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Effects of choline on sodium arsenite-induced neural tube defects in chick embryos

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ABSTRACT

Arsenic passes through the placenta and accumulates in the neuroepithelium of embryo, whereby inducing congenital malformations such as neural tube defects (NTDs) in animals. Choline (CHO), a methyl-rich nutrient, functions as a methyl donor to participate in methyl group metabolism. Arsenic methylation has been regarded as a detoxification process and choline (CHO) is the major source of methyl-groups. However, whether CHO intake reverses the abnormal embryo development induced by sodium arsenite (SA) and the relationship between CHO intake and arsenite-induced NTDs are still unclear. In this study, we used chick embryos as animal model to investigate the effects of SA and CHO supplementation on the early development of nervous system. Our results showed that the administration of SA led to reduction in embryo viability, embryo body weight and extraembryonic vascular area, accompanied by a significantly increased incidence of the failed closure of the caudal end of the neural tube. CHO, at low dose (25 μg/μL), reversed the decrease in embryo viability and the increase in the failed closure of the caudal end of the neural tube, which were induced by SA. In addition, CHO (25 μg/μL) inhibited not only the SA-induced cell apoptosis by up-regulating Bcl-2 level, but also the global DNA methylation by increasing the expressions of DNMT1 and DNMT3a. However, less significant difference was found between the embryos co-treated with SA and CHO (50 μg/μL) and the ones treated with SA alone. Taken together, these findings suggest that low dose CHO could protect chick embryos from arsenite-induced NTDs by a possible mechanism related to the methyl metabolism.

1. Introduction

Arsenic, which is mainly found in inorganic forms, has become a ubiquitous environmental pollutant because it is widely and duratively used in industry and agriculture (Coppin et al., 2008; Wlodarczyk et al., 1996). Epidemiological studies have shown that arsenic exposure leads to an increased morbidity of certain cancer and reproductive toxicities (Bettley and O'Shea, 1975; Borzsonyi et al., 1992). Increasing evidence shows that arsenic is both a teratogen and a carcinogen (Barrett et al., 1989; Hamilton and Bloom, 1986; Hood and Bishop, 1972). Arsenic passes through the placenta and accumulates in the neuroepithelium of embryo, whereby inducing congenital malformations such as neural tube defects (NTDs) in animals (Chaineau et al., 1990; Hood et al., 1988). However, the molecular and cellular mechanisms of arsenic inducing NTDs remain to be determined.

Inorganic arsenic is metabolized to form mono- and dimethylarsenic compound utilizing S-adenosylmethionine (SAM) as the methyl donating cofactor (Thomas et al., 2001), and this methylation facilitates urinary arsenic excretion (Heck et al., 2007). Methyl donors deficiency significantly decreases the total urinary excretion of orally administered sodium arsenite and markedly modulates target organ arsenic-induced DNA damage (Tice et al., 1997).

Choline (CHO), a methyl-rich nutrient, functions as a methyl donor to participate in methyl group metabolism (Zeisel, 1981). Studies in animal models and epidemiological studies in humans show that CHO intake during pregnancy is particularly importance for the normal development and function of the central nervous system (Blusztajn, 1998; Shaw et al., 2004; Zeisel, 2006). Several studies have demonstrated that prenatal CHO deficiency causes deficits in certain memory tasks (Meck and Williams, 1997), whereas prenatal CHO supplementation leads to enhance memory and attention, and prevent age-related memory decline (Meck et al., 1988; Meck and Williams, 1999; Mellott et al., 2004). In rodents, CHO is required for normal neural tube closure in early pregnancy. CHO-modulated arsenic trioxide-induced prolongation of cardiac repolarization in guinea pig (Sun et al., 2006). However, whether

Abbreviations: CHO, choline; SA, sodium arsenite; NTDs, neural tube defects; SAM, S-adenosylmethionine; TUNEL, terminal deoxynucleotidyl transferase-mediated biotinylated dUTP nick end labeling; 5-mec, 5-mecthylcystidine; SCP1, Small C-terminal domain phosphatase 1.

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Table 1
The effects of SA with or without CHO on chick embryo viability.

Exogenous treatment	Survival rate of embryos (%) (n = 25)	Embryo body weight (mg) (n = 15)
Saline (control)	97.33 ± 2.3	18.8 ± 1.9
SA	72.00 ± 4.0**	10.4 ± 3.7**
CHO25 + SA	88.00 ± 4.0##	15.5 ± 4.4#
CHO50 + SA	69.00 ± 6.1	9.9 ± 3.1
CHO25	96.00 ± 4.0	18.2 ± 1.9
CHO50	93.33 ± 2.3	18.0 ± 1.7

Data presented as mean ± S.D.
* $P < 0.05$, compared with the control.
** $P < 0.01$, compared with the control.
# $P < 0.05$, compared with SA-treated alone.
$P < 0.01$, compared with SA-treated alone.

CHO intake reversed the abnormal embryo development induced by SA is still unclear.

Central nervous system (CNS) of the chick embryo is an excellent model system to study the mechanisms involved in the patterning of the Central Nervous System in early embryo development (Adiguzel, 2004). So chick embryo is used as an embryological model to study neural tube closure and to examine the effects of various chemical agents (such as cotinine, meloxicam and phenytoin) on early embryonic neural tube development (Cetinkal et al., 2010; Dalgic et al., 2009; Guney et al., 2003; Hamilton et al., 1998; Temiz et al., 2009).

In the present study, the chick embryo was selected as the animal model to study the effects of different dose CHO on sodium arsenite-induced NTDs. We demonstrated the embryos neurotoxicity induced by SA exposure and the roles of different dose CHO in this process. We also studied the possible molecular mechanism of CHO and SA in the nervous system development of chick embryos. This study may provide new insights into the relation of environmental pollutant and micronutrient during the early embryo development.

2. Materials and methods

2.1. Embryo treatment

White Leghorn chick eggs (Bovan strain) were purchased from Merial Vital Laboratory Animal Technology Co., Ltd. (Beijing, China). To begin embryonic development, chick eggs were placed in automatic tilting racks in an incubator (Grumpatch, Savannah, GA, USA) and incubated at 38 °C and 60% humidity. Chick embryos were treated with SA (100 nM, Sigma–Aldrich Corp., St. Louis MO, USA) with or without CHO (25 or 50 μg/μL; Sigma–Aldrich Corp., St. Louis MO, USA), CHO (25 or 50 μg/μL) alone or saline as the control group at Hamburger–Hamilton (HH) stages 6, 8 and 12 (Hamburger and Hamilton, 1951). 50 μL of SA, CHO or saline were directly injected into the center of the egg yolk via a small hole at the blunt end of the egg using an established protocol (Rosenquist et al., 1996). Embryos were harvested for analysis after incubation for 72 h (HH stage 18).

2.2. Embryo viability

Embryo viability was indirectly monitored by measuring embryo body weight (n = 15), extraembryonic vascular development (n = 10) and embryo survival rate (n = 25) at day 3 of incubation (theoretical HH stage 19–20). In our experiment we used 25 chick embryos to examine embryo viability, the embryos were divided into two groups, one group containing 10 embryos was used to analyze extraembryonic vascular development (n = 10) and the other group containing 15 embryos was used to analyze embryo body weight (n = 15). All the embryos were used to analyze survival rate (n = 25). The experiment was repeated at least three times. Live embryos were defined as those possessing a beating heart. Embryo survival rate was expressed as the ratio of live embryos' numbers vs. total embryos' numbers. The body weight of each embryo is recorded to do statistical analysis. Extraembryonic vascular was photographed using commercial digital camera (Cybershot, Sony, Tokyo, Japan) and the images were scanned into Adobe Photoshop (Microtek, Scanmaker III, Redondo Beach, CA). Relative extraembryonic vascular area was quantified using ImagePro software, as described by Hayek et al. (1991) which was referenced to the control groups.

2.3. Histologic methods

Embryos harvested at 72 h were fixed in 4% formaldehyde, embedded in paraffin wax and serially sectioned (4 μm thickness). Neural tube cross sections which placed on the lumbosacral region were stained with Hematoxylin–Eosin (H&E) for morphological assessments. All the sections were examined and recorded using 400× objective on Nikon TE 2000-U microscope (Nikon, Japan). The incidence of the failed closure of the caudal end of the neural tube was expressed as ratio of the failed closure of the caudal end of the neural tube of embryos vs. total embryos.

2.4. Terminal deoxynucleotidyl transferase-mediated biotinylated dUTP nick end labeling (TUNEL) staining

In whole-mount TUNEL staining, chicken embryos were fixed, dehydrated, and rehydrated through graded methanol concentrations into PBS containing 0.1% Tween-20 (PBST). In paraffin-embedded section TUNEL staining, sections from chick embryo neural tube were deparaffinized and rehydrated, then sections were treated with proteinase-K and refixed in methanol-free 4% formaldehyde. DeadEnd Colorimetric TUNEL System (Promega, Madison, WI, USA) was used for *in situ* visualization of DNA fragmentation in whole mount embryos and sections from chick embryo neural tube. Embryos or sections were incubated with a solution consisting of equilibration buffer, nucleotide mix and TdT enzyme at 4 °C overnight. The reac-

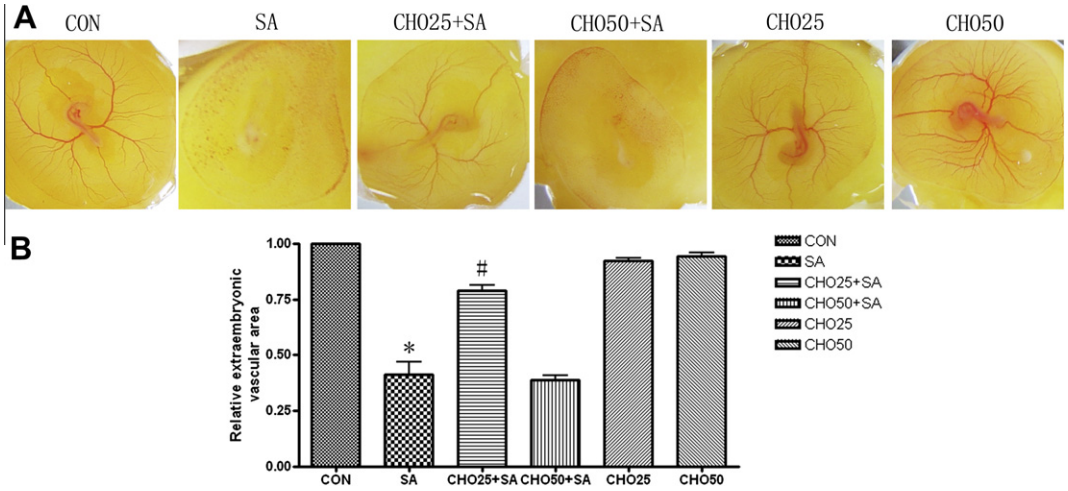

Fig. 1. Effects of CHO on the development of the extra-embryoonic vasculature in SA-induced chick embryos. Chick embryos were treated with SA (100 nM) with or without CHO (25 or 50 μg/μL) for 72 h. The images were scanned for quantification of relative extraembryonic vascular area by ImagePro software 5.01 (A) Representative of images revealed the development of the extra-embryoonic vasculature. CON: saline control group; SA: sodium arsenite (100 nM)-treated group; CHO25 + SA: choline (25 μg/μL) + sodium arsenite (100 nM)-treated group; CHO50 + SA: choline (50 μg/μL) + sodium arsenite (100 nM)-treated group; CHO25: choline (25 μg/μL)-treated group; CHO50: choline (50 μg/μL)-treated group; (B) Quantification of the relative area of extra-embryonic vasculature. Values are expressed as mean ± S.D. *$P < 0.05$, compared with the control; **$P < 0.01$, compared with the control; #$P < 0.05$, compared with SA-treated alone; ##$P < 0.01$, compared with SA-treated alone.