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Cadmium-induced neural tube defects and fetal growth restriction: Association with disturbance of placental folate transport

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ABSTRACT

Previous studies found that maternal Cd exposure on gestational day (GD)9 caused forelimb ectrodactyly and tail deformity, the characteristic malformations. The aim of the present study was to investigate whether maternal Cd exposure on GD8 induces fetal neural tube defects (NTDs). Pregnant mice were intraperitoneally injected with $CdCl_2$ (2.5 or 5.0 mg/kg) on GD8. Neither forelimb ectrodactyly nor tail deformity was observed in mice injected with $CdCl_2$ on GD8. Instead, maternal Cd exposure on GD8 resulted in the incidence of NTDs. Moreover, maternal Cd exposure on GD8 resulted in fetal growth restriction. In addition, maternal Cd exposure on GD8 reduced placental weight and diameter. The internal space of maternal and fetal blood vessels in the labyrinth layer was decreased in the placentas of mice treated with $CdCl_2$. Additional experiment showed that placental PCFT protein and mRNA, a critical folate transporter, was persistently decreased when dams were injected with $CdCl_2$ on GD8. Correspondingly, embryonic folate content was markedly decreased in mice injected with $CdCl_2$ on GD8, whereas Cd had little effect on folate content in maternal serum. Taken together, these results suggest that maternal Cd exposure during organogenesis disturbs transport of folate from maternal circulation to the fetuses through down-regulating placental folate transporters.

1. Introduction

Cadmium (Cd) is one of major occupational and environmental toxicants. Cd is frequently used in electroplating, pigments, paints, welding, and Ni-Cd batteries, where workers are exposed to Cd at a higher level (Beveridge et al., 2010). On the other hand, the general population is exposed to a low level of Cd via drinking water, food and cigarette smoking (Honda et al., 2010). Several epidemiological data demonstrate that maternal Cd exposure during pregnancy is associated with fetal growth restriction (Kippler et al., 2012; Menai et al., 2012; Wang et al., 2016). Animal experiments indicate that Cd is a potent teratogen in rodent animals (Barr, 1973; Thompson and Bannigan, 2008). Several studies showed that maternal Cd exposure at early limb development caused forelimb ectrodactyly and tail deformity, the characteristic malformations in fetuses (Hovland et al., 1999; Scott et al., 2005; Paniagua-Castro et al., 2007; Robinson et al., 2009). In addition, maternal Cd exposure during pregnancy resulted in fetal growth restriction in rodent animals (Ahokas et al., 1980; Ji et al., 2011). Nevertheless, the mechanism by which maternal Cd exposure induces fetal malformation and growth retardation remains obscure.

Increasing evidence demonstrated that placenta could deter most of Cd from passing from dams to fetuses. According to several earlier reports, only <0.1% of Cd was passed from dams to fetuses when pregnant mice were exposed to tracer levels of ^{109}Cd in drinking water (Whelton et al., 1993; Brako et al., 2003). Recently, we observed no significant elevation of blood Cd level in fetuses whose mothers were exposed to Cd during late pregnant period (Ji et al., 2011). These results indicate that Cd-induced fetal malformation and growth restriction cannot be completely attributed to its direct toxic effect on the fetuses. Indeed, the placenta is essential for the growth and development of the fetuses. Several studies demonstrates that the defects in placental function result in fetal growth restriction or even malformation and fetal demise (Watson and Cross, 2005). Therefore, we hypothesize that Cd induces fetal malformation and growth restriction through impairing placental development and function.

Previous studies focused on the effect of maternal Cd exposure at early limb development on forelimb structure. However, the aim of the present study was to explore the effect of maternal Cd exposure at neural tube development on neural tube formation and its mechanism. We found that maternal Cd exposure on gestational day (GD) 8 resulted in the incidence of neural tube defects (NTDs) and fetal growth restriction in mice. We demonstrate for the first time that maternal Cd exposure during organogenesis disturbs transport of folate from maternal circulation to the fetuses through down-regulating placental folate transporters.

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2. Materials and methods

2.1. Chemicals and reagents

$CdCl_2$ was from Sigma Chemical Co. (St. Louis, MO). TRI reagent was from Molecular Research Center, Inc. (Cincinnati, Ohio). RNase-free DNase was from Promega Corporation (Madison, WI). All other reagents were purchased from Sigma Chemical Co. (St. Louis, MO) if not otherwise stated.

2.2. Animals and treatments

The ICR mice (8–10 week-old; male mice: 28–30 g; female mice: 24–26 g) were purchased from Beijing Vital River whose foundation colonies were all introduced from Charles River Laboratories, Inc. The animals were allowed free access to food and water at all times and were maintained on a 12-h light/dark cycle in a controlled temperature (20–25 °C) and humidity (50 ± 5%) environment for a period of 1 week before use. For mating purposes, four females were housed overnight with two males starting at 21:00 h. Females were checked by 7:00 h the next morning, and the presence of a vaginal plug was designated as gestational day (GD) 0. The present study consisted of three independent experiments. Experiment 1. To investigate Cd-induced neural tube defects in mice, pregnant mice were divided randomly into three groups. In Cd group, pregnant mice were intraperitoneally (i.p.) injected a single dose of $CdCl_2$ (2.5 or 5.0 mg/kg) between 08:00 and 09:00 h on GD8. The saline-treated pregnant mice served as controls. The doses of $CdCl_2$ used in the present study were determined by preliminary experiments. The critical period of neural tube development is on GD8. In order to establish the mouse model of Cd-induced NTDs, the time of Cd exposure was chosen on GD8. For mechanical studies, a single dose of Cd by intraperitoneal injection was chosen in the current study. All animals were inspected daily for clinical signs and determined whether a pregnancy loss had occurred according to clinical signs and maternal weight. The dams were sacrificed on GD18. The uterine horns were exposed and weighed. Live, dead and resorbed fetuses were counted. Placentas were collected for histological examination. Live fetuses were sexed, weighed, and examined for external morphological malformations. Experiment 2. To investigate the effects of maternal Cd exposure during pregnancy on placental folate transport, twenty-four pregnant mice were divided randomly into two groups. In Cd group, pregnant mice were ip. injected with a single dose of $CdCl_2$ (5.0 mg/kg) between 08:00 and 09:00 h on GD8. The saline-treated pregnant mice served as controls. Pregnant mice were sacrificed 24 h after Cd injection. Maternal serum and embryo were collected for measurement of folate contents. Experiment 3. To investigate the effects of maternal Cd exposure during pregnancy on the expression of placental folate transporters, forty-eight pregnant mice were divided randomly into eight groups. In Cd group, all pregnant mice were ip. injected with a single dose of $CdCl_2$ (5.0 mg/kg) between 08:00 and 09:00 h on GD8. The saline-treated pregnant mice served as controls. Cd-treated pregnant mice were sacrificed at different time points (2, 12, 24, 48 and 72 h) after Cd injection. Normal saline-treated pregnant mice were killed on GD8, GD9, GD10 and GD11. Placentas were collected for real-time RT-PCR and Western blotting. All procedures on animals followed the guidelines for humane treatment set by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University.

2.3. Histology examination

Freshly collected placentas were fixed in 4% paraformaldehyde and embedded in paraffin. Paraffin-embedded placentas were serially sectioned. Hematoxylin and eosin (H&E) stained placental sections were analyzed for vascular space quantification according to the previous study (Neres et al., 2008). In each section, 5 fields were randomly selected in the labyrinthine region at magnification ×400. We performed an image analysis using the public domain NIH Image J Program. Briefly, the images were given a color threshold to cover the internal space of maternal and fetal blood vessels in the labyrinth layer after noise removal. The blood sinusoids area in the labyrinthine region was estimated from the analysis of two nonconsecutive sections in each placenta. The coverage percentage was calculated as the ratio between the number of pixels covered by the area defined by the threshold and the overall number of pixels in the image. The reported results in the present study represent the average results for six placentas from six pregnant mice in each group.

2.4. Isolation of total RNA and real-time RT-PCR

Total RNA was extracted using TRI reagent (MRC, Inc.). RNase-free DNase-treated total RNA (1.0 μg) was reverse-transcribed with AMV (Pregmega). Real-time RT-PCR was performed with a LightCycler® 480 SYBR Green I kit (Roche Diagnostics GmbH) using gene-specific primers as listed in Table 1. The amplification reactions were carried out on a LightCycler® 480 Instrument (Roche Diagnostics GmbH) with an initial hold step (95 °C for 5 min) and 50 cycles of a three-step PCR (95 °C for 15 s, 60 °C for 15 s, 72 °C for 30 s). The comparative C_T method was used to determine the amount of target, normalized to an endogenous reference (*Gapdh*) and relative to a calibrator ($2^{-\Delta\Delta Ct}$) using the Lightcycler®480 software (Roche, version 1.5.0).

2.5. Western blotting

Mouse placentas were homogenized in lysis buffer containing 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulphate (SDS) and 1 mM phenylmethylsulfonyl fluoride. The homogenates were then centrifuged at 15,000*g* for 15 min. Supernatants from each sample were added to a gel loading buffer (100 mM Tris, pH 6.8, 20% glycerol, 200 mM DTT, 4% SDS, 0.03% bromophenol blue) and boiled for 10 min. Proteins (20 μg per sample) in loading buffer were subjected to electrophoresis in 12.5% SDS-polyacrylamide gel (PAGE) for 3 h. The gel was transferred electrophoretically onto a polyvinylidene fluoride membrane (Immobilon-P; Millipore powdered milk in Dulbecco's PBS (DPBS)). The membranes were blocked by non-fat milk for 2 h at room temperature, and then incubated with primary antibodies PCFT or β-actin for 2 h at room temperature. After washes in DPBS containing 0.05% Tween-20 four times for 10 min each and PBS for 10 min once, the membranes were incubated with goat anti-rabbit or goat anti-mouse IgG antibody for 1.5 h at room temperature. The membranes were then washed four times in DPBS containing 0.05% Tween-20 for 10 min each and PBS for 10 min once, followed by signal development using an enhanced chemiluminescence (ECL) detection kit from Pierce (Pierce Biotechnology, Rockford, IL, USA).

2.6. Measurement of folate

Maternal sera and embryos on GD9 were collected. Maternal serum was centrifuged at 4000 rpm for 10 min and stored at −80 °C until the

Table 1
Primers for real-time RT-PCR.

Gene	Sequences	Product length
Gapdh	Forward:5′-ACCCCAGCAAGGACACTGAGCAAG-3′ Reverse:5′-GGCCCCTCCTGTTATTATGGGGGT-3′	109
Pcft	Forward: 5′-CTACCCTACCTCACCAGCCT-3′ Reverse: 5′-GCAAACGCAAAGACCACCAT-3′	119
Rfc-1	Forward: 5′-TGGGTGTTGTAGTCTGCGTG-3′ Reverse: 5′-CACTCCACCTTGCACTACCC-3′	114
Frα	Forward: 5′-GTGGAGACAAAGAAGCCCGA-3′ Reverse: 5′-CTCCACTCCCTGCTTAGGGT-3′	104

measurement of folate. Embryo was homogenized in 3 ml saline. The homogenate was then centrifuged at 4000 rpm for 10 min and stored at −80 °C until the measurement of folate. The levels of folate were measured by electrochemiluminescence immunoassay, using a kit from Roche (Roche Diagnostics GmbH, Mannheim, Germany) following manufacturer's instructions.

2.7. Statistical analysis

The litter was considered the unit for statistical comparison among different groups. Fetal malformation was calculated per litter and then averaged per group. For fetal weight and crown-rump length, the means were calculated per litter and then averaged per group. All quantified data were expressed as mean ± SEM at each point. ANOVA and the Student-Newmann-Keuls post hoc test were used to determine differences among different groups. Differences were considered to be significant only for $P < 0.05$.

3. Results

3.1. Cd-induced neural tube defects

No pregnant mice were dead after pregnant mice were injected with $CdCl_2$. No significant difference on diet intake was observed between Cd-treated pregnant mice and controls (6.2 ± 0.6 g per day vs 6.7 ± 0.4 g per day). No significant difference on weight gain was observed between Cd-treated pregnant mice and controls (Data not shown). All pregnant mice completed the pregnancy. No abortion and preterm delivery were observed among groups. The effects of maternal Cd exposure on pregnant outcomes were analyzed. As shown in Table 2, no significant difference on the number of fetal resorptions per litter was observed among different groups. As expected, maternal Cd exposure on GD8 significantly elevated the number of dead fetuses per litter (Table 2). The effects of maternal Cd exposure on external malformations were evaluated. Unexpectedly, neither forelimb ectrodactyly nor tail deformity, Cd-induced characteristic malformation, was observed when pregnant mice were injected with on $CdCl_2$ on GD8. Interestingly, maternal Cd exposure on GD8 resulted in the incidence of neural tube defects (NTDs). Among mice injected with 2.5 mg/kg of $CdCl_2$, 33.3% (4/12) of litters were with NTDs. Surprisingly, 75% (12/16) of litters were with NTDs among mice injected with 5.0 mg/kg of $CdCl_2$ (Fig. 1A). Anencephaly, exencephaly and encephalomeningocele are three of the most common NTDs. Further analysis showed that 3.6% of fetuses per litter were with either anencephaly or exencephaly or encephalomeningocele among dams injected with 2.5 mg/kg of $CdCl_2$. In addition, 20% of fetuses per litter were with anencephaly, exencephaly or encephalomeningocele among dams injected with 5.0 mg/kg of $CdCl_2$ (Fig. 1B).

3.2. Cd-induced fetal growth restriction

The effects of maternal Cd exposure on fetal weight and crown-rump length were evaluated. As shown in Fig. 2A, maternal Cd exposure on GD8 reduced fetal weight in a dose-dependent manner. In addition, maternal Cd exposure on GD8 significantly reduced crown-rump length (Fig. 2B). Difference on fetal weight and crown-rump length was then analyzed between fetuses with NTDs and without NTDs. As shown in Fig. 2C, the fetuses with NTDs were lighter than fetuses without NTDs. In addition, the fetuses with NTDs were shorter than fetuses without NTDs (Fig. 2D).

3.3. Maternal Cd exposure impairs placental development

The effects of maternal Cd exposure on placental weight were analyzed. As shown in Fig. 3A, maternal Cd exposure on GD8 reduced placental weight in a dose-dependent manner. In addition, maternal Cd exposure on GD8 significantly reduced placental diameter (Fig. 3B). To investigate whether maternal Cd exposure impairs placental development, a computerized morphometry method was used to analyze cross-sectional areas of blood sinusoids in placental labyrinthine region. As expected, the internal space of fetal and maternal blood vessels was reduced in placentas of Cd-treated mice (Fig. 3C and D).

3.4. Maternal Cd exposure disturbs placental folate transport

To test whether maternal Cd exposure on GD8 disturbs placental folate transport from maternal circulation into the embryos, folate content in maternal serum and embryo was measured 24 h after Cd injection. As shown in Fig. 4A, there was no significant difference on folate content in maternal serum between Cd-treated mice and controls. Interestingly, embryonic folate content was obviously decreased in Cd-treated mice (Fig. 4B).

3.5. Maternal Cd exposure down-regulates expression of PCFT in mouse placenta

The effects of maternal Cd exposure on the expression of placental proton-coupled folate transporter (PCFT) were analyzed. As shown in Fig. 5A, the level of placental *Pcft* mRNA was markedly decreased as early as 2 h after Cd injection. Interestingly, the level of placental *Pcft* mRNA remained decreased at 24 h after Cd injection. Correspondingly, the protein level of placental PCFT was persistently reduced, beginning at 24 h and remaining decreased at 72 h after Cd injection. In addition, the effects of maternal Cd exposure on the expression of placental folate receptor alpha (*Frα*) and reduced folate carrier (Rfc)1 were analyzed. As shown in Fig. S1A and B, maternal Cd exposure on GD8 had little effect on the expression of *Frα* and *Rfc1* mRNA in the placenta.

4. Discussion

Several studies demonstrated that maternal Cd exposure at early limb development resulted in forelimb ectrodactyly and tail deformity, a relatively specific malformation in rodent animals (Lutz and Beck, 2000; Robinson et al., 2009; Robinson et al., 2011; Wang et al., 2012). The present study showed that neither forelimb ectrodactyly nor tail deformity was observed when pregnant mice were injected with $CdCl_2$ on GD8. Instead, maternal Cd exposure on GD8 caused NTDs including anencephaly, exencephaly and encephalomeningocele. An earlier report by our laboratory showed that maternal Cd exposure at late gestational stage resulted in fetal growth restriction in mice (Ji et al.,

Table 2
Effects of maternal cadmium (Cd) exposure during pregnancy on pregnancy outcomes.

	Control	Cd-L	Cd-H
Litters	12	12	12
Live fetuses per litter	12.5 ± 0.4	12.9 ± 0.3	11.4 ± 0.9
Resorptions per litter	0.5 ± 0.2	0.3 ± 0.1	0.6 ± 0.2
Dead fetuses per litter	0.3 ± 0.1	0.3 ± 0.1	1.4 ± 0.4*

In control, Cd-L and Cd-H group, pregnant mice on GD8 were intraperitoneally (i.p.) injected with normal saline (NS), $CdCl_2$ (2.5 mg/kg), and $CdCl_2$ (5 mg/kg), respectively. Data were expressed as mean ± SEM.
* $P < 0.05$ as compared with the control.

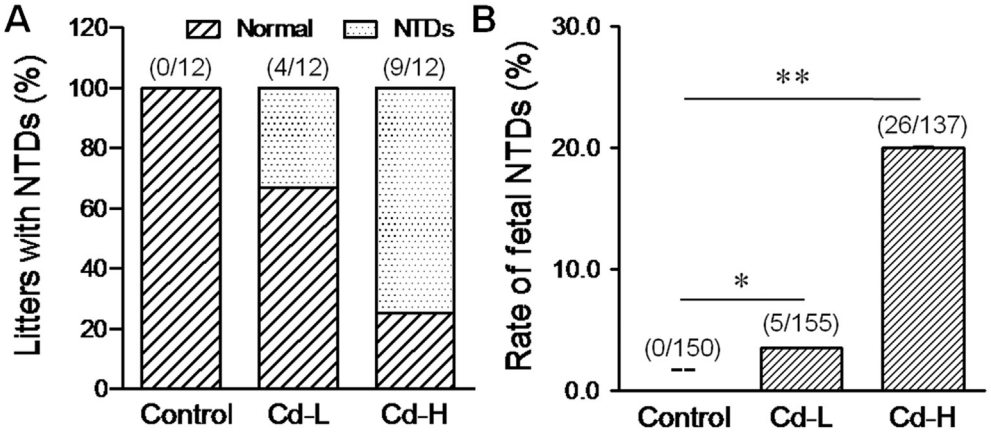

Fig. 1. Maternal Cd exposure results in fetal NTDs. Pregnant mice were i.p. injected with a single dose of $CdCl_2$ (2.5 or 5.0 mg/kg) between 08:00 and 09:00 h on GD8. Saline-treated pregnant mice served as controls. Fetal NTDs were examined on GD18. (A) Rate of litters with NTDs. Divide litters with NTDs by total litters per group equals to rate of litters with NTDs; (B) Rate of fetal NTDs per litter. Divide fetuses with NTDs per litter by total live fetuses per litter equals to rate of fetal NTDs. Data were expressed as mean ± SEM from twelve pregnant mice per group. $^{*}P < 0.05$, $^{**}P < 0.01$.

2011). The present study further analyzed the effects of maternal Cd exposure during organogenesis on fetal weight and crown-rump length in mice. We found that maternal Cd exposure on GD8 significantly reduced fetal weight and crown-rump length in a dose-dependent manner. Surprisingly, the fetuses with NTDs were lighter than fetuses without NTDs. In addition, the fetuses with NTDs were shorter than fetuses without NTDs. These results further expand Cd-induced developmental toxicity.

Several studies demonstrate that placenta deters most of Cd from passing from dams to the fetuses (Ji et al., 2011). Thus, Cd-induced fetal NTDs and growth restriction cannot be completely attributed to its direct toxic effect on the fetuses. Indeed, placenta is essential for sustaining the growth of the fetus during gestation (Yung et al., 2008; Cetin and Alvino, 2009; Scifres and Nelson, 2009). In the present study, we investigated the effects of maternal Cd exposure on GD8 on placental development. Our results showed that maternal Cd exposure on GD8 reduced placental weight and diameter in a dose-dependent manner. The labyrinth is the site of oxygen and nutrient exchange between the mother and the fetus. Increasing evidence indicates that placental transport efficiency depends on the surface area for exchange, thickness of the interhaemal membrane and density of transporter proteins inserted into the trophoblast membranes (Burton and Fowden, 2012). The present study showed that the internal space of maternal and fetal blood vessels in the labyrinth layer was markedly decreased

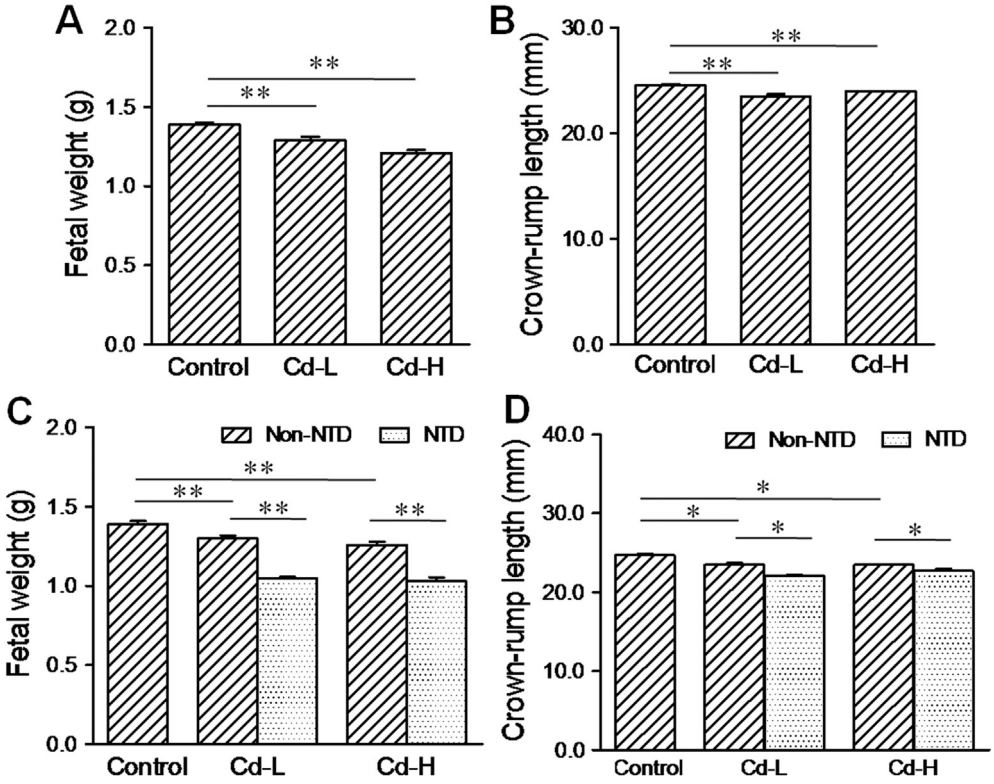

Fig. 2. Maternal Cd exposure induces fetal growth restriction. Pregnant mice were i.p. injected with a single dose of $CdCl_2$ (2.5 or 5.0 mg/kg) between 08:00 and 09:00 h on GD8. Saline-treated pregnant mice served as controls. (A and B) Fetal weight and crown-rump length were measured on GD18. (A) Fetal weight; (B) Crown-rump length. (C and D) Fetal weight and crown-rump length were compared among Cd-exposed NTD fetuses, Cd-exposed non-NTD fetuses and controls. Data were expressed as mean ± SEM from twelve pregnant mice per group. $^{*}P < 0.05$, $^{**}P < 0.01$.

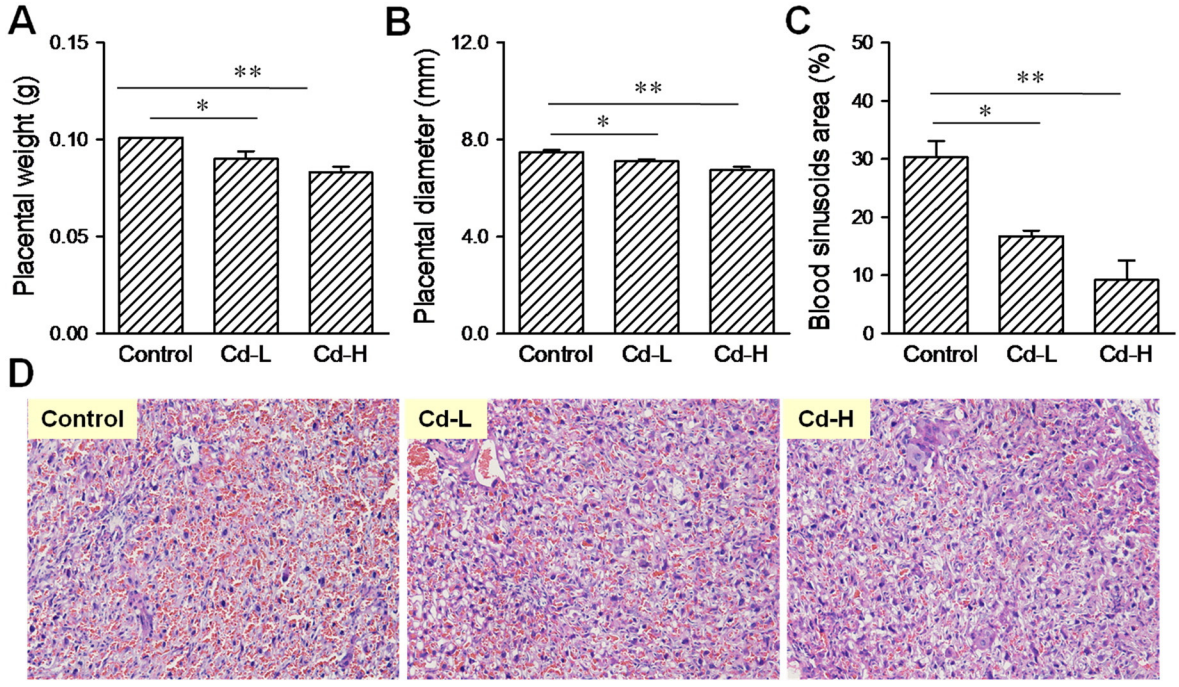

Fig. 3. Maternal Cd exposure impairs placental development. Pregnant mice were i.p. injected with a single dose of $CdCl_2$ (2.5 or 5.0 mg/kg) between 08:00 and 09:00 h on GD8. Saline-treated pregnant mice served as controls. Placentas were collected on GD18. (A and B) The weight and diameter of placentas were measured. (A) Placental weight; (B) Placental diameter. (C) The percentage of blood sinusoid area. Vascular area in the labyrinthine region was estimated from two nonconsecutive sections in each placenta using the public domain NIH Image J Program. Blood sinusoid area (%) was calculated as the ratio between the number of pixels covered by the area defined by the threshold and the overall number of pixels in the image. (D) Representative sections in mouse placenta from control, Cd-L and Cd-H groups. Placental cross-sections were stained with H & E. Original magnification: 200×. Data were expressed as mean ± SEM from twelve pregnant mice per group. $^{*}P < 0.05$, $^{**}P < 0.01$.

in the placentas of mice treated with $CdCl_2$. Thus, we guess that Cd-induced NTDs and fetal growth restriction are partially attributed to its impairment in placental development and the reduction of placental transport capacity.

Increasing evidence demonstrates that maternal folate deficiency during organogenesis is major etiology for fetal NTDs (Fleming and Copp, 1998; Czeizel et al., 2013). Several cohort studies found that maternal folate deficiency during pregnancy was associated with fetal growth restriction (van Eijsden et al., 2008; Baker et al., 2009). An earlier animal experiment showed that maternal folate deficiency during pregnancy significantly increased the incidence of fetal loss, intrauterine growth retardation and heart defects in mice (Li et al., 2005). Thus, it is especially interesting whether maternal Cd exposure during pregnancy influences folate metabolism in dams and fetuses. To demonstrate this hypothesis, the present study analyzed the effects of maternal Cd exposure on folate content in maternal serum and embryo. We showed that maternal Cd exposure on GD8 had little effect on folate content in maternal serum. Interestingly, embryonic folate content was markedly decreased when dams were injected with $CdCl_2$ on GD8. These results indicate that maternal Cd exposure during organogenesis disturbs placental folate transport from maternal circulation into the fetuses.

The mechanism by which Cd inhibits placental folate transport remains obscure. Increasing evidence demonstrates that the transport of folate from maternal circulation into the fetuses depends, to a great extent, on folate receptors, mainly FR-α, and folate transporters, such as PCFT and RFC1 (Zhao et al., 2011). Indeed, FR-α, PCFT and RFC1 are

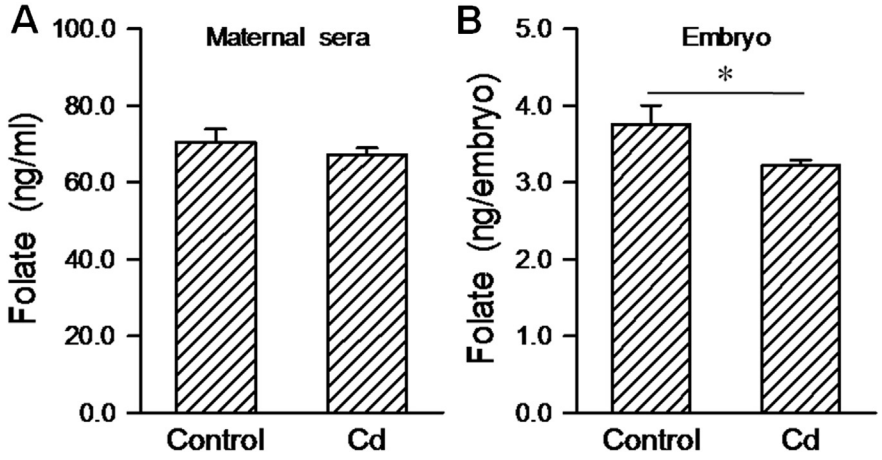

Fig. 4. Maternal Cd exposure disturbs placental folate transport from maternal circulation into the embryos. Pregnant mice were i.p. injected with a single dose of $CdCl_2$ (2.5 or 5.0 mg/kg) between 08:00 and 09:00 h on GD8. Saline-treated pregnant mice served as controls. All pregnant mice were sacrificed 24 h after Cd injection. (A) Folate contents in maternal serum. (B) Folate contents in embryos. Data were presented as mean ± SEM from twelve pregnant mice per group. $^{*}P < 0.05$.

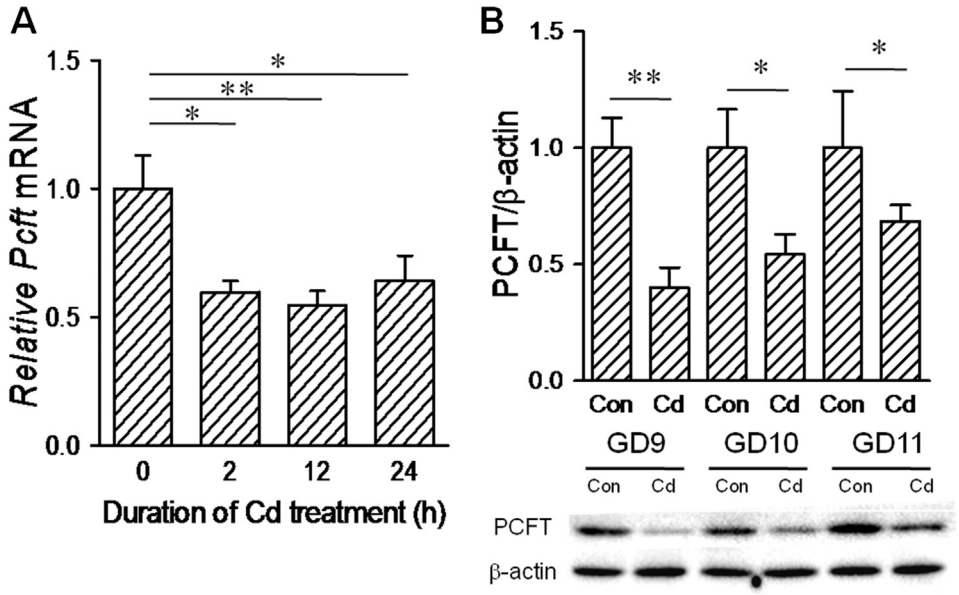

Fig. 5. Maternal Cd exposure down-regulates the expression of PCFT in mouse placenta. Pregnant mice were i.p. injected with a single dose of $CdCl_2$ (2.5 or 5.0 mg/kg) between 08:00 and 09:00 h on GD8. Saline-treated pregnant mice served as controls. Placentas were collected at different time points (2, 12, 24, 48 and 72 h) after Cd injection. (A) *Pcft mRNA*. The mRNA level of Pcft was determined using real-time RT-PCR; (B) PCFT protein. The protein level of PCFT was determined using Western blotting. All data were expressed as mean ± SEM from six pregnant mice per group $^{*}P < 0.05$, $^{**}P < 0.01$.

highly expressed in human and rodent placentas during early embryonic development (Solanky et al., 2010; Cherukad et al., 2012). According to a recent report, epigenetic alterations in folate transport genes were observed in the placentas from fetuses with NTDs (Farkas et al., 2013). The present study investigated the effects of maternal Cd exposure during organogenesis on the expression of placental folate transporters. Although there was no significant difference on the expression of placental *Fr*-α and *Rfc1* among different groups, placental *Pcft* mRNA was markedly down-regulated when dams were injected with $CdCl_2$ on GD8. In addition, placental PCFT protein, a critical folate transporter, has been detected during the critical period of neural tube development (GD9, GD10 and GD11). Our results showed that PCFT protein was persistently reduced, beginning on GD9 and remaining decreased on GD11. These results suggest that maternal Cd exposure during organogenesis disturbs placental folate transport from maternal circulation into the fetuses through down-regulating placental folate transporters.

In summary, the present study investigated the effects of maternal Cd exposure during organogenesis on placental and fetal development. We found that that maternal Cd exposure on GD8 resulted in the incidence of NTDs and fetal growth restriction in mice. We observed that maternal Cd exposure impaired placental development and reduced the internal space of maternal and fetal blood vessels in the labyrinth layer. We demonstrate for the first time that maternal Cd exposure during organogenesis disturbs placental folate transport from maternal circulation into the fetuses through down-regulating placental folate transporters.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

Transparency document

The Transparency document associated with this article can be found, in the online version.

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