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Maternal cadmium exposure reduces placental zinc transport and induces fetal growth restriction in mice

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ABSTRACT

Cadmium (Cd) is linked with increased risk of fetal growth restriction (FGR). Nevertheless, the mechanism remains unknown. This study established a mouse model of Cd-induced FGR through two exposure methods. Pregnant mice were either administered with $CdCl_2$ (5, 50 and 250 ppm) throughout pregnancy through drinking water or intraperitoneally injected with $CdCl_2$ (4.5 mg/kg) on GD9. As expected, fetal weight and crown-rump length were reduced in a gender-independent manner. Interestingly, *Mt1* and *Mt2*, two metallothionein genes, were up-regulated in maternal liver. Correspondingly, Cd accumulated mainly in maternal liver and kidney, and only trace amounts of Cd could pass from dam to placentas and fetuses. Further analysis showed that placental Zn concentration was elevated. Conversely, embryonic Zn concentration was reduced. Moreover, placental *Znt1* and *Znt2*, two zinc transporters, were down-regulated in Cd-exposed mice. These results suggest that maternal Cd exposure during pregnancy reduces placental Zn transport and induces fetal growth restriction.

1. Introduction

Cadmium (Cd) is an important occupational and environmental toxicant. Workers in electroplating, pigments, paints, welding and Ni–Cd batteries are frequently exposed to Cd at significantly higher level than the general population [1]. The general population is usually exposed to a low dose of Cd via drinking water, food and cigarette smoking [2]. Epidemiological investigations showed that Cd level in blood and seminal plasma was associated with male infertility and poor semen quality in humans [3–7]. At a high dose, Cd induces germ cell apoptosis in testes [8–13]. In addition, Cd (2.0 mg/kg, i.p.) inhibits the synthesis of testosterone in mouse testis [14,15].

An epidemiological report demonstrated that maternal urinary Cd level during pregnancy was negatively associated with birth weight and head circumference [16]. According to another birth cohort study, Cd level in maternal blood was positively associated with fetal growth restriction (FGR) [17]. In mice, maternal Cd exposure in mid- gestation resulted in a relatively specific forelimb ectrodactyly [18–23]. Moreover, maternal Cd exposure in middle and late gestational age induced FGR in mice [15,24,25]. Nevertheless, the molecular mechanism for Cd-induced FGR remains obscure.

The main objective of this study was to explore the effects of maternal Cd exposure during pregnancy on placental Zn transport and its mechanism. We established a mouse model of Cd-induced FGR through two different exposure methods. We showed that maternal Cd exposure through drinking water or ip injection significantly reduced fetal weight and crown-rump length in a gender-independent manner. We demonstrated that Cd accumulated mainly in maternal liver and kidney, whereas only trace amounts of Cd were found in placenta and fetus. We found that maternal Cd exposure during pregnancy reduced placental zinc (Zn) transport from maternal circulation to the fetuses through down-regulating the expression of Zn transporters.

2. Methods

2.1. Chemicals and reagents

Cadmium chloride ($CdCl_2$) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). TRI reagent was from Molecular Research Center, Inc. (Cincinnati, OH, USA). AMV RT kits and RNase-free

Abbreviations: Cd, cadmium; FAAS, flame atomic absorption spectroscopy; GFAAS, graphite furnace atomic absorption spectrometry; FGR, fetal growth restriction; MT, metallothionein; ZIPs, zinc iron permeases; Zn, zinc; ZnTs, zinc transporters.

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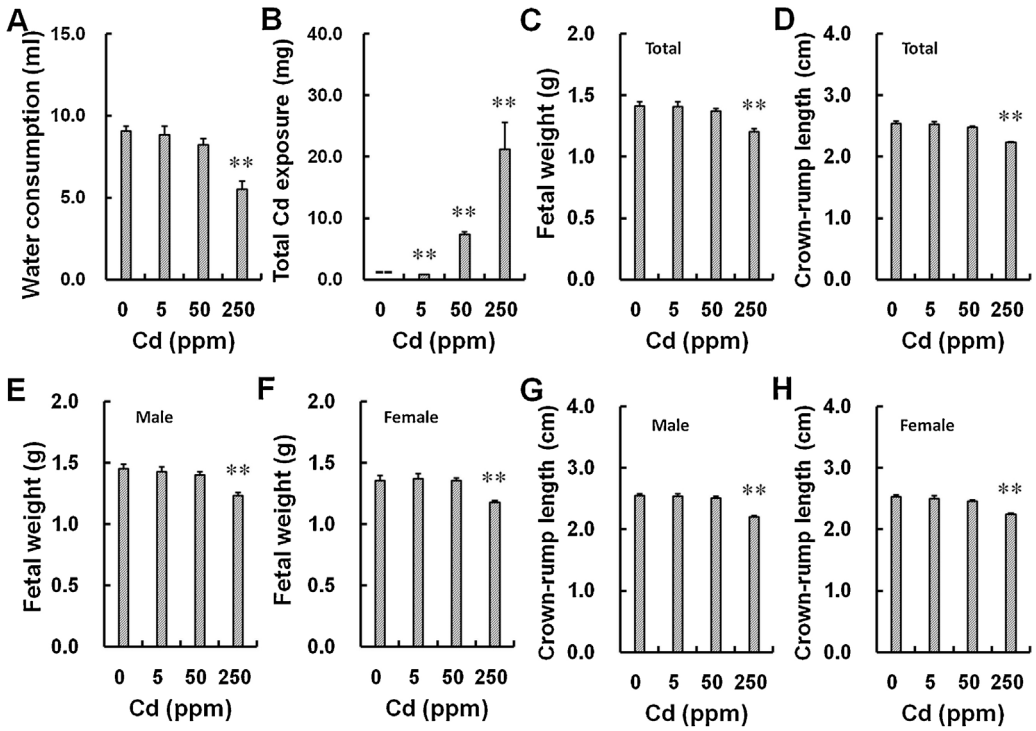

Fig. 1. Effects of maternal Cd exposure during pregnancy on maternal water consumption and fetal growth. Pregnant mice were administered with $CdCl_2$ (5, 50 and 250 ppm) throughout pregnancy through drinking water. All dams were sacrificed on GD18. Live fetuses were weighed and the crown-rump lengths were measured. (A) Maternal daily water consumption. (B) Total Cd exposure throughout pregnancy. (C) Fetal weight. (D) Crown-rump length. (E) The weight of male fetuses. (F) The weight of female fetuses. (G) The crown- rump length of male fetuses. (H) The crown-rump length of female fetuses. All data were expressed as mean ± SEM (n=10). $^{**}P < 0.01$ vs controls.

DNase were from Promega Co. (Madison, WI, USA). LightCycler® 480 SYBR Green I Master kit was from Roche Diagnostics Co. (Indianapolis, IN, USA). Ultrapure HNO_3 was from Aladdin Reagents Co., LTD (Shanghai, China). Matrix modifiers colloid palladium was from Xinda Measuring & Control Technology Co., Ltd (Colpd™, Chengdu, China).

2.2. Animals and experimental procedures

Adult CD-1 mice (8–10 week-old; male mice: 32–36 g; female mice: 26–28 g) were purchased from Beijing Vital River (Beijing, China) whose foundation colonies were all introduced from Charles River Laboratories, Inc. All mice were allowed free access to food (Beijing Keao Xieli Feed Co., LTD., Beijing 100107) and ultrapure water at all times and were housed in a room with controlled lighting (12-h light/12 -h dark cycle) and temperature (20–25 °C) for a period of one week before use. For mating purposes, four females were housed overnight with two males starting at 21:00. Females were checked at 7:00 am the next morning, and the presence of a vaginal plug was designated as gestational day (GD) 0. Four pregnant mice were housed per cage. The present study consisted of two independent experiments. *Experiment 1.* Forty pregnant mice were randomly divided into four groups. All pregnant mice were administered with different concentrations of $CdCl_2$ (0, 5, 50 and 250 ppm, dissolved in ultrapure water) through drinking water throughout pregnancy. The dose of $CdCl_2$ used in the current study referred to a previous study with minor revision [26]. Maternal toxicity was assessed according to maternal weight and general signs. Maternal water consumption was measured. All dams were sacrificed on GD18. The uterine horns were incised and weighed. Live fetuses were counted. The gender of fetal mice was determined by anogenital distance (AGD). Male and female fetuses per litter were weighed. Crown-rump length was measured. *Experiment 2.* Sixty pregnant mice were randomly divided into two groups. In the Cd-treated group, pregnant mice were intraperitoneally (i.p.) injected with $CdCl_2$ (4.5 mg/kg) on GD 9. Normal saline-treated pregnant mice served as controls. The dose of $CdCl_2$ used in the current study referred to our previous study [23]. Forty dams were sacrificed at different time points (0, 2, 12 and 24 h) after Cd injection. Maternal serum, maternal liver, maternal kidney, placenta and embryo were collected and stored at −80 °C for measurement of Cd and Zn. Twenty dams were sacrificed on GD18. The uterine horns were exposed and weighted. Live and dead fetuses were counted. Live fetuses were weighed. Maternal serum, maternal liver, maternal kidney, placenta, fetal serum and fetal liver were collected and stored at −80 °C for measurement of Zn and Cd. Maternal liver and placenta were collected for real-time RT-PCR. Placental cross sections were stained with Hematoxylin & Eosin (H&E). The present study was approved by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University. All animal experimental procedures were performed in accordance with the guidelines for humane treatments established by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University.

2.3. Cd measurement

The levels of Cd in maternal serum, maternal liver, maternal kidney, placenta, embryo, fetal serum and fetal liver were measured by graphite furnace atomic absorption spectrometry (GFAAS; model: TAS-990; Purkinje General Instrument Co., Ltd, Beijing, China) coupled with a deuterium-lamp background correction system. All samples were prepared and analyzed according to a slightly modified method as previously described [15]. For serum samples, maternal serum and fetal serum were diluted with 1% HNO_3 according to 1:4 (v/v). Matrix modifiers colloid palladium were added to each standard, blank and sample dilution. For tissue sam-