Effects of *Angelicae Gigantis Radix* on Noise Stress-induced c-Fos Expression in Rats

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Previous studies reported that exposure to noise during pregnancy adversely influenced the development of the fetus and neonate. In Oriental medicine, medications based on Angelicae gigantis radix have been known to be of efficacy in the treatment of various diseases. c-Fos, an immediate early gene whose expression is sometimes used as a marker for stimulus-induced changes in the metabolic activity of neurons. In the present study, the influence of postnatal Angelicae gigantis radix administration on c-Fos expression in the each region of hippocampus of offspring rats with prenatal noise stress during pregnancy was investigated. From the present results, exposure to the prenatal stress during pregnancy enhanced c-Fos expression, whereas exposure to postnatal Angelicae gigantis radix suppressed c-Fos expression in the offsprings with prenatal noise stress during pregnancy. Based on the present study, Angelicae gigantis radix may provide new therapeutic opportunities as an agent to counteract the effects of prenatal noise stress- induced hippocampal dysfunction, and may be useful in the treatment of psychiatric problems in children of mothers who have experienced noise stress during pregnancy.

Key words : Angelicae gigantis radix, prenatal noise stress, c-Fos, hippocampus

Introduction

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[1]. 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[2,3]. In addition, prenatal noise stress is known to influence learning and memory capabilities of the offspring by altering neuronal activity in the hippocampus[4].

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[5-7].

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[8-10]. Recently, it was reported that prenatal stress produces region-selective changes in expression of inducible transcription factors (ITFs) and metabolism in the brain[11,12].

Angelicae gigantis radix, which belongs to the Umbelliferae family, is one of the best known Oriental medicinal herbs, and have used for invigorating blood circulation[13]. In addition, medications based on Angelicae gigantis radix possesses a variety of pharmacological effects including immunoregulation, anti-oxidation, anti-tumor, anti-irradiation injury, promotion of hematopoiesis[14,15]. In the present study, the influence of postnatal Angelicae gigantis radixadministration 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 immunohistpchemistry.

Materials and Methods

1. Animals and treatments

The experimental procedures were performed in

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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= 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 14 days after mating, female rats were randomly divided into five groups: the control group, the noise-treated group, the noise- and 10 mg/kg Angelicae gigantis radix-treated group, the noise- and 50 mg/kg Angelicae gigantis radix-treated group, and the noise- and 100 mg/kg Angelicae gigantis 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[16]. 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 radixat the respective dose once a day for 7 days; they were sacrificed 6 weeks after birth. To obtain the aqueous extract of Angelicae gigantis radix, 200 g of Angelicae gigantis 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 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 ㎛ thickness were made using a freezing microtome (Leica, Nussloch, Germany).

3. c-Fos immunohistocheistry

c-Fos immunostaining was performed according to a protocol described by He et al[17]. 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% H_2O_2 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 $P < 0.05$.

Results

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

The number of c-Fos-positive cells in the control group was 51.47 ± 5.93/mm^2, and this number was increased significantly, to 165.03 ± 19.23/mm^2, in the prenatal noise stress-treated group compared to the control group. But this figure rose to 99.13 ± 2.55/mm^2 for the noise- and 10 mg/kg Angelicae gigantis radix-treated group, to 70.34 ±5.51/mm^2 for the noise- and 50 mg/kg Angelicae gigantis radix-treated group. This trend was slightly reversed in the noise- and 100 mg/kg Angelicae gigantis radix-treated group, in which the number of c-Fos-positive cells observed was 98.62 ± 13.56/mm^2 (Fig. 1).

2. Number of c-Fos-positive cells in the CA2 and CA3 regions of hippocampus

The number of c-Fos-positive cells in the control group was 11.33 ± 0.67/mm^2, and this number was increased significantly, to 65.67 ± 7.60/mm^2, in the prenatal noise stress-treated group

compared to the control group. But this figure rose to 43.56 ± 6.44/mm^2 for the noise- and 10 mg/kg Angelicae gigantis radix-treated group, to 37.33 ± 3.27/mm^2 for the noise- and 50 mg/kg Angelicae gigantis radix-treated group. This trend was slightly reversed in the noise- and 100 mg/kg Angelicae gigantis radix-treated group, in which the number of c-Fos-positive cells observed was 51.67 ± 4.42/mm^2 (Fig. 2).

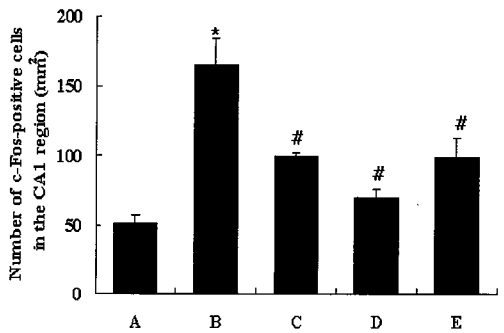

Fig. 1. Mean number of c-Fos-positive cells in the CA1 region in each group. * represents P < 0.05 compared to the control group. # represents P < 0.05 compared to the noise-treated group. A, the control group; B, the noise-treated group; C, the noise- and 10 mg/kg Angelicae gigantis radix-treated group; D, the noise- and 50 mg/kg Angelicae gigantis radix-treated group; E, the noise- and 100 mg/kg Angelicae gigantis radix-treated group.

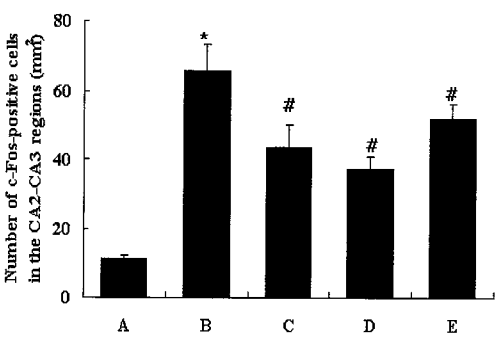

Fig. 2. Mean number of c-Fos-positive cells in the CA2 and CA3 regions in each group. * represents P < 0.05 compared to the control group. # represents P < 0.05 compared to the noise-treated group. A, the control group; B, the noise-treated group; C, the noise- and 10 mg/kg Angelicae gigantis radix-treated group; D, the noise- and 50 mg/kg Angelicae gigantis radix-treated group; E, the noise- and 100 mg/kg Angelicae gigantis radix-treated group.

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

The number of c-Fos-positive cells in the control group was 86.82 ± 9.91/mm^2, and this number was increased significantly, to 116.49 ± 12.40, in the prenatal noise stress-treated group compared to the control group. But this figure rose to 55.26 ± 4.79/mm^2 for the noise- and 10 mg/kg Angelicae gigantis radix-treated group, to 47.70 ± 6.78/mm^2 for the noise- and 50 mg/kg Angelicae gigantis radix-treated group. This trend was slightly reversed in the noise- and 100 mg/kg Angelicae gigantis radix-treated group, in which the number of c-Fos-positive cells observed was 59.77 ± 9.33/mm^2 (Fig. 3).

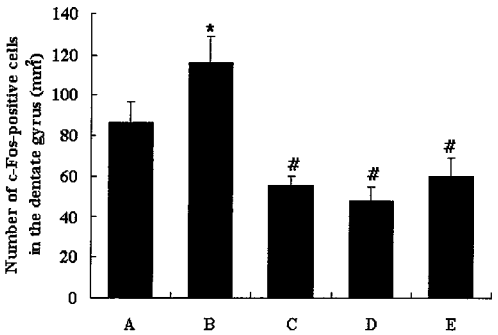

Fig. 3. Mean number of c-Fos-positive cells in the dentate gyrus in each group. * represents P < 0.05 compared to the control group. # represents P < 0.05 compared to the noise-treated group. A, the control group; B, the noise-treated group; C, the noise- and 10 mg/kg Angelicae gigantis radix-treated group; D, the noise- and 50 mg/kg Angelicae gigantis radix-treated group; E, the noise- and 100 mg/kg Angelicae gigantis radix-treated group.

Discussion and Conclusion

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 has beenshown that prenatal stress, including both noise stress[4] and restrained stress[18] during pregnancy, suppress the formation of hippocampus in a variety of mammalian species. In addition, Coe et al.[4] 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.

c-Fos is induced by a variety of stimuli such as brain injury. In various studies, c-fos gene expression has been associated with delayed neuronal cell death, and it has been shown that prolonged c-fos induction precedes neuronal death[19,20]. Preston et al.[21] reported that the c-Fos protein plays a causative role in the initiation of apoptosis. In previous study, prenatal stress induces apoptotic neurodegeneration in cerebellum and hippocampus of rats[22]. Saljo et al.[23] suggested that exposure to short-lasting impulse noise causes induction of apoptosis in the hippocampus of adult rat brain. From the present results, it was demonstrated that prenatal noise stress significantly increases the number of Fos-positive cells in the various hippocampal regions.

Recently, alternative and complementary approaches such as natural, herbal, and nutritional supplements, and physical

manipulations have been increasing popularity. Herbs of Angelicae gigantis radix family not only has regulatory effects on cytokines, complements, immunocompetent cells such as lymphocyte, macrophage but also show manifold immunological status, drug dose and drug administration surroundings[15,24]. In addition, a purified extract from a mixture of ten oriental herbs including Angelicae gigantis radixprotects against restraint stress-induced susceptibility in mice[25]. However, no study on the effect of Angelicae gigantis 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 Angelicae gigantis 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, Angelicae gigantis 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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