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Short communication

Early lifetime zinc supplementation protects zinc-deficient diet-induced alterations

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Abstract:
Preclinical and clinical data indicate the involvement of zinc in the pathophysiology and therapy of depression. A relationship between zinc-deficiency and depression symptoms was recently proposed. The present study investigated alterations in spontaneous locomotor activity and zinc concentrations in the serum, hippocampus and frontal cortex; these alterations were induced by subjecting rats to a zinc-deficient diet, prior subjected after birth to zinc-supplemented diet. Body weight was significantly reduced in animals subjected to the four-week zinc-deficient diet compared to those subjected to the zinc-adequate diet. The two-week zinc-deficient diet induced a significant increase in locomotor activity in all measured time periods (5, 30 and 60 min by 44–62%). The four-week zinc-deficient diet did not affect locomotor activity, while the six-week zinc-deficient diet resulted in a 45% increase in the 5 min time period. Serum zinc concentrations were significantly reduced (by 29%) in animals subjected to the four-week zinc-deficient diet but not in those subjected to the two- or six-week zinc-deficient diets. The zinc-deficient diet did not influence the zinc concentration in the examined brain regions regardless of the length. These results indicate that post-birth supplementation with zinc may protect zinc-deficient diet-induced rapid alterations in zinc homeostasis.

Key words:
zinc, supplementation, deficiency, locomotor activity, concentration, serum, brain

Introduction

Zinc, a divalent trace bio-metal, is essential for the physiological function of many biochemical processes [4, 27, 28, 48]. One of the main roles of zinc is the modulation of signal transmission by neurotransmitters in the endocrine and immunological systems [6, 7, 10, 19, 20, 29, 39]. In recent years, evidence for antidepressant-like activity of zinc has emerged from screening tests (e.g., forced swim test and tail suspension test) and models (e.g., olfactory bulbectomy, chronic unpredictable stress and chronic mild stress)

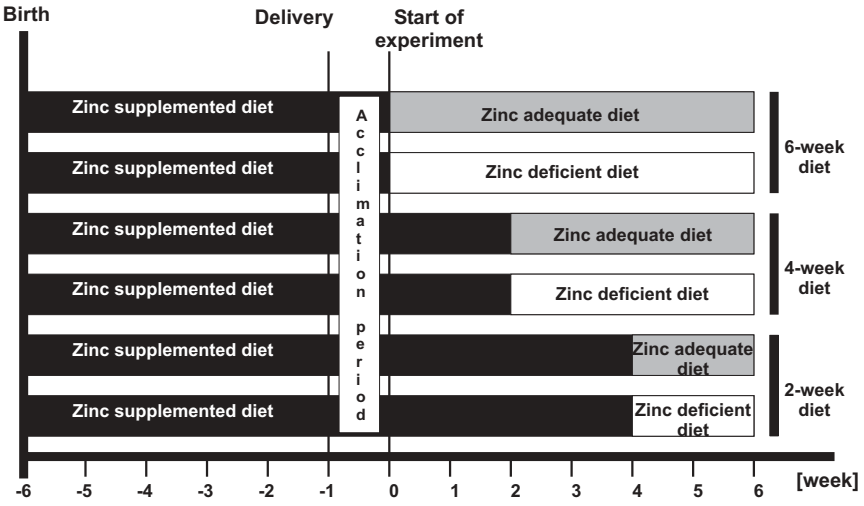

Fig. 1. Schedule of feeding procedure of rats subjected to the experiments

of depression [2, 12, 13, 23, 30, 33]. Moreover, zinc enhances the efficacy of antidepressant drugs [2, 5, 12, 35, 38].

Disturbances in zinc homeostasis have been described in many diseases, including neuropsychiatric illnesses [29, 43, 52, 53]. Depressive patients exhibit low zinc serum levels, which may be related to the severity of depressive symptoms [8, 15–18, 21, 31]. Moreover, successful antidepressant therapy may normalize low zinc concentrations in the serum [16, 18, 31]. Enhancement of antidepressant therapy by zinc supplementation has been demonstrated in clinical trials [22, 32].

In previous studies, zinc-deficient diets induced behavioral and neurochemical changes related to depression [3, 34, 40, 41, 43, 47, 51]; however, these changes could be abrogated by concurrent zinc supplementation (see [43] for review).

Our study investigated the effect of post-birth zinc supplementation followed by zinc deprivation on spontaneous locomotor activity and zinc concentrations in the serum, hippocampus and frontal cortex of rats.

Materials and Methods

Experimental animals and diet

Male Wistar rats (~150 g, ~5 weeks old) were obtained from a commercial source (Charles River Laboratories, Germany), where they were fed a standard non-purified diet containing ~180 mg Zn/kg. The rats were maintained in a temperature-controlled facility with a 12-h light:dark cycle (lights on at 07:00 h) and free access to food and water. After delivery, animals were fed with the aforementioned standard non-purified diet for a seven-day acclimation period, after which the rats were randomly assigned to one of the six experimental groups. A zinc-deficient or zinc-adequate (control) diet was initiated at six, eight and ten weeks of age in the six-, four- and two-week experiments, respectively (Fig. 1). Control (33.5 mg Zn/kg) and zinc-deficient (max. 0.2 mg Zn/kg) pelleted diets (egg white-based semipurified experimental diets based on the AIN-76 and AIN-76C mixtures) were purchased from MP Biomedicals (France). Animals were weighed daily. At the end of the experiment, when all animals were 12 weeks old, locomotor activity was measured. Twenty-four hours later, the animals were decapitated, blood was collected and their brains were removed. Hippocampi and frontal cortices were dissected and immediately frozen over solid CO_2. The frozen tissues were stored at –80°C before the assay.

All procedures were conducted according to the NIH Animal Care and Use Committee guidelines and approved by the Ethical Committee of the Institute of Pharmacology, PAS, Kraków, Poland.

Locomotor activity

Rat locomotor activity was recorded individually for each animal in Opto-Varimex cages (Columbus Instruments, USA) linked on-line to a compatible IBM-PC. Each cage (43 × 44 × 25 cm) was surrounded with a 15 × 15 array of photocell beams located 3 cm from

the floor surface, Interruptions of these photobeams resulted in horizontal activity defined as distance traveled (in cm). Locomotor activity was recorded for 5 min and analyzed using Auto-track software (Columbus Instruments, USA).

Total reflection X-ray radiation fluorescence (TXRF) method

Energy dispersive X-ray fluorescence analysis is a well-known method for the elemental analysis of industrial, environmental and biological materials. These materials are analyzed in powder, filter, pellet or liquid form. Sometimes, chemical preconcentration is required and combined with the measurements. X-ray radiation from the tube or radioisotope excites atoms in the sample, and secondary X-rays are then emitted and recorded as a spectrum by Si(Li) detectors. Spectral lines are characteristic for each element present in a sample.

The geometry of the total reflection X-ray fluorescence measurements was invented and developed mainly for the trace analysis of liquid samples. The primary beam from the X-ray tube is directed onto an optically flat surface of the reflector at an angle smaller than the critical angle of the total reflection. A small amount of the sample (a few microliters) is deposited on the optically flat surface of the so-called reflector made of pure Si or SiO_2. Prior to analysis, the solid sample must be dissolved; a pressure-acid digestion using nitric acid is the most popular method of dissolution. The sample is then spiked with a known amount of an internal standard, dried and measured in TXRF geometry. Each reflector is cleaned and checked before the analysis. Because of the small amount of the sample and the modification of the X-ray beam, the background of the spectrum is reduced and the detection limits are lower than that of conventional EDXRF spectrometry. Quantification is performed using a universal calibration curve; each element is measured with an internal standard and relative sensitivity is calculated.

Brain hippocampi and frontal cortices were digested with the nitric acid (suprapure). Serum samples were diluted with distilled water (3:1). As an internal standard, gallium was added to a final concentration of 5 mg/l in for serum and 30 mg/kg for tissues. A multifunctional X-ray spectrometer was used in measurements. This spectrometer was built in the Faculty of Physics and Applied Computer Science, AGH. It contains a TXRF module from the Atomic Institute (Vienna, Austria) equipped with a long fine focus Mo X-ray tube operating at 55 kV, 30 mA and a Si(Li) detector. Counting time was equal to 1000 s. Axil software was used for quantification.

Anodic stripping voltammetric (ASV) method

We used an ASV procedure described previously [25]. Multipurpose Electrochemical Analyzer M161 with an M164 electrode stand (both MTM-ANKO, Poland) was used for all voltammetric measurements. We used a conventional three-electrode quartz cell (volume 10 ml), which consisted of the following: a controlled growth mercury electrode (CGME) as the working electrode, Ag/AgCl/3 M KCl with additional electrolytic key filled with 1 M KNO_3 as a reference and a platinum wire as the auxiliary electrode. The stripping was performed in differential pulse (DP) mode. Two milliliters of 0.05 M KNO_3 was added to the electrochemical cell as a blank and the solution was purged with 99.995% pure argon for at least 5–7 min. A preconcentration step was carried out with the stirred solution for a period of t_{acc} = 20 s at an E_{acc} = –1.05 V *vs.* the Ag/AgCl electrode at a fresh mercury drop. After a rest period of 5 s, the DP voltammogram was recorded in the anodic direction from –1.05 to –0.8 V with a potential scan rate of 25 mV/s and a pulse amplitude of –50 mV. The voltammogram for the blank solution was used to characterize the electrochemical cell and supporting electrolyte purity. Next, 20 µl of sample solution was added to the cell while maintaining an argon atmosphere over the solution and DP voltammograms were recorded. The quantitative determinations of zinc ions were performed using the standard additions method (three concentrations). Three curves were recorded and averaged for each concentration. All samples were measured using the same conditions. The MTM-ANKO *EAGRAPH* software enabled electrochemical measurements, data acquisition and advanced processing of the results.

Data analysis

Statistical significance of the results was estimated by one-way ANOVA (and Tukey's *post-hoc* test) or Student *t*-test. All results are presented as the mean ± SEM; $p < 0.05$ was considered as statistically significant.

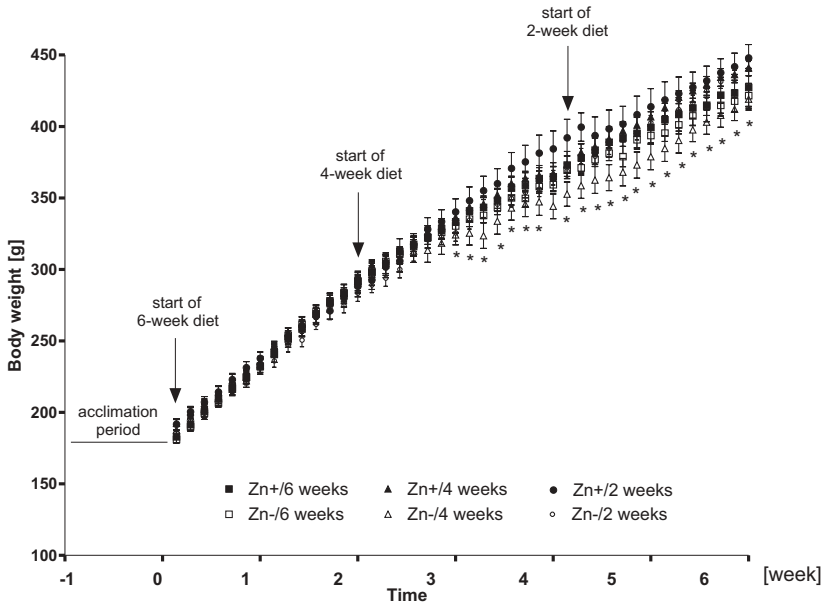

Fig. 2. Mean body weight (± SEM) of rats fed with the zinc-adequate diet (Zn+, n = 6) and the zinc-deficient diet (Zn–, n = 7) during the course of the study. *p < 0.05 Zn–/4-week *vs.* Zn+/4-week group

Results and Discussion

Disturbed zinc homeostasis has been reported in the pathophysiology and therapy of depression (see [36] for review). The mechanisms involved in pathological and therapeutic zinc action in this illness are under extensive experimental and clinical examination (e.g., [1, 5, 32, 34, 37], see [36] for review).

Zinc-deficiency leads to aberrant zinc homeostasis, which affects the central nervous system [43]. Recently, numerous reports have implicated zinc-deficiency in the development of behavioral and neurochemical alterations [34, 42, 44, 46, 50]. Most of them indicate that such alterations are related to depression symptoms [43, 46, 47, 51].

The present study demonstrated a gradual increase in body weight over time, which was significantly diminished in rats fed with a zinc-deficient diet for four weeks compared to rats receiving a control-adequate diet (Fig. 2). This effect emerged during the first week and persisted to fourth week of zinc-deficient diet administration; however, it was not robust (6–11%) (Fig. 2). A longer six-week zinc-deficient diet did not influence body weight (Fig. 2). Thus, it seems that the duration of prior zinc supplementation and/or age of the animals at the beginning of feeding with the zinc-deficient diet are involved in these results.

The two-week zinc-deficient diet induced a significant increase in locomotor activity during all measured time periods (44–62% increase over 5, 30 or 60 min; Fig. 3). The four-week zinc-deficient diet had no effect on this parameter, while the six-week zinc-deficient diet resulted in a 45% increase in activity at over the 5 min period (Fig. 3). Increased locomotor activity resulting from the two-week zinc-deficient diet may indicate the enhancement of psychostimulatory processes (e.g., induced by unblocking of the NMDA glutamate receptors [27]). As the adaptation to zinc-deficiency proceeds, compensational mechanisms normalize such behavior. However, increased exploratory behavior (5 min measurement) of rats following the 6-week zinc deficient diet may indicate a more complex mechanism, which would require further study.

Serum zinc concentrations were significantly reduced (by 29%) in animals fed for 4 weeks with the zinc-deficient diet (in comparison to the 4-week control diet), but not in animals subjected to the 2- or 6-week zinc-deficient diet (Tab. 1). Thus, even the reduction in serum zinc concentration induced by the 4-week zinc-deficient diet was normalized during the prolonged duration of this diet by some blood homeostatic mechanism (Tab. 1). Previously, it was shown that only one week of zinc deprivation was necessary to induce more than 50% reduction in the serum zinc concentrations of animals not previously supplemented with zinc [45]. To examine the effect of zinc deprivation on brain zinc homeostasis, we measured the total zinc concentration in hippocampus and frontal cortex by two methods: TXRF [11] and ASV [49]. These methods are very precise and sensitive, and

they are able to detect even small changes in zinc concentrations [9, 14, 24, 26]. No alterations in zinc concentration were found in the hippocampus or frontal cortex following any of the zinc-deficient diet time periods as measured by either TXRF or ASV (Tab. 2). Previously, Takeda et al. [41, 42] demonstrated that a 4-week zinc deprivation decreases hippocampal extracellular and presynaptic (histochemically reactive) zinc pools. Because we did not assess these zinc pools in our study investigation, they should be the subject of future studies.

Our data demonstrate that rats fed with a zinc-supplemented diet after birth are more resistant to a zinc-deficiency-induced reduction in weight and zinc serum concentration. The fact that a reduction in body weight was induced only one week after the introduction of a zinc-deficient diet suggests that during the first eight weeks of its life, the rat is most sensitive to alterations in zinc homeostasis, regardless of prior zinc supplementation. Thus, this age seems to be the most appropriate to achieve zinc-deficiency as a possible model of depression in rats.

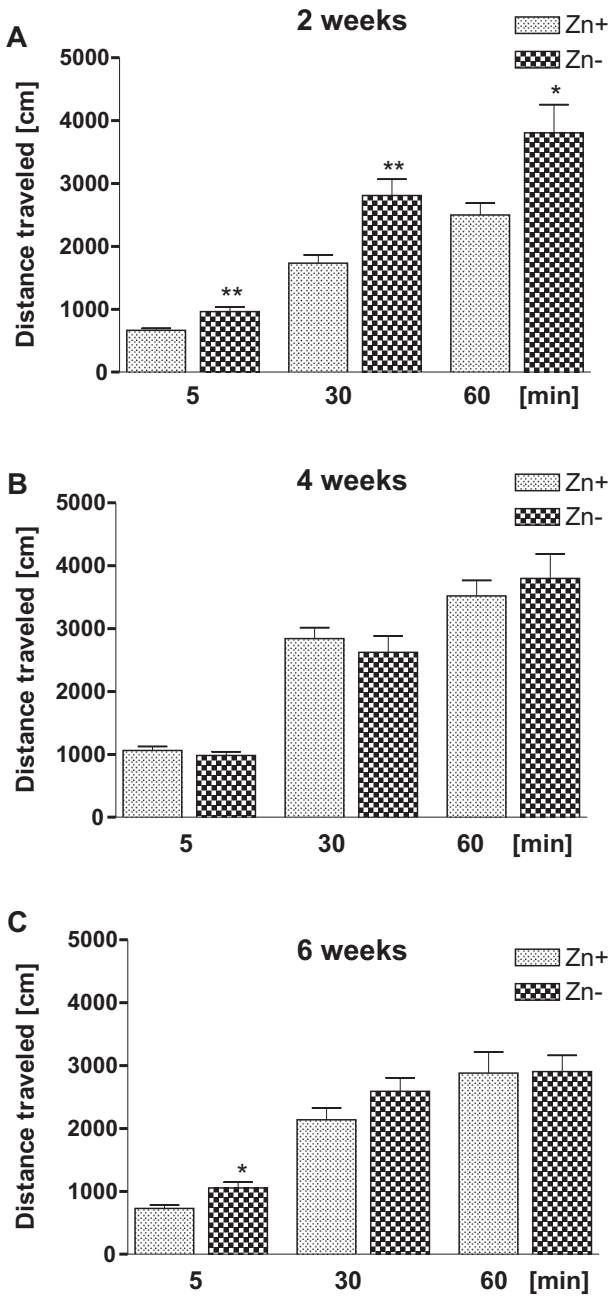

Fig. 3. Effect of feeding rats with zinc-deficient diet (Zn–) on locomotor activity. Results are expressed as the mean ± SEM of 4–5 animals per group. * $p < 0.05$ *vs.* control zinc-adequate diet (Zn+) group

Tab. 1. A time course showing the effect of feeding rats with zinc-deficient diet on serum zinc concentrations

Weeks of feeding	Control	Zn-deficient
2	1.54 ± 0.19	1.81 ± 0.18
4	1.88 ± 0.01	1.34 ± 0.12*
6	1.46 ± 0.09	1.61 ± 0.30

Results are expressed in µg Zn/ml as the mean ± SEM of 3 rats per group. * Student *t*-test t(4) = 4.402, p = 0.0117

Tab. 2. A time course showing the effect of feeding rats with a zinc-deficient diet on zinc concentrations in the brain as measured by two analytical methods

Weeks of feeding	TXRF method		ASV method	
	Control	Zn-deficient	Control	Zn-deficient
	Hippocampus			
2	11.8 ± 0.8	11.8 ± 0.7	10.1 ± 1.4	10.6 ± 1.8
4	12.8 ± 1.4	10.5 ± 0.9	13.7 ± 3.0	10.8 ± 3.3
6	14.4 ± 0.8	12.6 ± 0.6	11.5 ± 1.0	9.4 ± 1.3
	Frontal cortex			
2	13.4 ± 0.7	13.7 ± 0.3	16.2 ± 1.6	16.1 ± 0.5
4	13.6 ± 0.1	12.8 ± 0.5	15.9 ± 2.0	15.0 ± 1.1
6	13.8 ± 0.7	13.5 ± 0.8	17.9 ± 1.5	16.8 ± 2.0

Results are expressed as µg Zn/g tissue as the mean ± SEM of 3–5 rats per group

Our results suggest that post-birth supplementation with zinc may protect against the rapid alterations in zinc homeostasis induced by a zinc-deficient diet.

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