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Disruption of the zinc metabolism in rat fœtal brain after prenatal exposure to cadmium

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ABSTRACT

This study was carried out to investigate the effects of maternal Cd and/or Zn exposure on some parameters of Zn metabolism in fetal brain of Wistar rats. Thus, female controls and other exposed by the oral route during the gestation period to Cd (50 mg $CdCl_2$/L) and/or Zn ($ZnCl_2$ 60 mg/L) were used. The male fetuses at age 20 days of gestation (GD20) were sacrificed and their brains were taken for histological, chemical and molecular analysis. Zn depletion was observed in the brains of fetuses issued from mothers exposed to Cd. Histological analysis showed that Cd exposure induces pyknosis in cortical region and CA1 region of the hippocampus compared to controls. Under Cd exposure, we noted an overexpression of the genes coding for membrane transporter involved in the intracellular incorporation of Zn (ZIP6) associated with inhibition of that encoding the transporters involved in the output of the Zn into the extracellular medium (ZnT1 and ZnT3). A decrease in the expression of the gene encoding the neuro-trophic factor (BDNF) associated with overexpression of the encoding the metal regulatory transcription factor 1 (MTF1), factor involved in the homeostasis of Zn, was also noted in Cd group. Interestingly, Zn supply provided a total or partial restauration of the changes induced by the Cd exposure. The depletion of brain Zn contents as well as the modification of the profile of expression of genes encoding membrane Zn transporters, suggest that the toxicity of Cd observed in fetal brain level are mediated, in part, by impairment of Zn metabolism.

1. Introduction

The exposure of developing brain to neurotoxic metals affect cerebral function leading in long-lasting or permanent deficits in cerebral function that can be reflected at different ages as alterations in motor function or coordination and/or altered intellectual function with alterations in learning ability and/or memory [1–3]. Heavy metals including cadmium (Cd) have received attention as both environmental contaminants and potential neurotoxicological hazards [4–6]. The sources of human exposure to Cd include primary metal industries, production of certain batteries, intake of contaminated food or water, and inhalation of tobacco smoke or polluted air [7]. Cd accumulates and is toxic for many organs, including kidneys, lungs, and testis. Cd also reaches the central nervous system (CNS) causing neurological alterations in humans and animals. In animals models, several researches have shown that perinatal exposure of Cd result in behavioral changes, hypernociception, olfactory dysfunction, impaired learning ability and mental retardation [8–11]. Furthemore, Cd exposure during gestation and lactation has been reported to alter enzymes activities in rat brain [12,13]. In humans, Cd is a known embryotoxic and teratogenic [14]. In fact there is growing evidence of associations between Cd and adverse birth outcomes, such as small for gestational age and preterm birth [15–19]. In addition, to its impact on growth parameters, Cd is an established brain disorder [20], thus there is a diversity of models of neural cells (astrocytes, microglia, and neurons) exposed to Cd point to reactive oxygen species (ROS) production, cell death, and disturbance of cell signaling pathways as a foremost mechanism of Cd neurotoxicity [20].

Although the damage induced by Cd was recognized decades ago, the precise mechanisms underlying its neurotoxicity remain unclear. The interaction between Cd and essential trace elements, such as zinc (Zn) could be one of the reasons for Cd-induced neurotoxicity. In fact, interactions between Zn and Cd can take place at different stages of absorption, distribution in the organism, excretion of both metals and at

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Table 1
Cadmium (Cd) and zinc (Zn) levels in the brain at the 20th of gestation of control fetuses (C) and fetuses from mothers treated with Cd and/or Zn during gestation. Values are expressed as mean ± SE from 6 samples in each group.

Groups		C	Cd	Zn	Cd + Zn
Brain	**Cd** (ng/g dry weight)	nd	nd	nd	nd
	Zn (µg/g dry weight)	25.44 ± 2.57	17.46 ± 1.81[a]	23.33 ± 0.89[b]	20.60 ± 2.59[ab]
Liver	**Cd** (ng/g dry weight)	nd	24.8 ± 3.99[ac]	nd	16.0 ± 4.09[abc]
	Zn (µg/g dry weight)	62.3 ± 6.53	33.2 ± 7.27[a]	67.8 ± 9.27[b]	48.4 ± 7.86[abc]

C: Control group; **Cd:** Cadmium group; **Zn:** Zinc group; **CdZn:** Cadmium and Zinc group; nd: not detected; **a:** $p < 0.05$ when compared to Controls (C) animals; **b:** $p < 0.05$ when compared to Cd-exposed animals; **c** $p < 0.05$ when compared to Zn-exposed animals.

Table 2
Developmental parameters of control fetuses (C) and fetuses from mothers treated with Cd and/or Zn during gestation.

Finding	C	Cd	Zn	Cd + Zn
Body weight (b.w) (g)	3.45 ± 0.16	3.33 ± 0.05	3.50 ± 0.12	3.31 ± 0.14
Cranio-caudal lenght (cm)	5.03 ± 0.29	4.89 ± 0.33	4.78 ± 0.34	4.82 ± 0.25
Relative brain weight (g/ 100 g b.w)	4.80 ± 0.26	5.13 ± 0.77	4.13 ± 0.76	4.63 ± 0.91

Values are expressed as mean ± SE from 6 samples from each group.
C: Control group; **Cd:** Cadmium group; **Zn:** Zinc group; **CdZn:** Cadmium and Zinc group; **b.w**: Body weight.

the stage of Zn biological functions [21]. In the brain, Zn is necessary for not only brain development but also for brain function. Evidences from experimental animals indicate that nutritional Zn deficiency cause teratogenic and CNS disturbances in animals [22,23], specifically by reducing synaptic activity [24]. Several studies indicate that Cd gains access to cells by mimicking Zn at the site of Zn transporters [25,26]. While Cd and Zn interactions during early life have been reported in several others works, the interaction between Cd and Zn has been little explored in the brain especially during fetal development. The parallelism existing between negative effects of Cd exposure and Zn deficiency on brain development led to hypothesis that Cd neurotoxicity is mediated by Cd-induced alteration of Zn metabolism. Recently, we have demonstrated that the toxic effects of Cd observed during development are mediated by the disruption of Zn metabolism, which is established in mothers during pregnancy causing Zn deficiency in fetuses [26–28]. Therefore, the current study is a continuous report, in which we used the same conditions of our previous work in order to investigate the involvement of the disruption of Zn metabolism in Cd-induced neurotoxicity in fetal brain.

2. Material and methods

2.1. Animals and experimental design

Parental generation of male and female Wistar rats was subjected to 2-week acclimatization period. The animals were housed in individual stainless steel cages at 23 ± 1 °C and exposed to 12-h light-dark cycle. They had access to a standard rodent laboratory diet and drinking water *ad libitum*. The animals were housed according to the EEC 609/86 Directives regulating the welfare of experimental animals.

After acclimatization, male and female rats were mated to obtain the first-generation offspring. During mating, rats were separated after positive identification of a vaginal sperm plug, after which a designation of gestational day zero (GD0) was made. At GD 0, pregnant rats were housed individually in plastic cages and randomly divided into four groups (**C:** Control group; **Cd:** Cadmium group; **Zn:** Zinc group; **CdZn:** Cadmium and Zinc group). A control group of animals received tap water and three experimental groups received Cd (50 mg/L Cd as $CdCl_2$), Zn (60 mg/L Zn as $ZnCl_2$) or Cd + Zn (50 mg/L Cd + 60 mg/L Zn) in their drinking water during gestation. Cd and Zn doses and manner of administration were chosen on the basis of our previous research group [27,28] and of available literature [21,29]. At GD20, the dams were killed under ether anesthesia and their fetuses were carefully removed. The number and body weight of male fetuses were recorded and their brain as well as their livers were removed and treated as described below.

2.2. Analytical procedures

2.2.1. Histology

Brains were immersed and fixed in Bouin's fixative (75% saturated picric acid, 20% formalin, and 5% acetic acid) for 24 h. They were, after that, dehydrated in ascending grades of alcohol before paraffin inclusion. Brains sections (5 µm) were obtained, stained with hematoxylin and eosin, and examined under a PrimoStar light microscope (Zeiss) for histopathological evaluation. Photographs were taken using the AxioCam ERc5s camera and the Zen software (Blue Edition 2012).

2.2.2. Measurement of cadmium and zinc concentrations

Brain destined for Cd and Zn analyses were oven-dried (60 °C) to constant weight and digested with concentrated nitric acid (Merck, 65%) at 120 °C. When fumes were white and the solution was completely clear, the samples were cooled to room temperature and the tubes were filled to 5 ml with ultra-pure water. Samples were analyzed to determine Zn and Cd concentrations using flame atomic absorption spectrometry. These measures were implemented using a ZEEnit 700-Analytik-Jena, Germany equipped with deuterium and Zeeman background correction. Samples were analyzed in triplicate and the variation coefficient was usually less than 10%. Concentrations of the metals were expressed as micrograms per gram dry weight.

2.2.3. Metallothionein levels measurement

MTs protein levels in the brains of rats were determined using a spectrophotometric assay for MTs using Ellman's reagent (0.4 mM5,5′-dithio-nitro-benzoate (DTNB) in 100 mM KH_2PO_4) at pH 8.5 in a solution containing 2 M NaCl and 1 mM EDTA [30]. Total protein content in a sample of the homogenate was measured by the Bradford method [31], at 595 nm, using bovine serum albumin as standard.

2.2.4. SOD activity measurement

SOD activity was measured by the method of [32]. It is based on the inhibitory effect exerted by SOD against the autoxidation of pyrogallol.

2.2.5. Gene expression

Total RNA was extracted from about 10-mg frozen brain tissues of