fraction was extracted three times with 100 ml ethylacetate and was separated with ethylacetate (EtOAc fr.) and water. The water fraction was extracted three more times with 100 ml n-BuOH, filtered and concentrated yielding a 1.4 g saponin fraction. Finally, 10.2 g of the H_2O fraction was obtained by the decompression followed by the condensation of the remaining supernatants (Fig. 1).

Cells and Chemicals

SK-N-SH (neuroblastoma cells) and C6 (rat glioma cells) were obtained from ATCC (Manassas, VA). These cells were cultured at 37°C in 5% CO_2 and in RPMI 1640 (C6) or DMEM medium (SK-N-SH) containing 10% FBS, 100 IU/ml penicillin and 100 g/ml streptomycin. Unless otherwise indicated, all chemicals were purchased from Sigma (St. Louis, MO). RPMI 1640 medium, DMEM medium and fetal bovine serum (FBS) were purchased from GIBCO (Grand Island, NY). The NCAM antibody was purchased from Santacruz Biotechnology (Santa Cruz, CA). All the tissue culture reagents and

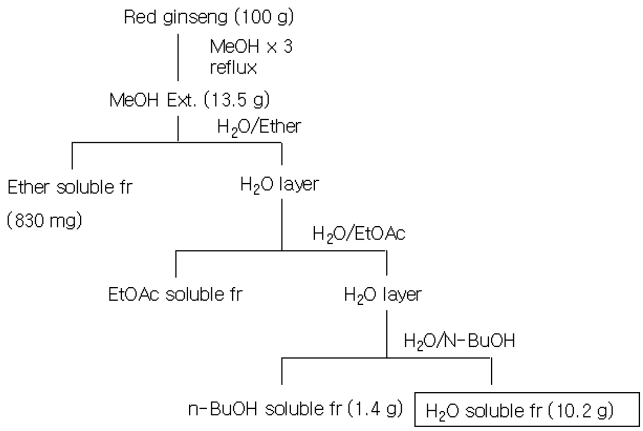

Fig. 1. Various solvent fraction of red ginseng.

NSRG were assayed for any endotoxin contamination using the Limulus lysate test (E-Toxate, Sigma), and the endotoxin levels were found to be < 10 pg/ml.

MTT assay for cell proliferation

Cell proliferation was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay adapted from Mosmann (1983)[8]. Briefly, the cells were seeded in 96-well plates at a suitable densities of cells per well with various concentrations of NSRG for 24 h. The enzyme activity of viable cells was measured by addition MTT to each well. After additional incubation for 4 h, the amount of formazan was determined by absorbance at 540 nm using a microplate reader (Menlo Park, CA).

Nitrite determination

The cells were treated with various doses of NSRG for 24 h and the accumulation of nitrite in culture supernatants was measured using the assay system described by Ding *et al*[9]. 100 μL aliquots of culture supernatants were mixed with an equal volume of Griess reagent (mixture at 1:1 of naphthylethylenediamine dihydrochloride and 1% sulphanilamide in 5% H_3PO_4) and incubated at room temperature for 10 min. Nitrite concentration was calculated from a $NaNO_2$ standard curve.

Western blot analysis

The level of NCAM expression was measured by Western blot analysis. After treatment with NSRG in the absence or presence of acrylamide for 24 h, cells were lysed in sodium dodecylsulfate polyacrylamide gel electrophoresis buffer. The protein concentration was determined using a Bio-Rad protein assay (Bio-Rad Lab, Hercules, CA) with BSA (Sigma) as the standard. The whole lysates (20 μg) were resolved on a 7.5% SDS-polyacrylamide gel, transferred to an immobilon polyvinylidene difuride membrane (Amersham, Arlington heights, IL) and probed with the appropriate antibodies. The blots were then developed using an enhanced chemoluminescence (ECL) kit (Amersham). In immunoblotting experiments, the blots were reprobed with the anti-β-actin antibody as a control for the protein loading.

Statistical analysis

The data is represented as a mean ± S.E. The statistical difference between the groups was determined using a one-way analysis of variance (ANOVA) with a Student-Newman-Keuls test. A P value < 0.05 was considered significant.

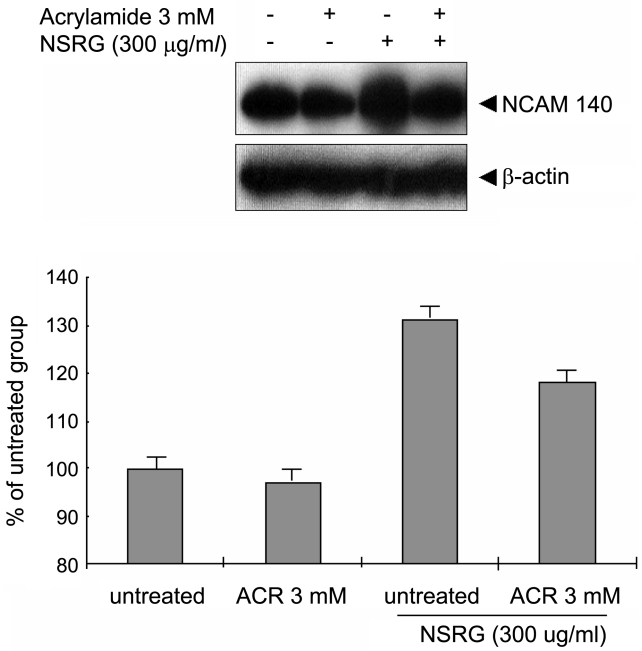

Fig. 2. The effect of NSRG on NCAM expression in acrylamide (ACR)-treated neuroblastoma cells. SK-N-SH neuroblastoma cells (2×10^6 cells/well) were treated with NSRG (300 μg/ml) in the absence or presence of acrylamide (3 mM) for 24 h. Total protein was extracted and subjected to Western blot anaysis for NCAM expression in protein level. Immunoreactive bands were quantitated by densitometric analysis. The control value was set as 100% and presented as a bar graph. Similar observations were obtained in two other experiments.

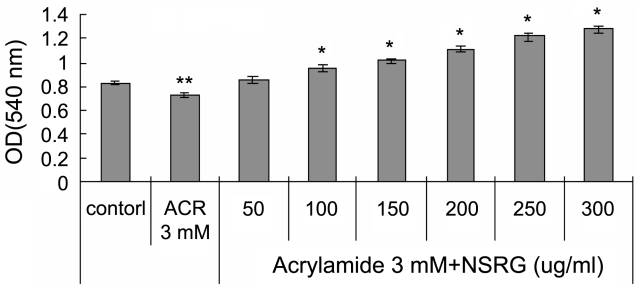

Fig. 3. The effect of NSRG on proliferation in acrylamide (ACR)-treated neuroblastoma cells. SK-N-SH neuroblastoma cells (5×10^4 cells/well) were treated with various concentrations of NSRG in the presence of acrylamide (3 mM) for 24 h. Cell proliferation was determined by MTT assay. Optical density was measured at 540 nm. The data represents the mean ± SE of quadruplicate experiments. *p<0.05; significantly different from acrylamide- treated. **; Significantly different from control (no treatment).

RESULTS AND DISCUSSION

Effect of NSRG on NCAM expression impaired by ACR

ACR is a well-known industrial neurotoxic chemical that can induce neurodegeneration. We determined the protective effect of NSRG on the expression of NCAM suppressed by ACR (3 mM). As shown in Fig. 2, ACR-induced suppression is significantly inhibited by NSRG. In addition, the data showed that NSRG significantly increased the NCAM expression in neuronal cells (Fig. 2: lane 3). Since NCAM has been implicated as having a role in cell-cell adhesion, neurite outgrowth, synaptic plasticity, and learning and memory[7], these data suggest that NSRG has a poteintial neuroprotective effect.

Effect of NSRG on ACR-induced suppression of proliferation in neuron cells

Since our previous results indicated that NSRG had a protective effect on NCAM expression, this study next examined whether or not NSRG has a proliferative effect on neuron cells impaired by ACR. Our data showed that NSRG enhanced SK-N-SH proliferation impaired by ACR in a concentration-dependent manner (Fig. 3). The enhanced effect of NSRG on the proliferation of SK-N-SH may give a considerable promise for preventing and treating neurodegenerative disorders due to cell death.

Effect of NSRG on proliferation and NO production in C6 cell

Recent studies have indicated that brain cells significantly affect the pathological state and/or regulate the regenerative state and remodeling of the brain by producing a variety of biologically active molecules including free radicals such as superoxide and nitric oxide (NO)[10]. In addition, under pathological conditions, excessive formation of NO is associated with tissue damage observed in arthritis, type 1 diabetes, septic shock and Alzheimer's disease[11,12]. Activated glial cells are the source of nitric oxide that is largely produced by inducible NO synthase (iNOS) in response to a series of proinflammatory cytokines including TNF-α, IFN-γ, and interleukin-1β which are present in a large amount in Alzheimer's brain patients[11,13]. Because of the neuronal toxicity of nitric oxide, inhibition of iNOS expression may be a promising treatment for Alzheimer's disease[14]. Therefore, the effects of NSRG on NO production are examined in C6 glioma cells. As shown in Fig. 4, NSRG has an inhibitory

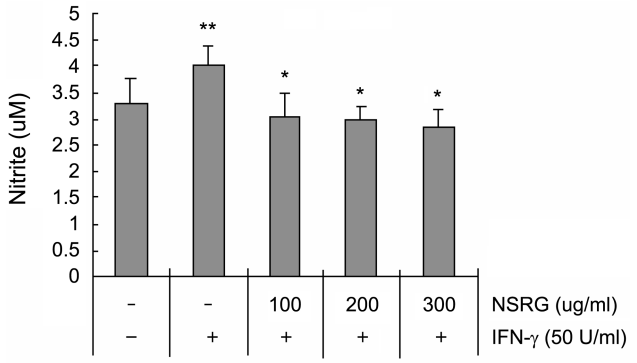

Fig. 4. The effect of NSRG on production of nitrite in IFN-γ-stimulated C6 glioma cells. C6 glioma cells (5×10^4 cells/well) were treated with various concentrations of NSRG for 24 h in the absence or presence of IFN-γ (500 u/ml). Cell proliferation was determined by MTT assay. For NO determination, culture supernatants were collected and the levels of nitrite were measured as described in materials and method. The data represents the mean ± SE of quadruplicate experiments. *$p<0.05$; significantly different from IFN-γ-treated. $p<0.05$. **; Significantly different from control (no treatment).

effect on the production of NO in IFN-γ-treated C6 cells. These results agree with previous report showing that acidic polysaccharides from Panax ginseng have immunomoduatory effects involved in NO production[15]. Thus, our data suggest that inhibitory effects of NSRG on NO production induced by IFN-γ in C6 cells could provide the opportunity to develop a new therapeutic agent for neuronal disease.

In summary our results indicate the preventive effect of NSRG on both cell proliferation and NCAM expression impaired by ACR in neuron cells. Moreover, NSRG has a inhibitory effect of NO production. Therefore, NSRG might be useful as potential neuroprotective therapy against neurodegenerative disorders by neurotoxic materials or neuroinflammatory conditions.

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