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인삼약침이 소음스트레스로 인한 태아쥐의 NOS 신경세포 발현에 미치는 영향

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Effect of Ginseng radix herb-acupuncture on noise stress-induced NOS expression in the offspring rats

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Abstract

목적 : 인삼은 전통적으로 학습과 기억능력 증진 및 각종 질병치료에 우수한 효과가 있는 것으로 알려져 있다. 본 연구에서는 이러한 인삼약침이 소음스트레스가 유발된 태아쥐의 해마 및 시상하부에서 nitric oxide synthase에 미치는 영향을 관찰하였다.

방법 : 인삼약침이 소음스트레스에 미치는 영향을 연구하기 위하여 태아기에 소음스트레스를 유발하고 생후 4주후 7일간 인삼약침을 투여한 후 NADPH-d 조직화학법을 시행하였다.

결과 : 소음스트레스는 태아쥐의 해마 및 시상하부에서 NOS 발현이 유의하게 증가하였다. 2. 인삼약침은 해마 CA1에서만 소음스트레스에 의해서 증가된 NOS 발현을 유의하게 억제하였다. 3. 인삼약침은 시상하부 PVN, DMH 및 LHA에서 소음스트레스에 의해서 증가된 NOS 발현을 유의하게 억제하였다.

결론 : 본 연구를 통하여 인삼약침이 소음스트레스가 유발된 태아쥐의 해마 및 시상하부에서 증가된 NOS 발현을 유의하게 억제시켜 태아의 스트레스 자극에 인삼약침치료가 유의한 효과가 있음을 확인하였다.

Key words : Ginseng radix; prenatal noise stress; nitric oxide synthase; hippocampus; hypothalamus

1. Introduction

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, anti-aging, and anti-oxidant actions[1-3].

It was reported that exposure to prenatal

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stress alters an individual's developmental trajectory through altered early brain development. Various physical and emotional stresses applied during pregnancy result in low birth-weight of the offspring, increased risk of premature delivery, and a higher incidence of neonatal abnormality[4]. Delayed motor and cognition development was also observed in the offspring of stressed pregnant rats[5]. In late gestation, the fetus can hear sound from the outside of the mother[6]. 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[7,8]. In addition, stress is associated with activation of the hypothalamic-pituitary-adrenal (HPA) axis. Various stress related inputs converge upon the neurons located in the paraventricular nucleus (PVN). In fact, this nucleus has a pivotal role in the control of pituitary-adrenocortical activity in response to stress.

The 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. In various studies, prenatal stress by restraint[9], alcohol[10] and noise[11] is known to influence learning and memory capabilities of the offspring by altering neuronal activity in the hippocampus and related structures[12].

Nitric oxide (NO), endogenously generated from L-arginine by NO synthase (NOS), is a free radical with signaling functions in the central nervous system (CNS). It has been known to play important roles implicated in numerous physiological and pathological processes in the brain[13]. Nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) is a histochemical marker specific for NOS in the CNS. Neurons containing NADPH-d have been reported to be relatively resistant to various toxic insults and neurodegenerative disorders.

In the present study, the influence of postnatal Ginseng radix herb-acupuncture on neuronal development, in particular with respect to NOS expression in the each regions of hippocampus and hypothalamus of offspring rats with prenatal noise stress during pregnancy was investigated via NADPH-d histochemistry.

2. Materials & Methods

2.1. 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 ±

10g, 8 weeks old) were used in this study. Female rats (n = 40) were allowed to mate with male rats (n = 40) 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℃) 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 14th days after mating, female rats were randomly divided into six groups (experimental 1) : the control group, the 50 mg/kg Ginseng radix-treated group, the 100 mg/kg Ginseng radix-treated group, the noise-treated group, the noise- and 50 mg/kg Ginseng radix-treated group, and the noise- and 100 mg/kg Ginseng radix-treated group and divided into five groups (experimental 2) : the control group, the noise-treated group, the noise- and 10 mg/kg Ginseng radix-treated group, and the noise-and 50 mg/kg Ginseng radix-treated group, and the noise- and 100 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 h once a day until delivery[14].

After birth, the offspring in each group was left undisturbed together with the respective mother for 28 days, and then, offspring rats were acupunctured at Chung-Wan (CV_{12}) acupoint once a day for 7 consecutive days at the respective doses; 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℃ concentrated with a rotary evaporator, and lyophilized. The resulting powder, weighing 20 g (a collection rate of 10%), was diluted with saline.

2.2. 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 ㎛ thickness were made using a freezing microtome (Leica, Nussloch, Germany).

2.3. NADPH-d histochemistry

Sections were then stained for NADPH-d activity according to a previously described protocol[15]. In brief, free-floating sections were incubated at 37℃ for 1 h in 100 mM PB containing 0.3% Triton X-100, 0.1 mg/ml nitroblue tetrazolium, and 0.1 mg/ml β-NADPH. The sections were then washed

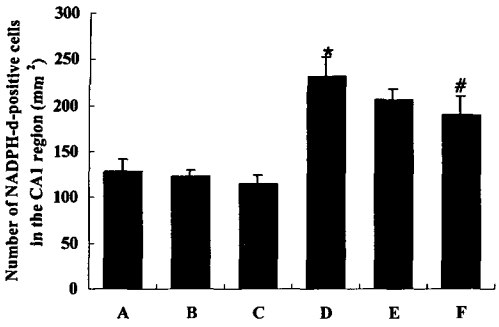

Fig. 1. Mean number of NADPH-d-positive cells in the CA1 region in each group.
* represents $P < 0.05$ compared to the control group.
# represents $P < 0.05$ compared to the noise-treated group.
A, the control group
B, the 50 mg/kg Ginseng radix-treated group
C, the 100 mg/kg Ginseng radix-treated group
D, the noise-treated group
E, the noise- and 50 mg/kg Ginseng radix-treated group
F, the noise- and 100 mg/kg Ginseng radix-treated group

three times with PBS and mounted onto gelatin-coated slides. The slides were air dried overnight at room temperature, and coverslips were mounted using Permount®.

2.4. Data analysis

To score the number of NADPH-d-positive cells in each area of the hippocampus, cell counting was performed using Image-Pro®Plus computer-assisted image analysis system (Media Cyberbetics Inc., Silver Spring, MD, USA) attached to a light microscope (Olympus, Tokyo, Japan). The staining intensities of the processed sections were assessed in a quantitative fashion according to a microdensitometrical method based on opticval density using and image analyzer (Multiscan, Fullerton, CA).

2.5. Statistical analysis

Statistical analysis was performed by Student's t-test. The results were presented as the mean ± standard error mean (S.E.M.). Differences were considered significant for $P < 0.05$.

3. Results

3.1. Number of NADPH-d-positive cells in CA1 region of hippocampus

The number of NADPH-d-positive cells in the CA1 region was about 128.92 ± 12.59/mm^2 in the control group, 123.04 ± 7.54/mm^2 in the 50 mg/kg Ginseng radix-treated group, and 114.71 ± 10.16/mm^2 in the 100 mg/kg Ginseng radix-treated group. This number was significantly increased to 231.86 ± 20.69/mm^2 in the noise-treated group, but was dose-dependently decreased again to 206.37 ± 11.57/mm^2 in the noise- and 50 mg/kg Ginseng radix-treated group, and 189.71 ± 20.10/mm^2 in the noise- and 100 mg/kg Ginseng radix-treated group (Fig. 1).

3.2. Number of NADPH-d-positive cells in CA2 and CA3 regions of hippocampus

The number of NADPH-d-positive cells in the CA2 and CA3 regions was about 30.40

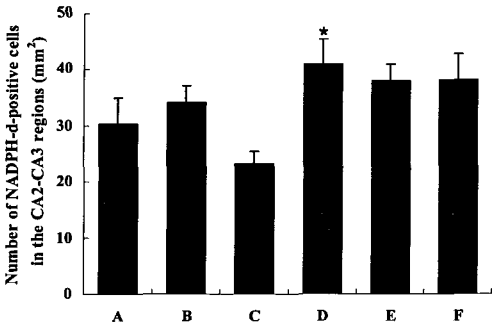

Fig. 2. Mean number of NADPH-d-positive cells in the CA2 and CA3 regions in each group.
* represents $P < 0.05$ compared to the control group.
# represents $P < 0.05$ compared to the noise-treated group.
A, the control group
B, the 50 mg/kg Ginseng radix-treated group
C, the 100 mg/kg Ginseng radix-treated group
D, the noise-treated group
E, the noise- and 50 mg/kg Ginseng radix-treated group
F, the noise- and 100 mg/kg Ginseng radix-treated group

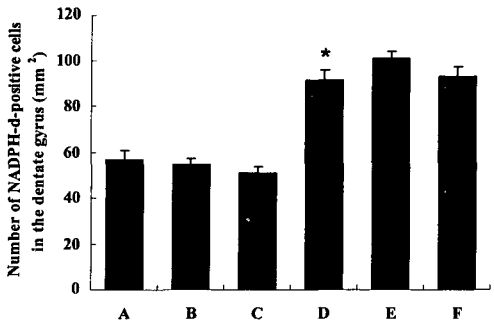

Fig. 3. Mean number of NADPH-d-positive cells in the dentate gyrus region in each group.
* represents $P < 0.05$ compared to the control group.
# represents $P < 0.05$ compared to the noise-treated group.
A, the control group
B, the 50 mg/kg Ginseng radix-treated group
C, the 100 mg/kg Ginseng radix-treated group
D, the noise-treated group
E, the noise- and 50 mg/kg Ginseng radix-treated group
F, the noise- and 100 mg/kg Ginseng radix-treated group

± 4.50/mm^2 in the control group, 34.00 ± 3.11/mm^2 in the 50 mg/kg Ginseng radix-treated group, and 23.20 ± 2.30/mm^2 in the 100mg/kg Ginseng radix-treated group. This number was significantly increased to 41.20 ± 4.33/mm^2 in the noise-treated group, but was dose-dependently decreased again to 38.00 ± 3.13/mm^2 in the noise- and 50 mg/kg Ginseng radix-treated group, and 38.20 ± 4.67/mm^2 in the noise- and 100 mg/kg Ginseng radix-treated group (Fig. 2).

3.3. Number of NADPH-d-positive cells in the dentate gyrus region of hippocampus

The number of NADPH-d-positive cells in the dentate gyrus region was about 56.54 ± 5.89/mm^2 in the control group, 54.19 ± 3.71/mm^2 in the 50 mg/kg Ginseng radix-treated group, and 50.79 ± 5.90/mm^2 in the 100 mg/kg Ginseng radix-treated group. This number was significantly increased to 91.88 ± 9.23/mm^2 in the noise-treated group, but was dose-dependently decreased again to 100.79 ± 7.73/mm^2 in the noise- and 50 mg/kg Ginseng radix-treated group, and 93.19 ± 20.10/mm^2 in

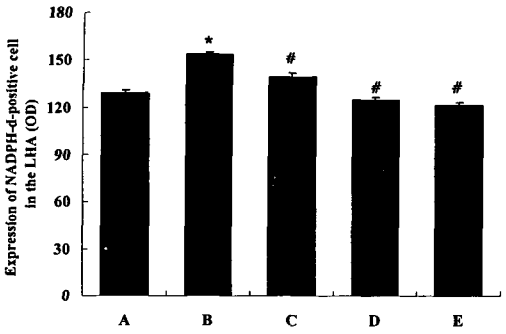

Fig. 4. Mean densities of NADPH-d-positive cells in the lateral hypothalamic area (LHA) region in each group.
* represents P < 0.05 compared to the control group.
# represents P < 0.05 compared to the noise-treated group.
A, the control group
B, the noise-treated group
C, the noise- and 10 mg/kg Ginseng radix-treated group
D, the noise- and 50 mg/kg Ginseng radix-treated group
E, the noise- and 100 mg/kg Ginseng radix-treated group

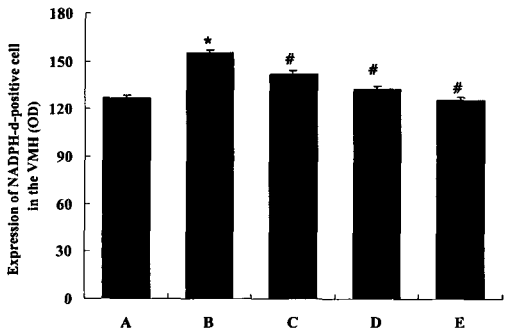

Fig. 5. Mean densities of NADPH-d-positive cells in the ventromedial hypothalamic nucleus (VMH) region in each group.
* represents P < 0.05 compared to the control group.
# represents P < 0.05 compared to the noise-treated group.
A, the control group
B, the noise-treated group
C, the noise- and 10 mg/kg Ginseng radix-treated group
D, the noise- and 50 mg/kg Ginseng radix-treated group
E, the noise- and 100 mg/kg Ginseng radix-treated group

the noise- and 100mg/kg Ginseng radix-treated group (Fig. 3).

3.4. The density of NADPH-d-positive cells in lateral hypothalamic area (LHA) region of hypothalamus

The density of NADPH-d-positivity in the hypothalamic LHA region was 129.42 ± 1.90 in the control group. This was increased significantly to 153.76 ± 1.47, in the noise stress-treated group compared to the control group; but the densities dropped to 139.37 ± 2.56 in the noise- and 10 mg/kg Ginseng radix-treated group, to 124.84 ± 1.86 in the noise- and 50 mg/kg Ginseng radix-treated group, and to 121.92 ± 1.61 in the noise- and 100 mg/kg Ginseng radix-treated group (Fig. 4).

3.5. The density of NADPH-d-positive cells in ventromedial hypothalamic nucleus (VMH) region of hypothalamus

The density of NADPH-d-positivity in the hypothalamic VMH region was 127.11 ± 1.27 in the control group. This was increased significantly to 155.47 ± 1.31, in the noise stress-treated group compared to the control group; but the densities dropped to 141.92 ± 3.18 in the noise- and 10 mg/kg Ginseng rad-

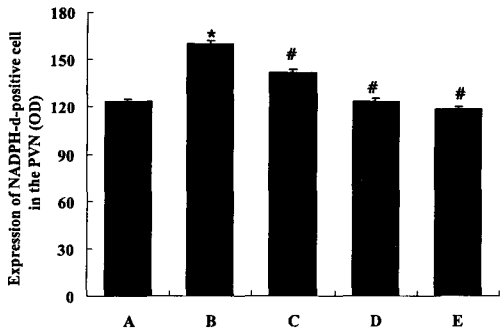

Fig. 6. Mean densities of NADPH-d-positive cells in the periventricular nucleus (PVN) region in each group.
* represents $P < 0.05$ compared to the control group.
# represents $P < 0.05$ compared to the noise-treated group.
A, the control group
B, the noise-treated group
C, the noise- and 10 mg/kg Ginseng radix-treated group
D, the noise- and 50 mg/kg Ginseng radix-treated group
E, the noise- and 100 mg/kg Ginseng radix-treated group

ix-treated group, to 132.42 ± 2.41 in the noise- and 50 mg/kg Ginseng radix-treated group, and to 125.71 ± 1.57 in the noise- and 100 mg/kg Ginseng radix-treated group (Fig. 5).

3.6. The density of NADPH-d-positive cells in periventricular nucleus (PVN) region of hypothalamus

The density of NADPH-d-positivity in the hypothalamic PVN region was 123.41 ± 3.05 in the control group. This was increased significantly, to 160.61 ± 0.60, in the noise stress-treated group compared to the control group; but the densities dropped to 141.65 ± 4.06 in the noise- and 10 mg/kg Ginseng radix-treated group, to 123.87 ± 2.11 in the noise- and 50 mg/kg Ginseng radix-treated group, and to 118.84 ± 1.39 in the noise- and 100 mg/kg Ginseng radix-treated group (Fig. 6).

4. 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 was reported that prenatal stress reduced the density of the pyramidal neurons, decreased the total hippocampal volume, and induced the synaptic loss in the hippocampus[4,9]. In addition, Coe et al.[11] suggested that prenatal environment can alter behavior, dysregulate neuroendocrine systems, and affect the hippocampal structure of primates in a persistent manner through suppression of neurogenesis.

NO is diffusible free radical that has recognized as a biological messenger involved in several physiological and pathological functions[16]. In the CNS, NO has been implicated in various neurophysiological functions including feeding, anxiety, immune response, and synaptic plasticity[17-20]. Moreover, NO has also been implicated in the physiological processes of learning and memory formation and administration of NOS inhibitors results in learning

disability and memory deficits[21,22]. In the pathologic conditions, NO acts as a major toxic mediator when overproduced[23,24]. Sustained overproduction of NO is known to induce neurodegenerative change[25] and cell death[26]. Patients with Alzheimer disease showed increased in iNOS expression in the hippocampus, frontal and entorhinal cortex (Heneka et al., 2001). Traumatic brain injury showed transient increase in NOS activity[27]. In the cerebral cortex of epilepsy patients, particularly in those with a long seizure history, the number and labeling intensity of NOS-positive neurons was higher than normal[28]. The present results showed that the exposure to the noise during pregnancy caused the increase of the NOS expression in the hippocampus and hypothalamus of offspring rat.

The aqueous extracts of Ginseng radix are composed of a mixture, ginsenosides, trace minerals, and a variety of complex carbohydrates as well as peptides. Ginsneg radix was medicinal herb for treatment of various neurodegenerative disorders such as ischemia[3], Alzheimer's disease[29], and Parkinson's disease[30]. In addition, Ginseng radix herb-acupuncture was associated with improvement of learning and memory. Jin et al.[31] reported that Ginseng radix alleviates scopolamine-induced learning disability and improves spatial working memory in mice. Nishijo et al.[32] 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.[33] 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 and hypothalamic neurons containing NOS in the offspring rats with prenatal noise stress during pregnancy has been made yet. The present results demonstrated that postnatal Ginseng radix herb-acupuncture showed to suppress increments of NOS in the hippocampus and hypothalamus 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 and hypothalamic dysfunction through NOS change, and may be useful in the treatment of psychiatric problems in children of mothers who have experienced noise stress during pregnancy.

5. Conclusion

In the present study, the effect of Ginseng radix herb-acupuncture on the NOS-positive cells in the hippocampus and hypothalamus of offspring rats with prenatal noise stress during pregnancy was elucidated using

NADPH-d histochemistry assay.

The results are as follows:

1. In the CA1 region, the noise- and 100 mg/kg Ginseng radix-treated group decreased significantly compared to the noise treated group.
2. In the CA2 and CA3 regions, the noise- and Ginseng radix-treated groups are no significant decrease to the noise treated group.
3. In the dentate gyrus regions, the noise- and Ginseng radix-treated groups are no significant decrease to the noise treated group.
4. In the LHA region, the noise- and Ginseng radix-treated groups decreased significantly compared to the noise treated group.
5. In the VMH region, the noise- and Ginseng radix-treated groups decreased significantly compared to the noise treated group.
6. In the PVN region, the noise- and Ginseng radix-treated groups decreased significantly compared to the noise treated group.

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