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Effects of maternal mild zinc deficiency and zinc supplementation in offspring on spatial memory and hippocampal neuronal ultrastructural changes

XiaoDan Yu Ph.D., M.D. [a,*], LongMei Jin M.D. [b], XiaoHua Zhang M.D. [b], XiaoGang Yu M.D. [a]

[a] MOE-Shanghai Key Laboratory of Children's Environmental Health, Shanghai Institute for Pediatric Research, XinHua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China
[b] Minhang District Maternal and Child Health Hospital, Shanghai, China

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ABSTRACT

Objective: Knowledge about the hippocampal morphologic mechanisms of learning and memory for maternal mild zinc deficiency during pregnancy/lactation followed by zinc supplementation of pups after weaning is limited. This study examined the effects of zinc deficiency and zinc supplementation on cognition and hippocampal neurons.
Methods: One-day pregnant rats were randomly divided into four groups ($n = 12$): control (CO), pair-fed (PF), zinc-deprived (ZD), and oral zinc-supplemented (OZS). The CO and PF groups were fed a control diet (zinc 25 μg/g diet), and the others were fed a mildly zinc-deficient diet (zinc 2 μg/g diet) during pregnancy and lactation. After weaning (day 21), offspring in the OZS group were switched to a control diet. After 35 d, the behavioral function of the offspring was tested with the Morris water maze test. The ultrastructure of the hippocampal CA3 area was observed under a transmission electron microscope.
Results: Compared with the CO and PF groups, rats in the ZD group spent more time finding the latent platform and swam longer distances ($P < 0.05$). The time used finding the platform and the swimming distance in the OZS group were similar to those in the CO and PF groups ($P > 0.05$). In addition, apoptotic neuronal changes in the hippocampus were observed in the ZD group, whereas the reversal of neuronal morphologic changes was observed in the OZS group.
Conclusion: The changes in hippocampal neuron morphology were consistent with the changes in the learning and memory ability of mildly zinc-deficient and zinc-supplemented offspring.

Introduction

Severe zinc deficiency is considered rare, whereas mild or moderate zinc deficiency is more widespread [1]. It is estimated 82% of pregnant women worldwide have a zinc intake lower than the recommended dietary intake, and this may approach 100% in developing countries [2]. Zinc is one of the most abundant divalent metal ions in the central nervous system and is stored mainly in the hippocampal zinc-rich mossy fiber pathway, which is important for maintaining cognitive function [3,4]. Zinc is important for myelination and for the release of the neurotransmitters γ-aminobutyric acid and glutamate, which are key modulators of neuronal excitability [5]. The developing nervous system is a prime target for the disruptive effects of zinc deficiency, because the brain undergoes its most rapid period of maturation during fetal life. Studies have shown a correlation between maternal zinc status and neonatal and infant behavior and cognitive function [6]. Few intervention studies in human populations have suggested that improving maternal zinc status through prenatal supplementation might improve fetal neurobehavioral development [6]. However, the limited studies on the effects of zinc supplementation on cognitive recovery in zinc-deprived animal offspring have reported conflicting results [7,8]. Thus, more research is needed to allow definitive conclusions.

In contrast, the neuronal pathomechanism of zinc supplementation remains unknown. In the central nervous system, zinc appears to be under strict homeostatic control [9]. Zinc deficiency occurring early in life results in lower hippocampal and cerebral zinc concentrations [10]. An extensive decreased zinc concentration leads to the activation of apoptosis in different cells including neurons [11–13], although the typical apoptotic

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* Corresponding author. Tel.: +0086-21-2507-8868; fax: +0086-21-2507-8857.
E-mail address: shahd1230@yahoo.com.cn (X. Yu).

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changes have been observed in neurons in different experimental models in vivo [14,15] and in vitro [16,17]. However, no data are available regarding the effects of zinc supplementation on the neuronal morphologic changes in offspring deprived of zinc during gestation and lactation. Thus, the objective of this study was to explore the effects of maternal mild zinc deficiency during pregnancy and lactation followed by zinc supplementation in offspring on cognition and hippocampal neuronal morphology.

Materials and methods

Diets

Rats were fed an egg-white protein-based semipurified experimental diet based on AIN-93 recommendations [18]. The experimental diets differed only in zinc content, containing zinc 25 μg/g of diet (control diet) or 2 μg/g diet (mildly zinc-deficient diet) as zinc carbonate, which was confirmed by atomic absorption spectrophotometry (Thermo Electron, Waltham, Massachusetts, USA).

Rats

This study complied with the Guide for the Use and Care of Laboratory Rats and was administered under the auspices of the Animal Resource Services of Xinhua Hospital affiliated to the Medical School of Shanghai Jiaotong University, which is accredited by the Chinese Association for the Accreditation of Laboratory Animal Care. Twenty-four virgin Sprague-Dawley rats (220–250 g) were obtained from a commercial source (Bikei Animal Company, Shanghai, China). The rats were maintained in stainless-steel hanging cages in a temperature-controlled facility with a 12-h dark/light cycle. After the consumption of a standard non-purified diet (Bikei Animal Dietary, Shanghai, China) for a 5-d acclimation period, the rats were fertilized. One-day pregnant rats were randomly divided into four groups: control (CO), pair-fed (PF), zinc-deprived (ZD), or oral zinc-supplemented (OZS). Each group had six pregnant rats. The CO and PF groups were fed the control diet (zinc 25 μg/g), with the PF group receiving only the daily average amount of food eaten by the ZD group. The ZD and OZS groups were fed a zinc-deficient diet (zinc 2 μg/g) during pregnancy and lactation. After weaning (day 21), two male pups with similar weight were kept from each dam; thus, each group had 12 male pups. Pups in the CO and PF groups continued receiving the control diet, whereas pups in the ZD group continued receiving the zinc-deficient diet. The OZS pups were switched to the control diet. All four groups were fed deionized water ad libitum (Fig. 1). Dietary intakes were measured every day and body weights were measured twice per week. When the pups were 35 d old, the experiments were performed as described below.

Morris water maze

Beginning on postnatal day 35, the rats received 5 d of training to test their capacity for learning and memory acquisition using a Morris water maze (MWM), as previously described [19]. Briefly, for the place-navigation test (spatial learning acquisition), each animal was subjected to two trials per day for 4 consecutive days. Each trial consisted of placing the rat in water so that it faced the wall of the pool at one of four starting locations (north, east, south, or west) in a random order. The rat was allowed to search the platform for a maximum of 120 s. If an animal did not find the platform in 120 s, it was gently lifted up and placed onto the platform for 20 s before being returned to the cage. The escape latency (duration before finding the platform) and swim paths were automatically recorded by a video/computer system. The escape latency, path length, and swim speed were recorded as indices of learning and memory capacity.

Sample collection and analyses

After weaning, maternal blood was drawn from the right orbital vein through a syringe for the measurement of the serum zinc level. At the end of the MWM tests, rats were anesthetized by a short inhalation of ether, and then blood was drawn from the right orbital vein through a syringe for the measurement of the serum zinc level. Blood samples were centrifuged (3000 rpm/5 min; Labofuge 400r, Heraeus Instrument, Hanau, Germany) and serum samples were kept at −85°C. Serum 0.1 mL was digested in acid-washed vials with 0.9 mL of HNO_3 1 mol/L for 48 h. The samples were centrifuged and the supernatant was collected and measured by atomic absorption spectrophotometry (Thermo M6).

Tissue preparation and processing for electron microscopy

After removal of the blood samples, all pups were deeply anesthetized with ether and were perfused transcardially with 100 mL of physiologic saline, followed by 100 to 150 mL of 3.5% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.2–7.4) at room temperature. After perfusion, the brains were removed and sliced into 50-μm-thick sections. Further fixation for electron microscopy was performed by the immersion of slices in 2.5% glutaraldehyde in the same 0.1 M Na-cacodylate buffer for 24 h. The tissue was postfixed with 1% osmium tetroxide and 0.01% potassium dichromate in the same buffer for 1 to 2 h at room temperature. The tissue was dehydrated in graded aqueous solutions of ethanol from 40% to 96% (each for 10 min) and then 100% acetone (three changes, each for 10 min). Specimens were infiltrated with a mixture of 50% epoxy resin and 50% pure acetone for 30 min at room temperature. Each slice was placed on an Aclar film (Honeywell International Inc., Morristown, New Jersey, USA), covered with a capsule containing pure epoxy resin (Epon 812/AralditeM epoxy resins, Polysciences Inc., Warrington, PA, USA) for 1 h at 60°C, and polymerized overnight at 80°C. Slices in blocks were then coded and all further analyses were carried out with the investigator blinded to the experimental status of the tissue. Ultrathin sections (5 nm) including the CA3 hippocampal area were prepared with a Reichert ultramicrotome (Lecia, Kista, Sweden). Sections were counterstained with saturated ethanolic uranyl and then viewed using a JEM-1230 electron microscope (JEOL, Tokyo, Japan) operated at an accelerating voltage of 80 kV. Then, electron micrographs of this specimen were taken at a magnification of 4000× to examine the histopathology of the neuron. Fifteen to 20 photographs were taken of each specimen [20].

Statistical analysis

All data for performance, body weight, and serum zinc were analyzed using analysis of variance with repeated measurements. The level of statistical significance was set at $P < 0.05$. Statistical analyses were performed using SPSS 13.0 for Windows (SPSS, Inc., Chicago, IL, USA). All data were presented as mean ± standard deviation.

Results

Zinc deficiency affected serum zinc concentrations and growth of rats

In the ZD group, maternal rats had poor appetites after being fed the zinc-deficient diet for about 8 to 9 d (Fig. 2A) and then showed zinc-deficiency symptoms including diarrhea, indifference, and growth retardation (compared with the CO and PF groups, $P < 0.05$). After weaning, the concentration of maternal serum zinc in the ZD group decreased significantly (compared with the PF and CO groups, $P < 0.05$). In addition,

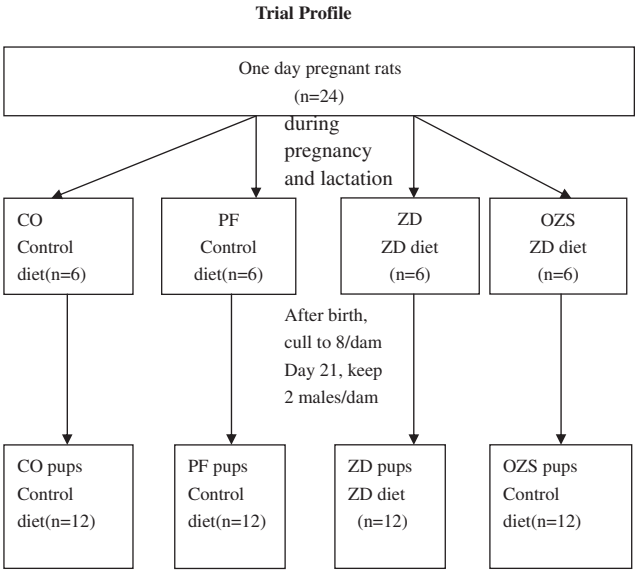

Fig. 1. Trial profile showing the number of dams and pups in each group that were fed a zinc-deficient diet (zinc 2 μg/g) or a control diet (zinc 25 μg/g). CO, control; OZS, oral zinc-supplemented; PF, pair-fed; ZD, zinc-deprived.