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Effects of Aqueous Extract of Schizandrae Fructus on Lead-Induced Change of Monoamine Neurotransmitters in Hippocampus

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ABSTRACT

The effects of aqueous extract of Schizandrae Fructus (AESC) on lead (Pb)-induced changes of monoamine neurotransmitters in the hippocampus (HIP) of adult rats were investigated. Male Sprague-Dawley rats were received intraperitoneal (i.p.) administration of Pb acetate (5 mg/kg/d) for 28 days and sacrificed 7 days after the last administration. Concentrations of norepinephrine (NE), dopamine (DA), serotonin (5-HT), 5-hydroxyindole acetic acid (5-HIAA) in HIP were measured by HPLC. There were significant decreases of NE, DA, 5-HT and 5-HIAA in Pb treated rats ($P < 0.05$), while pretreatment with AESC (100 mg/kg/d or 300 mg/kg/d, p.o., 2 h before Pb) greatly inhibited the decrease of monoamine transmitters, respectively ($P < 0.05$). Also, AESC (300 mg/kg/d) significantly increased the reduction of glutathione contents and superoxide dismutase activities in HIP induced by chronic Pb. These results suggest that AESC ameliorates Pb-induced depletion of monoamine neurotransmitters in HIP through its antioxidant activity.

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Ⅰ. Introduction

It is well documented that chronic exposure to lead (Pb) results in a wide range of neurobehavioral and cognitive dysfunctions, such as behavioral abnormalities, decreased hearing, learning impairment and impaired memory in humans and experimental animals[1,2]. Many experimental studies have proposed that these brain dysfunctions probably come from long-lasting adverse effects of Pb on neurotransmitter systems in brain. Several lines of evidence indicate that chronic Pb exposure influences monoaminergic systems, producing a widespread decrease in neurotransmitter levels in brain[3,4].

The hippocampus (HIP), an important brain structure responsible for acquisition of spatial learning is susceptible to Pb-induced neurotoxicity. Therefore, the HIP frequently serves as an excellent site where the neurobiological mechanisms of Pb-induced neurotoxicity are investigated[5]. Jett et al. showed that the direct injection of Pb into the hippocampus of normal adult rats was able to produce a deficit in the acquisition of the water maze learning task[6]. The HIP is heavily innervated by monoaminergic afferents from several brain stem regions. Physiological and pharmacological studies showed that monoamine neurotransmitter systems in HIP play critical roles in the regulation of cognitive processes and locomotor activity[7]. For example, noradrenergic afferents from the locus coerulus to the HIP is closely associated with the spatial learning impairment induced by repeated administration of ethanol[8].

Due to the abundance of polyunsaturated fatty acids and high rate of oxygen utilization, the tissues in brain are highly vulnerable to oxidative stress. Much evidence suggested that cellular damage coming from attack of free radicals can be involved in the Pb-induced depletion of neurotransmitters[9]. In the previous study we demonstrated that chronic administration of Pb significantly decreased levels of monoamines in mice brain through disruption of the prooxidant/ antioxidant balance[4].

The Schizandrae Fructus, one of the widely used Oriental herbs, has been used to treat several human diseases including insomnia and amnesia[10]. It has been reported the therapeutic role of Schizandrae Fructus in central nervous disorders is closely associated with its effects on neurotransmitter systems. Gomisin A, an important component of Schizandrae Fructus greatly improved scopolamine -induced cognitive impairments through inhibiting acetylcholinesterase activity[11].

It has already been documented the antioxidant activity of Schizandrae Fructus plays a leading role in its therapeutic actions[12]. Schizandrin, another important component of Schizandrae Fructus greatly prevented the cold stress-induced increase of malonic dialdehyde in rat liver homogenate

and inhibited non-enzymatic ascorbate-dependent lipid peroxidation in liver homogenate in vitro[13].

Previously we demonstrated aqueous extract of Schizandrae Fructus (AESC) ameliorated Cd-induced depletion of monoamine neurotransmitters in brain through its antioxidant activity[14]. In the present study we investigated the effects of AESC on Pb-induced changes of monoamine neurotransmitters in the HIP and the possible mechanism involved in it.

II. Materials and methods

1. Preparation of AESC

The dried Schizandrae Fructus was purchased from a local market and ground to fine powder, and consecutively extracted under reflux with water for 1 h. The obtained water extract was evaporated under reduced pressure at temperature of 37 °C and lyophilized.

2. Reagents

Pb acetate, sodium octanesulfonic acid, acetonitrile, tetrahydrofurane, norepinephrine (NE), dopamine (DA), serotonin (5-HT), and 5-hydroxyindole acetic acid (5-HIAA) were purchased from Sigma Co. (St. Louis, MO, USA). All other drugs were of analytical or HPLC grade.

3. Animals and experimental design

Adult male Sprague-Dawley rats (250-270 g) were obtained from the Laboratory Animal Center in Yanbian Medical College of Yanbian University (Yanji, China). The rats were individually housed in a controlled environment during all experimental treatments. Food and water were provided ad libitum and the rats were maintained on a 12-hour light/dark cycle. All animal procedures were approved by the Institutional Animal Care and Use Committee and were accomplished in accordance with the provisions of the NIH "Guide for the Care and Use of Laboratory Animals."

The rats were divided into four groups. Group 1: distilled water (D.W.) + saline (W + S), Group 2: D.W. + Pb acetate (W + Pb), Group 3: AESC (100 mg/kg/d) + Pb acetate (AESC100 + Pb), Group 4: AESC (300 mg/kg/d) + Pb acetate (AESC300 + Pb). The rats were given oral administration (p.o.) of D.W. or AESC (100 mg/kg/d or 300 mg/kg/d, dissolved in D.W). Two hours after AESC the rats were also intraperitoneally (i.p.) received saline or Pb acetate (5 mg/kg/d, dissolved in saline) for 4 weeks. One week after the last treatment, the rats were killed and the brain removed quickly for the dissection of HIP to prepare tissue homogenates.

4. Monoamines analysis

HIP samples were sonicated in 1 ml of 0.1 M HClO4 for 30 s, and centrifuged for 15 min at 26,000 g, 4ºC. Then, a 20 μl supernatant aliquot was injected directly into the HPLC with a coulmoetric detector (Coulochem II; ESA, Bedford, MA, USA). The HPLC system consisted of a C18 reverse-phase column (5 u ODS; Altex, Ann Arbor, MI, USA) and an electrochemical transducer with a glassy carbon electrode set at 350 mV. The mobile phase was 0.163 M citric acid, pH 3.0, containing 0.02 mM EDTA with 0.69 mM sodium octanesulfonic acid as an ion-pairing reagent, 4% (v/v) acetonitrile and 1.7% (v/v) tetrahydrofurane. Peaks and values of NE, DA, 5-HT and 5-HIAA in samples were identified and calculated by comparing their retention times

and peak heights with those of standards. Results were reported as ng/g wet tissue. The protein concentration in brain homogenate was determined by the method of Lowry et al.[15].

5. Determination of antioxidant activities

The levels of reduced glutathione (GSH) in the HIP homogenates were determined by the method of Moron et al.[16] based on the reaction with Ellman's reagent (19.8 mg DTNB in 100 ml of 0.1% sodium citrate). The activities of superoxide dismutase (SOD) were also measured spectrophotometrically in the HIP homogenates by the method of Kakkar et al.[17].

6. Statistical analysis

All data were expressed as mean ± SEM, and analyzed statistically by one-way ANOVA followed by Tukey's multiple comparison tests using SPSS software (Student's version). $P < 0.05$ was considered statistically significant.

Ⅲ. Results

1. Effects of AESC on Pb induced neurotoxicity

Administration of Pb acetate (5 mg/kg/d, i.p. for 28 days) to rats led to damage to central nervous system evidenced by significant decreases of NE, DA, 5-HT and 5-HIAA in HIP as compared to those of saline treated rats ($P < 0.05$). Pretreatment with AESC (100 mg/kg/d or 300 mg/kg/d, p.o., 2 h before Pb) greatly inhibited the decreased levels of NE, DA, 5-HT and 5-HIAA in a dose dependent way(Fig. 1)($P < 0.05$).

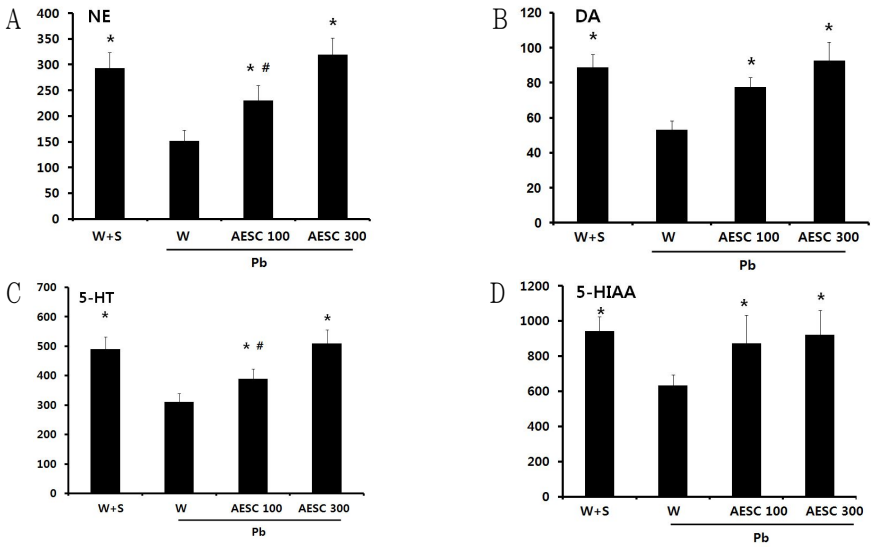

Fig. 1. Effects of AESC on Pb-induced changes of concentrations of monoamines in the rat hippocampus.

NE = norepinephrine (A); DA = dopamine (B); 5-HT = serotonin (C); 5-HIAA = 5-hydroxyindole acetic acid (D). Data are presented as mean ± SEM ng/g wet tissue in the hippocampus from rats treated daily with Pb acetate (5 g/kg/d, i.p.) for 28 days and sacrificed 7 days after the last administration. The numbers in parentheses indicate the number of rats in each group. *: $P < 0.05$, compared to W + Pb; #: $P < 0.05$, compared to AESC300 + Pb (ANOVA, followed by the post hoc Tukey test).

2. Effects of AESC on Pb produced damage to antioxidant defense system in HIP

The content of GSH and activity of SOD in the HIP are important biochemical parameters to estimate the capacity to defense oxidative stress. Administration of Pb to rats resulted in significant reduction of GSH contents and SOD activities in the rats HIP as compared to those in saline treated rats ($P < 0.05$), while pretreatment with AESC (300 mg/kg/d, p.o.) reversed the alterations of GSH and SOD(Table 1)($P < 0.05$).

Table 1. Effects of AESC on Pb-induced changes of concentrations of monoamines in the rat hippocampus.

groups	GSH (μg/mg protein)	SOD (activity unit)
W + S (8)	5.70 ± 0.53*	15.72 ± 2.21*
W + Pb (8)	3.18 ± 0.35	7.83 ± 1.11
AESC300 + Pb (8)	5.01 ± 0.61*	14.49 ± 2.11*

Data are presented as mean ± SEM, eight rats per group. The activities of SOD are expressed as follows: one unit of activity was taken as the enzyme reaction, which gave 50% inhibition of nitroblue tetrazolium reduction in 1 min/mg protein from cortex of rats. *: $P < 0.05$, compared to W + Pb (ANOVA, followed by the post hoc Tukey test).

Ⅳ. Discussion

NE, DA and 5-HT are most important classic neurotransmitters, and the alteration of their concentrations in brain is a hallmark indicating dysfunction of central nervous system. Previous study we demonstrated 20 days of intraperitoneal injection of Pb acetate (10 mg/kg/d) markedly decreased monoamine neurotransmitters 10 days after the last dose of Pb in mice brain[4]. In the present study we observed the significant reduction of monoamines in HIP exposure to chronic Pb. These results were also identical to similar studies done by other investigators[18].

Two potential mechanisms may underlie the depletion of monoamines in the HIP in Pb-intoxicated rats. One is down-regulation of biosynthesis of monoamines, the other is up-regulation of elimination of neurotransmitters. 5-HIAA is a metabolized form of 5-HT, in the present study, both of 5-HT and 5-HIAA were significantly decreased in Pb treated rats as compared with saline treated rats, it indicates the decrease of 5-HT in brain induced by Pb may come from the down-regulation of biosynthesis. This hypothesis was supported by the study done by McIntosh et al.. In their study McIntosh et al. demonstrated that there was significant down-regulation of activities of tyrosine hydroxylase (the key enzyme of biosynthesis of DA and NE) in the rat brain after chronic intake of Pb[18]. Meanwhile, in the same study, McIntosh et al. also observed enhancement of activities of phenylethanolamine- N-methyl transferase (the enzyme responsible for biosynthesis of epinephrine from NE) in Pb-treated rats[18]. In previous study we also observed that there was significant increase of activities of acetylcholinesterase in the mouse brain when chronically exposed to Pb[4]. These evidences offer support for the notion that decreased levels of monoamines in the brain

induced by Pb may come from over-elimination of neurotransmitters.

The deficiency of antioxidant molecules can lead to the alteration of levels of neurotransmitters in brain since most of enzymes involved in biosynthesis and elimination of neurotransmitters in brain are vulnerable to oxidative stress. Several studies show that the capacity of antioxidant defense system is adversely affected by Pb[9]. The decreased levels of GSH and reduced activities of SOD are important biomarkers to show deficiency of tissue antioxidant capacity. In the present study there was significant decrease of GSH concentrations and SOD activities in rats treated with Pb. These results are also consistent with the data obtained in other studies[19].

Recently extracts from traditional oriental herbs have been received great attention in treating disorders in central nervous system induced by intake of heavy metals. The beneficial effects of extracts from oriental herbs on heavy metal neurotoxicities are attributed to improved antioxidant activity, which potentially reduces generation of the active free radicals harmful to all cells and proteins, further to protect neurotransmitter systems in brain[4,14].

Numerous studies have shown that Schizandrae Fructus is full of components with antioxidant activities. Ko et al. demonstrated that extract of Schizandrae Fructus decreased the elevation of malonic dialdehyde and enhanced GSH status in liver induced by CCl_4[20]. Also dibenzocyclooctenes, lignans from Schizandrae Fructus exhibited a protective action against oxidative stress-associated ageing-related brain ischemia[21].

In the previous study, we demonstrated that AESC can improve Cd-induced monoamines depletion in the rat brain through promotion of antioxidant systems in brain. In the first part of this study, we observed prophylactic administration of AESC (100 mg/kg/d, 300 mg/kg/d, p.o.) before Pb dose-dependently inhibited the decrease of NE, DA, 5-HT and 5-HIAA in the rat HIP. In consistent with this, in the second part of this study, we also showed AESC (300 mg/kg/d) significantly ameliorated the deficiency of GSH and SOD in HIP induced by Pb.

In summary, this study investigated the effect of aqueous extract of Schizandrae Fructus on Pb-induced decrease of monoamine neurotransmitters in the HIP and its possible mechanism. AESC (100 mg/kg/d, 300 mg/kg/d) greatly increased the reduction of neurotransmitters in the HIP and significantly reversed the decrease of GSH contents and SOD activities induced by Pb. It indicates that preventive administration of AESC prior to Pb can protect antioxidant system in brain against the damage coming from Pb intake, further block the decrease of monoamine neurotransmitters in brain. This study may provide a clue to the role of Schizandrae Fructus in treating Pb-induced neurotoxicity.

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