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Research Report

Fetal origin of adverse pregnancy outcome: The water disinfectant by-product chloroacetonitrile induces oxidative stress and apoptosis in mouse fetal brain

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Abstract

Epidemiological studies indicate a relationship between water disinfectant by-products (DBP) and adverse pregnancy outcomes (APO) including neural tube defects. These studies suggest that fetal brain may be vulnerable to DBP during early stages of development. Therefore, we examined several molecular markers commonly known to indicate chemical-induced neurotoxicity during fetal brain development following prenatal exposure to the DBP; chloroacetonitrile (CAN).

Pregnant mice, at gestation day 6 (GD6), were treated with a daily oral dose of CAN (25 mg/kg). At GD12, two groups of animals were treated with an i.v. tracer dose of [2-^{14}C]-CAN. These animals were sacrificed at 1 and 24 h after treatment and processed for quantitative in situ micro-whole-body autoradiography. The remaining groups of animals continued to receive CAN. At GD18, control and treated animals were weighed, anesthetized, and fetuses were obtained and their brains were removed for biochemical and immunohistochemical analyses.

Whole-body autoradiography studies indicate a significant uptake and retention of [2-^{14}C]-CAN/metabolites (M) in fetal brain (cerebral cortex, hippocampus, cerebellum) at 1 and 24 h. There was a 20% reduction in body weight and a 22% reduction in brain weight of fetuses exposed to CAN compared to controls. A significant increase in oxidative stress markers was observed in various fetal brain regions in animals exposed to CAN compared to controls. This was indicated by a 3- to 4-fold decrease in the ratio of the reduced to oxidized form of glutathione (GSH/GSSG), increased lipid peroxidation (1.3-fold), and increased 8-hydroxy-2-deoxyguanosine levels (1.4-fold). Cupric silver staining indicated a significant increase in the number of degenerating neurons in cortical regions in exposed animals. In animals exposed to CAN there was increase in nuclear DNA fragmentation (TUNEL staining) detected in the cerebral cortex and cerebellum (2-fold increase in apoptotic indices). Caspase-3 activity in cerebral cortex and cerebellum of treated animals were also increased (1.7- and 1.5-fold, respectively). In conclusion, this study indicates that CAN/M crossed the placenta and accumulated in fetal brain tissues where it caused oxidative stress and neuronal apoptosis. These events could predispose the fetus to altered brain development leading to APO as well as behavioral and learning and memory deficits.

Theme: Disorders of the nervous system: developmental disorders
Topic: Neurotoxicology

Keywords: Adverse pregnancy outcome; Water disinfectant by-product; Neurotoxicity; Chloroacetonitrile; Oxidative stress; Apoptosis

Abbreviations: APO, adverse pregnancy outcome; CAN, chloroacetonitrile; DBP, disinfectant by-products; HAN, haloacetonitriles; GSH, L-γ-glutamyl-L-cysteinyl-glycine; GSSG, glutathione disulfide; IMWBA, in situ micro-whole-body autoradiography; ROS, reactive oxygen species; 8-OHdG, 8-hydroxy-2-deoxyguanosine; VYS, visceral yolk sac

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1. Introduction

Chlorination of drinking water results in the formation of a variety of DBP including trihalomethanes, haloacetic acids, and haloacetonitriles (HAN, X_nCH_n CN) such as chloroacetonitrile (CAN, $ClCH_2$-CN). Epidemiological studies indicate that DBP induce susceptibility to sporadic forms of APO [12,18,22,23,31,33,53] manifested as congenital CNS anomalies such as neural tube defects (NTD) [34]; intra-uterine growth restriction [35,23]; skeletal defects [40]; very low birth weight [28,45] pre-term delivery [11,23]; and spontaneous abortion [5,53,57]. Significant association between APO and DBP was found in women who consumed more than five glasses of water per day where DBP concentrations were about 75 μg/l [62]. 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.

Important signals to divide and differentiate inside the womb start at the earliest stages of embryonic development. Shifts in either of these processes or their initiation signals can lead to altered structures and functions throughout gestation and probably throughout the entire lifespan. Optimal brain development is considered the key factor in normal fetal outcome [38]. In addition, the structure and function of various other organs are controlled by the brain [50]. Alteration in brain development is well known to result in multiple signals that lead to APO [19,59].

Beginning early on in the second week of gestation in rodents (GD7 in mouse) and first month of gestation in humans, specific areas of the CNS begin to form in the forebrain, midbrain, and hindbrain [50]. There follows a sequence of developmental processes of proliferation, migration, differentiation, synaptogenesis, apoptosis, and myelination [50]. Perturbations in these processes during development result in long-term irreversible consequences that affect the structure and function of CNS and account for susceptibility to abnormal brain development [7]. Exposure to chemicals during various stages of brain development is known to induce adverse effects that span the embryonic, fetal, and offspring stages [8,9,25].

Environmental chemicals are known to alter mitochondrial oxidative phosphorylation and the subsequent generation of hydroxyl radicals [14,20] that oxidize cellular proteins, lipids, and DNA. Defects in mitochondrial function and excessive formation of reactive oxygen species (ROS) are responsible for neural cell death and a broad spectrum of neurological diseases. Thus, fetal brains, which consume large amounts of O_2, are prone to damage by ROS, which can also induce apoptosis. During critical periods of development, exposure to environmental chemicals may shift the tightly regulated balance of neurotrophic signals that regulate apoptosis, thereby causing shifts that result in altered cell number in particular brain regions leading to abnormal brain development [6,21,26,39,41,44,50].

HAN possess genotoxic and teratogenic activities [16,43]. Increased covalent interactions of CAN with fetal DNA following glutathione (GSH) depletion has been reported [1]. CAN caused mitochondrial damage and inhibited ATP production [46]. CAN and other HAN induce oxidative stress, resulting in the in vivo formation of 8-hydroxy-2-deoxyguanosine (8-OHdG) [3]. Low doses of HAN induce apoptotic cell death while high doses cause necrosis in mouse peritoneal macrophages [4,10,56,58].

These reports provide valuable clues on the mechanism of toxicity of these chemicals. They have, however, overlooked the molecular cascade of signals that precede the observed toxic endpoints. The major reason for the shortcoming is the use of high concentrations of the chemicals to ascertain toxicity. The current concern is that chemicals at low dose levels may cause molecular defects that disrupt developmental processes, possibly without observable physical malformation, leading to APO.

The disposition of environmental agents in organs provides important mechanistic information on the potential target organs of toxicity and establishes causal relationships. Therefore, the objective of this study is to examine the uptake of [2-^{14}C]-CAN/M in fetal mouse brain, the impact of uptake of CAN/M on redox balance and developmental apoptosis in fetal brain. We used repeated in utero exposure to low, non-teratogenic doses of CAN that do not produce detectable maternal or fetal signs of toxicity.

2. Methods

2.1. Chemicals

[2-^{14}C]-CAN (Specific activity 4.07 mCi/mmol) was obtained from Pathfinder Laboratories Inc. (St. Louis, MO). Radiochemical purity of [2-^{14}C]-CAN was determined by gas chromatographic analysis (Isothermal at 35 °C on a 30-cm SE-30 capillary column). All other chemicals were of the highest purity grade available. The experiments were conducted in well-ventilated laboratory hoods.

2.2. Animal care and treatment

All animal care and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee prior to initiation of experiments. Eight-week-old (22 g) timed pregnant mice (CD-1 strain, Charles River Laboratories, Wilmington, MA) were used in all experiments. Animals were maintained on a regular diet and water throughout the experimental period. Pregnant mice at gestation day 6 (GD6) were divided into six groups (6 mice/group), two control and four treatment groups. Treatment groups were given a daily oral dose of CAN (25 mg/kg) until GD18. Control groups received equal volume of vehicle. Of the treatment groups, two were used for disposition