Toxicology and Applied Pharmacology 269 (2013) 263–269

Contents lists available at SciVerse ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Metabolic changes and DNA hypomethylation in cerebellum are associated with behavioral alterations in mice exposed to trichloroethylene postnatally

Sarah J. Blossom [a,*], Craig A. Cooney [b], Stepan B. Melnyk [a], Jenny L. Rau [a], Christopher J. Swearingen [a], William D. Wessinger [c]

[a] Department of Pediatrics, University of Arkansas for Medical Sciences, College of Medicine, Arkansas Children's Hospital Research Institute, 13 Children's Way, Little Rock, AR 72202, USA
[b] Department of Research and Development, Central Arkansas Veterans Healthcare System, John L. McClellan Memorial Veterans Hospital, 4300 West 7th St., Little Rock, AR 72205-5484, USA
[c] Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, College of Medicine, 4301 West Markham St., Little Rock, AR 72205, USA

ARTICLE INFO

Article history:
Received 30 January 2013
Revised 16 March 2013
Accepted 18 March 2013
Available online 6 April 2013

Keywords:
Cerebellum
Trichloroethylene
Locomotor behavior
Oxidative stress
Methylation

ABSTRACT

Previous studies demonstrated that low-level postnatal and early life exposure to the environmental contaminant, trichloroethylene (TCE), in the drinking water of MRL+/+ mice altered glutathione redox homeostasis and increased biomarkers of oxidative stress indicating a more oxidized state. Plasma metabolites along the interrelated transmethylation pathway were also altered indicating impaired methylation capacity. Here we extend these findings to further characterize the impact of TCE exposure in mice exposed to water only or two doses of TCE in the drinking water (0, 2, and 28 mg/kg/day) postnatally from birth until 6 weeks of age on redox homeostasis and biomarkers of oxidative stress in the cerebellum. In addition, pathway intermediates involved in methyl metabolism and global DNA methylation patterns were examined in cerebellar tissue. Because the cerebellum is functionally important for coordinating motor activity, including exploratory and social approach behaviors, these parameters were evaluated in the present study. Mice exposed to 28 mg/kg/day TCE exhibited increased locomotor activity over time as compared with control mice. In the novel object exploration test, these mice were more likely to enter the zone with the novel object as compared to control mice. Similar results were obtained in a second test when an unfamiliar mouse was introduced into the testing arena. The results show for the first time that postnatal exposure to TCE causes key metabolic changes in the cerebellum that may contribute to global DNA methylation deficits and behavioral alterations in TCE-exposed mice.

Introduction

Trichloroethylene (TCE) is an organic solvent used as an industrial degreasing agent. Human TCE exposure can occur at all stages of life. TCE crosses the placenta and can be detected in breast milk (Beamer et al., 2012). Studies of school-aged children found detectable blood levels of TCE in approximately 6% of the subjects (Adgate et al., 2004; Sexton et al., 2005). One of the predominant non-cancer health effects associated with exposure to TCE is neurotoxicity (Bale et al., 2011; Chiu et al., 2006). However, the mechanisms underlying this toxicity, as well as the behavioral effects resulting from this exposure remain elusive.

Xenobiotics are known to impart long-lasting effects on organ systems especially if exposure occurs during development (Heindel, 2008). Epidemiologic studies documenting human exposure reported motor function deficits in occupationally exposed adults (Rasmussen et al., 1993). Individuals who ingested water from TCE-contaminated water wells and municipal water supplies showed higher mean scores for depression and mood disorders, and lower intelligence scores as compared to subjects who did not ingest contaminated water (Kilburn and Warshaw, 1993; Reif et al., 2003). In terms of developmental exposure, impaired motor coordination and behaviors characterized by inattention and hyperactivity in children of mothers exposed to solvents occupationally have been documented (Laslo-Baker et al., 2004; Till et al., 2001). Rodent studies by our lab and others have shown that during the perinatal and postnatal periods of development, the brain is particularly vulnerable to oxidative stress, impaired glutathione redox balance, and inflammation from exposure to environmental toxicants (Blossom et al., 2012; Stringari et al., 2006).

Oxidative stress and impaired glutathione anti-oxidant capacity can result from toxicant exposure and is linked to neurologic disorders. Glutathione is a tripeptide that functions as the major intracellular anti-oxidant against oxidative stress and plays an important role in the detoxification of reactive oxygen species in the brain (Biswas et al., 2006; Jain et al., 1991). Pro-oxidant environmental exposures have the potential to decrease the active reduced form of glutathione (GSH) and to increase the inactive oxidized disulfide form (GSSG) leaving the cell vulnerable to oxidative damage. Alterations in glutathione redox potential have been shown to modulate the fate of oligodendrocyte precursor cells

* Corresponding author. Fax: +1 501 364 2403.
E-mail address: blossomsarah@uams.edu (S.J. Blossom).

0041-008X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.taap.2013.03.025

(Noble et al., 2005), and maturing neurons (Maffi et al., 2008; McLean et al., 2005) suggesting that altered brain redox status and increased oxidative stress could hinder neural development and promote behavioral pathology.

We recently demonstrated that mice exposed to TCE in the drinking water postnatally from birth postnatal day (PND) 0 through PND 42 showed decreased GSH and an increase in the GSH/GSSG ratio commensurate with elevated levels 3-nitrotyrosine, a biomarker of oxidative protein damage, in the hippocampus (Blossom et al., 2012). These metabolic changes in the hippocampus were accompanied by alterations in the inter-related transmethylation pathway metabolites in the plasma. As shown in Fig. 1 one crucial pathway that intersects with the transsulfuration pathway leading to glutathione synthesis is the methionine cycle (Selhub, 2002). From a functional standpoint, deficits in transmethylation metabolites could lead to global DNA hypomethylation and ultimately impact cell differentiation, gene expression, and chromatin structure (Castro et al., 2003; Caudill et al., 2001; Feil, 2006; Yi et al., 2000).

The purpose of this study was to investigate the impact of postnatal TCE exposure on glutathione redox potential and biomarkers of oxidative stress in the cerebellum. In this study, methionine was examined in cerebellum rather than plasma. Mice were exposed to drinking water alone or two doses of TCE in the drinking water postnatally from birth until 6 weeks of age. Global DNA methylation was assessed in cerebellar tissue to examine the potential impact of impaired methyl metabolism with TCE exposure. Because the cerebellum is functionally important for coordinating motor activity, including exploratory and social approach behaviors, these behavioral parameters were evaluated in the present study. The results show for the first time that postnatal exposure to TCE causes key metabolic changes in the cerebellum that may contribute to global DNA methylation deficits and behavioral alterations in TCE-exposed mice.

Methods

Mice. Male MRL+/+ mice were used to evaluate TCE-induced neurotoxicity as described previously (Blossom et al., 2012). The MRL+/+ strain of mice were used based on this strain's sensitivity to TCE toxicity as described (Blossom and Doss, 2007; Blossom et al., 2006, 2008, 2008). MRL+/+ mice are by all accounts normal and are often used as the control strain for studies using MRL/lpr mice. MRL/lpr mice develop, in addition to autoimmune disease, several behavioral deficits and neuropathological changes with age and are considered to be a model of idiopathic neurological lupus (Ballok, 2007; Ballok et al., 2004; Sakic et al., 2005). Unlike control C57Bl/6 mice, young MRL+/+ mice have been used to study neurogenesis in response to pharmacological agents that target neuroplasticity (Balu et al., 2009; Hodes et al., 2010). Because many neurological disorders such as autism are accompanied by immune dysfunction and autoimmunity (Cabanlit et al., 2007; Comi et al., 1999; Molloy et al., 2006; Sweeten et al., 2003), MRL+/+ mice were used in the current study.

Only male, but not female offspring were evaluated in the current study. Females were not included based on our published findings showing male mice were much more sensitive than female mice to TCE-mediated neurologic effects involving oxidative stress (Blossom et al., 2008). In the current study, eight-week-old MRL+/+ breeder pairs were purchased from Jackson Laboratories (Bar Harbor, ME). The mice were acclimated to the animal facility for one week before breeding cages were established. During breeding the mice were housed in standard polycarbonate cages and had free access to drinking water and standard laboratory mouse chow (Harlan 7027). The pregnant mice were housed in separate cages, provided with nesting material (Ancare, Bellmore, NY) and checked twice daily for new born pups. The date the pups were born (PND 0) was recorded and the dams were then randomly assigned to treatment groups [0 (vehicle control), 0.01, and 0.1 mg/ml TCE]. TCE (purity 99+% from Sigma, St. Louis, MO) was suspended in drinking water with an emulsifier, 1% of ethoxylated castor oil (Alkamuls EL-620™ (Rhone-Poulenc, Cranbury, NJ)). Mice not receiving TCE received water with 1% ethoxylated castor oil as a vehicle control. There were a total of 10 dams per treatment group. Nine dams in the control group and 8 dams in the TCE-treated groups produced litters with sufficient numbers of male pups for our study. Dams received a freshly made solution of TCE in their drinking water every 2–3 days beginning at PND 0. At PND 21, the offspring were weaned and the male mice continued their exposure to vehicle or vehicle + TCE in the drinking water until PND 42. For each experimental study, one randomly selected male mouse from each litter was chosen thus the litter and not the individual mouse represented the experimental 'n.' As shown previously, water consumption in the dams and pups did not differ among treatment groups (Blossom et al., 2012). All studies

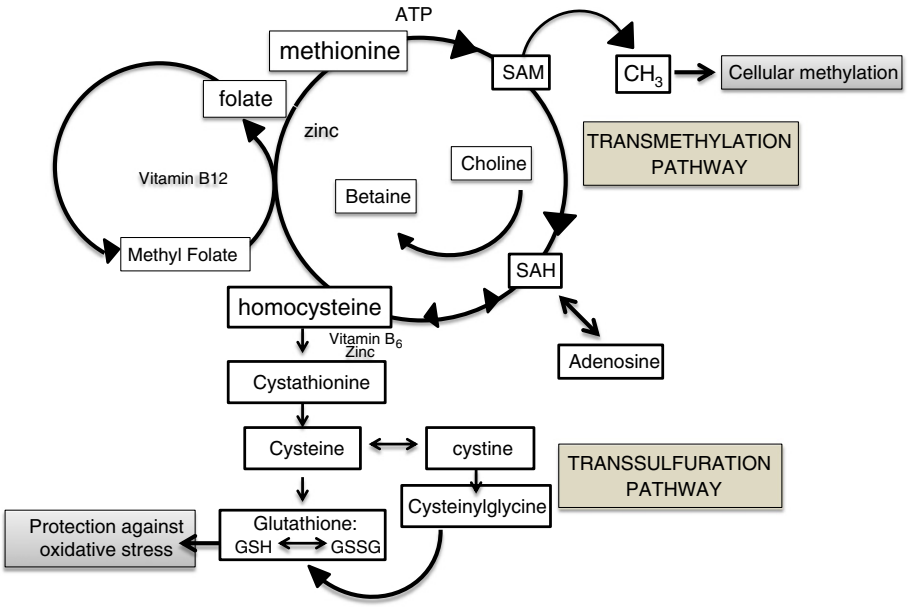

Fig. 1. Folate-dependent methionine transmethylation and transsulfuration pathways involved in redox potential and cellular methylation.