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Dichloroacetonitrile induces oxidative stress and developmental apoptotic imbalance in mouse fetal brain

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ABSTRACT

Dichloroacetonitrile (DCAN) is one of the disinfection by-products of chlorination of drinking water. Limited mechanistic studies exist on the developmental toxicity of haloacetonitriles (HANs). The present study was designed to investigate the potential adverse effects of maternal exposure to DCAN on mouse fetal brain. Based on initial dose–response experiment, DCAN (14 mg/kg/day) was administered orally to pregnant mice at gestation day (GD) 6, till GD 15. Maternal exposure to DCAN resulted in redox imbalance in fetal cortex and cerebellum, characterized by significant decrease in reduced glutathione (GSH), and elevation of malondialdehyde (MDA) level and superoxide dismutase (SOD) activity. Further, DCAN induced apoptosis indicated by significant enhancement of DNA fragmentation and active caspase-3 level in fetal cortex and cerebellum. Neuronal degeneration was indicated by positive cupric silver staining. In conclusion, maternal exposure to DCAN adversely affects mouse fetal brain as evidenced by induction of oxidative stress, apoptotic imbalance and neurodegeneration.

1. Introduction

Chlorination of water supplies is among the most important public health advances of the 20th century, eliminating once-common diseases such as cholera, dysentery and typhoid fever (Mughal, 1992). However, the formation of a variety of disinfection by-products (DBPs) due to interaction of chlorine with organic matter is a significant health hazard (Doyle et al., 1997). These chemicals include halomethanes, haloacetic acids, and haloacetonitriles (Muellner et al., 2007). Among HANs, dichloroacetonitrile (DCAN) possesses a major concern. It was previously reported to be teratogenic in pregnant Long-Evans rats and capable of inducing observed anomalies (Smith et al., 1989). DCAN can be formed in vivo, following human consumption of drinking water contaminated with chlorine residues (Mink et al., 1983). It has been also detected in swimming pools because of chlorination-mediated reactions that degrade nitrogen-containing compounds of human origin including urea, hair, saliva and skin (Kim et al., 2002).

Epidemiological studies have indicated a moderate evidence of relationship between HANs and sporadic forms of adverse pregnancy outcomes (APOs) (Bove et al., 2002). These are manifested as pre-term delivery (Bove et al., 1995); spontaneous abortion (Waller et al., 1998); neural tube defects (Klotz and Pyrch, 1999); skeletal defects (Magnus et al., 1999); low birth weight (Nieuwenhuijsen et al., 2000) and intra-uterine growth restriction (Graves et al., 2001). However, only limited mechanistic studies exist on the developmental toxicity of HANs (Lipscomb et al., 2009).

Exposure to environmental chemicals during early stages of brain development can induce adverse effects that span

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the embryonic, fetal, and offspring stages (Guerri, 1998). It is worthy noted that environmental chemicals can alter mitochondrial oxidative phosphorylation and the subsequent generation of reactive oxygen species (ROS) that are responsible for neural cell death and a broad spectrum of neurological diseases (Breen and Murphy, 1995). Fetal brains, that consume large amounts of oxygen, are prone to injury by ROS, leading to abnormal brain development (Aschner et al., 1999). Additionally, proper brain development requires apoptosis that systematically removes large number of neurons produced during ontogeny (Blaschke et al., 1996). Alterations in apoptotic balance by environmental chemicals may cause loss or retention of neurons and thus compromising neurological functions (Busciglio and Yankner, 2001).

Optimal brain development is considered a key factor for normal pregnancy outcome (Lanzi et al., 1990). In addition, the structure and function of various organs are controlled by the brain (Rice and Stan, 2002). Significant association between APOs and DBPs was found in women who consumed more than five glasses of water per day where DBPs concentrations were about 75 μg/l (Wright et al., 2004). In addition, dihaloacetonitriles were detected in drinking water at concentrations up to 42 μg/l (Trehy and Bieber, 1981). Thus, the development of an animal model exposed to known concentrations of individual DBP at various gestational stages is required in order to evaluate this causal relationship between maternal exposures to individual DBP and APO. Therefore, the present study was designed to investigate the potential adverse effects of maternal exposure to DCAN on fetal brain in mice, with respect to oxidative stress and apoptosis.

2. Experimental procedures

2.1. Chemicals

DCAN 98%, Ellman's reagent [5,5-dithio-bis (2-nitrobenzoic acid); DTNB], Folin reagent, reduced glutathione (GSH), thiobarbituric acid (TBA), trichloroacetic acid (TCA), silver nitrate and bovine serum albumin (BSA) were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). Kits for determining the activities of superoxide dismutase (SOD) and catalase were obtained from Randox Laboratories, Crumlin, UK. ELISA kit for caspase-3 activity was purchased from R&D Systems (Minneapolis, MN, USA). Genomic DNA Purification Kit was obtained from Fermentas (St. Leon-Rot, Germany). All other chemicals and solvents were of highest purity commercially available.

2.2. Animals

Mature male and female BALB/c mice, weighing 25–30 g, were obtained from the Animal Breeding Laboratory, VACSERA, Helwan, Egypt. Animals were housed in plastic cages in our animal facility at a temperature of 25 ± 2 °C, a relative humidity of 50 ± 10%. Mice were fed standard diet pellets and tap water was supplied *ad libitum*. All procedures of laboratory animal care in research were approved by the Local Ethical Committee, Ain Shams University, Cairo, Egypt. After a quarantine period of 10 days, one male and four females were cohabited overnight. The females were examined the following morning for copulatory plugs. The day on which a vaginal plug was found was designated as gestation day 0 (GD 0).

2.3. Experimental design

In order to evaluate the causal relationship between maternal exposures to DCAN and APO, an animal model was established following FDA guidelines for reproduction studies (Single-generation studies, segment II: assessment of developmental toxicity) (Branch, 2004). The average breeding time was 1 month. At GD 0, plug-positive female mice were randomly divided between four groups ($n = 10$ animals per group). Group I served as a control and was given corn oil (8 ml/kg/day) orally from GD 6 to GD 15. DCAN was dissolved in corn oil and given to groups II, III, and IV at respective doses of 7, 14 and 28 mg/kg/day orally in a dosing volume of 8 ml/kg from GD 6 to GD 15. These doses were tested in a dose–response experiment to help select a marginally non-lethal dose for developing fetuses. The tested doses are approximately equal to $LD_{1.25}$, $LD_{2.5}$ and LD_5 (Hayes et al., 1986). At the end of the experiment (GD 18), animals in all groups were sacrificed, and the abdomen of each mouse was opened. The numbers of total implants, resorptions, and live & dead fetuses were recorded. All live fetuses (males and females) were dissected from the uterus and evaluated for body weight. Live fetuses from each group (control and 14 mg/kg/day DCAN-treated group) were used for subsequent biochemical and histological analyses. Fetal cortices and cerebella from each litter were isolated and stored at −80 °C until homogenization and biochemical analyses. Representative fetal heads (encapsulated brains) from each group were fixed in Bouin's solution for histological examination of neurodegeneration.

2.4. Tissue samples collection and preparation

Generally, cortical and cerebellar tissues from each group were weighed and homogenized (1:10, weight/volume) with ice-cold phosphate-buffered saline (PBS, 0.01 M, pH 7.4). The homogenates were centrifuged at 4 °C for 15 min at 4000 × *g*. The supernatants were collected, divided into aliquots and used directly for biochemical analyses or kept frozen at −80 °C for subsequent measurements. For determination of non-protein thiols, tissue samples were homogenized in a solution containing 5% trichloroacetic acid and 5 mM EDTA at 4 °C. Each sample was centrifuged at 4000 × *g* for 15 min at 4 °C.

2.5. Biochemical assessment of oxidant/antioxidant parameters

Non-protein thiols (indicative to GSH content) were determined as described by Ellman (1959). Total glutathione was measured according to the method described by Tietze (1969). Lipid peroxides were determined as thiobarbituric acid reactive substances (TBARS), according to method of Uchiyama and Mihara (1978). The assay of SOD activity employs the use of xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitropheno)-5-phenyltetrazolium-chloride to form a red formazan dye. The superoxide dismutase activity was measured by the degree of