Influences of Postnatal *Ginseng radix* Administration on Prenatal Noise Stress–induced c–Fos Expression in the Hippocampus of Offspring Rats

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**Abstract**

Background: To elucidate the role of c–Fos expression in response to noise stress in the hippocampus of newborn rats, we investigated the influence of *Ginseng radix* administration on postnatal c–Fos expression and behavioral development.

Methods: Pregnant rats were divided into three groups: control, noise stress, and *Ginseng radix* treatment. The newborn rats were exposed to 85 dB noise daily for 4 weeks. The c–Fos expression was evaluated using immunohistochemistry and Western blot analysis.

Results: In the control group, c–Fos expression was detected in the CA1, CA2, and CA3 regions of the hippocampus. No significant difference in c–Fos expression was observed between the control and noise stress groups. However, the *Ginseng radix* treatment group showed a decrease in c–Fos expression compared to the control group.

Conclusion: The findings suggest that *Ginseng radix* administration may have a protective effect on c–Fos expression in the hippocampus of newborn rats exposed to noise stress.
I. INTRODUCTION

Stress has become an integral part of human life in this modern era. Among the innumerable stressors to which mankind is exposed, noise happens to be a commonly encountered stressor throughout the world. Even brief noise exposure is known to increase heart rate and peripheral vascular resistance, leading to a rise in blood pressure. On the other hand, experimental studies have demonstrated ultra structural modifications in rat cardiomyocytes mainly involving mitochondria. These subcellular alterations are related to an imbalance in calcium homeostasis, which is supposed to be sustained by increased catecholamine innervations.\(^1\,^2\)

Noise as a stressful stimulus is a widely accepted fact. However an effective agent to counter the noise stress-induced biochemical alterations remains elusive. *Ginseng radix*, the root of *Panax ginseng* C.A. Meyer (Araliaceae), is one of the most famous Oriental medical herbs and has several therapeutic applications. It is well documented that *Ginseng radix* possesses a number of pharmacological effects including hypotensive, cardiotonic, sedative, aphrodisiac, antiaging, and antioxidant actions.\(^3\,^4\)

It was suggested that exposure to prenatal stress alters an individual's developmental trajectory through altered early brain development. In late gestation, the fetus can hear sound from the outside of the mother.\(^5\)

Previous studies reported that exposure to noise during pregnancy adversely influenced the development of the fetus and neonate: increased antepartum fetal death and congenital anomaly in the central nervous system, impaired social behavior in juvenile stage, and a long-term alteration in the immune function.\(^6\,^7\) In addition, prenatal noise stress is known to influence learning and memory capabilities of the offspring by altering neuronal activity in the hippocampus.\(^8\)

Hippocampal formation is a brain region critically involved in learning and memory formation.

In humans, hippocampal damage impairs explicit memory, and in rodents, hippocampal damage impairs spatial and contextual learning which require the formation of relational representations among multiple cues.\(^9\,^{10}\)

*c-Fos* is an immediate early gene whose expression is sometimes used as a marker for stimulus-induced changes in the metabolic activity of neurons, being induced in the CNS under various conditions.\(^12\,^{14}\) Recently, it was reported that prenatal stress produces region-selective changes in expression of inducible transcription factors (ITFs) and metabolism in the brain.\(^13\,^{16}\) In the present study, the influence of postnatal *Ginseng radix* administration on neuronal changes, in particular with respect to *c-Fos* expression in the hippocampus of offspring rats with prenatal noise stress during pregnancy was investigated via *c-Fos* immunohistochemistry.
II. MATERIALS AND METHODS

1. Animals and treatments

The experimental procedures were performed in accordance with the guidelines of the National Institute of Health (NIH) and the Korean Academy of Medical Sciences. Male Sprague–Dawley rats (250 ± 10 g, 12 weeks old) and female Sprague–Dawley rats (180 ±10 g, 8 weeks old) were used in this study. Female rats (n = 20) were allowed to mate with male rats (n = 20) for 24 h. One day later, female rats were separated from the male rats and housed individually in a plastic home cage at the controlled temperature (20 ± 2 °C) and the light-dark cycle of 12 h of light and 12 h of darkness (light on from 07:00 h to 19:00 h). Food and water were made available ad libitum. After confirming of pregnancy on the 14 days after mating, female rats were randomly divided into four groups: the control group, the 10 mg/kg *Ginseng radix*–treated group, the noise–treated group, the noise– and 10 mg/kg *Ginseng radix*–treated group (n = 5 for each group). Starting on the 15th day of pregnancy, rats of the prenatal noise–treated groups were applied with the 95 decibel supersonic machine sound for 1 honce a day until delivery.17 After birth, the offspring in each group was left undisturbed together with the respective mother for 28 days, and then, offspring rats were administered per os (P.O.) with *Ginseng radix* at the respective dose once a day for 7 days; they were sacrificed 6 weeks after birth. To obtain the aqueous extract of *Ginseng radix*, 200 g of *Ginseng radix* was added to distilled water, and extraction was performed by heating at 80°C concentrated with a rotary evaporator, and lyophilized. The resulting powder, weighing 30 g (a collection rate of 15 %), was diluted with saline.

2. Tissue preparation

For the sacrificial process, animals were first weighed and overdosed with Zoletil 50 (10 mg/kg, i.p.; Vibac, Carros, France). After a complete lack of response was observed, the rats were transcardially perfused with 50 mM phosphate-buffered saline (PBS) and then with 4% paraformaldehyde in 100 mM phosphate buffer (PB) at pH 7.4. The brains were dissected, postfixed in the same fixative overnight, and transferred into a 30% sucrose solution for cryoprotection. Serial coronal sections of 40 µm thickness were made using a freezing microtome (Leica, Nussloch, Germany).

3. c-Fos immunohistochemistry

c-Fos immunostaining was performed according to a protocol described by He et al.18 Eight sections on average were selected from each brain region spanning from Bregma -3.30 mm to -4.16 mm. Free-floating tissue sections were incubated overnight with rabbit anti–Fos antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a dilution of 1:1000, and the sections were then incubated for 1 h with biotinylated anti-rabbit secondary antibody (Vector Laboratories, Burlingame, CA, USA). The sections were subsequently incubated with avidin–biotin–peroxidase complex (Vector Laboratories, Burlingame, CA,
USA) for 1 h at room temperature. Immunoreactivity was visualized by incubating the sections in a solution consisting of 0.05% 3,3-diaminobenzidine and 0.01% H2O2 in 50 mM Tris-buffer (pH 7.6) for approximately 3 min. As the negative control, brain sections from the experiment were likewise processed using normal goat serum as the primary antibody; no c-Fos-like immunoreactivity was observed.

4. Data analysis

To quantify the number of Fos-positive cells in each areas of the hippocampus, cell counting was performed through a light microscope (Olympus, Tokyo, Japan). The number of Fos-positive cells inside pyramidal cell layer was counted hemilaterally in each of the selected hippocampal regions.

5. Statistical analysis

Statistical significance of differences were determined by one-way analysis of variance (ANOVA) followed by Dunncan’s post-hoc analysis, and results were expressed as mean ±standard error mean (S.E.M.) of Fos-positive cells. Differences were considered significant for $P < 0.05$.

III. RESULTS

1. Number of c-Fos-positive cells in the CA1 region of hippocampus

The number of c-Fos-positive cells in the CA1 region of hippocampus was 50.00 ± 5.56/mm² in the control group, 47.06 ± 5.81/mm² in the 10 mg/kg Ginseng radix-treated group, 143.14 ± 14.63 in the prenatal noise stress-treated group, and 59.80 ± 2.71/mm² in the prenatal noise stress- and 10 mg/kg Ginseng radix-treated group. The number of prenatal noise stress-treated group was increased significantly compared to the control group. The number of 10 mg/kg Ginseng radix-treated group was not increased significantly compared to the control group. And, the number of the prenatal noise stress— and 10 mg/kg Ginseng radix-treated group was decreased significantly compared to the prenatal noise stress-treated group (Fig. 1-2).
2. Number of c-Fos-positive cells in the CA2 and CA3 regions of hippocampus

The number of c-Fos-positive cells in the CA2 and CA3 regions of hippocampus was 11.60 ± 0.72/mm² in the control group, 30.00 ± 3.88/mm² in the 10 mg/kg Ginseng radix-treated group, 56.00 ± 3.43 in the prenatal noise stress-treated group, and 23.20 ± 4.98/mm² in the prenatal noise stress- and 10 mg/kg Ginseng radix-treated group. The number of prenatal noise stress-treated group was increased significantly compared to the control group. The number of 10 mg/kg Ginseng radix-treated group was not increased significantly compared to the control group. And, the number of the prenatal noise stress- and 10 mg/kg Ginseng radix-treated group was decreased significantly compared to the prenatal noise stress-treated group (Fig. 3-4).

3. Number of c-Fos-positive cells in the dentate gyrus region of hippocampus

The number of c-Fos-positive cells in the dentate gyrus region of hippocampus was 75.39 ± 7.45/mm² in the control group, 63.87 ± 12.51/mm² in the 10 mg/kg Ginseng radix-treated group, 130.37 ± 9.57 in the...
IV. DISCUSSION

Stressful experiences during the development period may exert a long-term effect on the hippocampal functions and may induce various psychosomatic problems such as mental retardation and developmental disorders. Various prenatal stresses have been reported to induce structural abnormality in the hippocampal formation. It with delayed neuronal cell death, and it has been shown that prolonged c-fos induction precedes neuronal death.\textsuperscript{20,21} Preston et al. reported that the c-Fos protein plays a causative role in the initiation of apoptosis.\textsuperscript{22} In previous study, prenatal stress induces apoptotic neurodegeneration in cerebellum and hippocampus of rats.\textsuperscript{23} Saljo et al. suggested that exposure to short-lasting impulse noise causes induction of apoptosis in the hippocampus of adult rat brain.\textsuperscript{24} From the present results, it was demonstrated that prenatal noise stress significantly increases the number of Fos-positive cells in the various hippocampal regions.

The aqueous extracts of \textit{Ginseng radix} are composed of a mixture, ginsenosides, trace minerals, and a variety of complex carbohydrates as well as peptides. \textit{Ginseng radix} was medicinal herb for treatment of various neurodegenerative disorders such as ischemia,\textsuperscript{4} Alzheimer's disease,\textsuperscript{25} and Parkinson's disease.\textsuperscript{26} In addition, administration of \textit{Ginseng radix} was associated with improvement learning and memory. Jin et al. reported that \textit{Ginseng radix} alleviates

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure6}
\caption{Photomicrographs of Fos-positive cells in the dentate gyrus region in each group. Sections were stained for c-Fos-positive cells (reddish brown). Scale bar represents 100 um. A, the control group; B, the 10 mg/kg Ginseng radix-treated group; C, the noise-treated group; D, the noise- and 10 mg/kg Ginseng radix-treated group.}
\end{figure}
scopolamine-induced learning disability and improves spatial working memory in mice. Nishijo et al. suggested that Ginseng radix ameliorates learning and memory deficits in an amnesia animal model. In addition, Ginseng radix and its constituents are known to possess anti-neoplastic, anti-stress, and anti-oxidant activities. Evidence supporting the medicinal efficacy of Ginseng radix based on its protective property against free radical attack has been presented. Zhang et al. reported that extracts of Ginseng radix scavenge hydroxyl radicals and protect unsaturated fatty acids from decomposition caused by iron-mediated lipid peroxidation. However, no study on the effect of Ginseng radix on the expression of hippocampal neurons containing c-Fos in the offspring rats with prenatal noise stress during pregnancy has been made yet. The present results demonstrated that postnatal Ginseng radix administration shown to suppress increments of c-Fos in the CA1, CA2 and CA3, and dentate gyrus of offspring rats with prenatal noise stress during pregnancy. Based on the present study, Ginseng radix may provide new therapeutic opportunities as an agent to counteract the effects of prenatal noise stress-induced hippocampal dysfunction through c-Fos change, and may be useful in the treatment of psychiatric problems in children of mothers who have experienced noise stress during pregnancy.

V. Conclusion

Exposure to noise during pregnancy adversely influenced the development of the fetus and neonate. In the present study, the influence of postnatal Ginseng radix administration on neuronal changes, in particular with respect to c-Fos expression in the hippocampus of offspring rats with prenatal noise stress during pregnancy was investigated via c-Fos immunohistochemistry.

From the present results,

1. The number of c-Fos-positive cells in the CA1 region in the prenatal noise stress and 10 mg/kg Ginseng radix-treated group was decreased significantly compared to the prenatal noise stress-treated group.

2. The number of c-Fos-positive cells in the CA2 and CA3 regions of hippocampus of the prenatal noise stress and 10mg/kg Ginseng radix-treated group was decreased significantly compared to the prenatal noise stress-treated group.

3. The number of c-Fos-positive cells in the dentate gyrus region of hippocampus of the prenatal noise stress and 10mg/kg Ginseng radix-treated group was decreased significantly compared to the prenatal noise stress-treated group.

Based on the present study, Ginseng radix may provide new therapeutic opportunities as an agent to counteract the effects of prenatal noise stress-induced hippocampal dysfunction through c-Fos change, and may be useful in the treatment of psychiatric problems in children of mothers who have experienced noise stress during pregnancy.
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