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A role for glutathione, independent of oxidative stress, in the developmental toxicity of methanol

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ABSTRACT

Oxidative stress and reactive oxygen species (ROS) have been implicated in the teratogenicity of methanol (MeOH) in rodents, both in vivo and in embryo culture. We explored the ROS hypothesis further in vivo in pregnant C57BL/6J mice. Following maternal treatment with a teratogenic dose of MeOH, 4 g/kg via intraperitoneal (ip) injection on gestational day (GD) 12, there was no increase 6 h later in embryonic ROS formation, measured by 2′,7′-dichlorodihydrofluorescin diacetate (DCFH-DA) fluorescence, despite an increase observed with the positive control ethanol (EtOH), nor was there an increase in embryonic oxidatively damaged DNA, quantified as 8-oxo-2′-deoxyguanosine (8-oxodG) formation. MeOH teratogenicity (primarily ophthalmic anomalies, cleft palate) also was not altered by pre- and post-treatment with varying doses of the free radical spin trapping agent *alpha*-phenyl-N-*tert*-butylnitrone (PBN). In contrast, pretreatment with L-buthionine-(S,R)-sulfoximine (BSO), an inhibitor of glutathione (GSH) synthesis, depleted maternal hepatic and embryonic GSH, and enhanced some new anomalies (micrognathia, agnathia, short snout, fused digits, cleft lip, low set ears), but not the most common teratogenic effects of MeOH (ophthalmic anomalies, cleft palate) in this strain. These results suggest that ROS did not contribute to the teratogenic effects of MeOH in this in vivo mouse model, in contrast to results in embryo culture from our laboratory, and that the protective effect of GSH in this model may arise from its role as a cofactor for formaldehyde dehydrogenase in the detoxification of formaldehyde.

Introduction

The acute toxicity of methanol (MeOH) has been widely described in humans and animal models (Clary, 2003), but less is known about its developmental toxicity, for which the underlying mechanisms have yet to be fully determined (Wells et al., 2013). Only two cases of human fetal MeOH exposure have been reported, but no mechanistic conclusions could be drawn from these cases given the difficulty of obtaining accurate information (dose, duration, etc.) (Belson and Morgan, 2004; Hantson et al., 1997). MeOH can be found in fruits, vegetables and alcoholic beverages, is a product of aspartame metabolism, and is employed as an industrial solvent and in chemical syntheses. More recently, its use as an alternative fuel source raises added questions of its safety given increased exposure levels (Anderson et al., 1984; Reed and Lerner, 1973).

There are species differences in the metabolism of MeOH: humans metabolize MeOH to formaldehyde primarily using alcohol dehydrogenase (ADH1), whereas rodents rely on the peroxidative activity of the enzyme catalase, which also has antioxidative activity (Fig. 1) (Bradford et al., 1993; Cederbaum and Qureshi, 1982; Karinje and Ogata, 1990). Formaldehyde is short-lived and quickly converted to the acutely toxic metabolite formic acid (FA) through a similar mechanism in both species via the glutathione (GSH)-dependent enzyme formaldehyde dehydrogenase (ADH3) (Harris et al., 2004). FA is converted to the non-toxic metabolites carbon dioxide and water using a folate-dependent dehydrogenase (Johlin et al., 1987). In humans, it is thought that the accumulation of FA due to low endogenous folate, and therefore slower FA metabolism, causes the acute symptoms of MeOH toxicity, which include metabolic acidosis, ocular toxicity and death (Lanigan, 2001; Wallage and Watterson, 2008). Conversely, rodents do not appear to accumulate FA due in part to their higher endogenous folate stores, and to their additional ability to metabolize FA using catalase, which is said to explain why rodents do not exhibit the acute symptoms of MeOH toxicity as seen in humans (Harris et al., 2004; Roe, 1982).

Abbreviations: 8-oxodG, 8-oxo-2′-deoxyguanosine; ADH1, alcohol dehydrogenase; ADH3, formaldehyde dehydrogenase; BSO, L-buthionine-(S,R)-sulfoximine; DCFH-DA, 2′,7′-dichlorodihydrofluorescin diacetate; DMSO, dimethyl sulfoxide; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); EtOH, ethanol; FA, formic acid; GD, gestational day; GSH, reduced glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; HNE, hydroxynonenal; HPLC, high-performance liquid chromatography; ip, intraperitoneal; LC–MS/MS, liquid chromatography with tandem mass spectrometry; MeOH, methanol; Na-FA, sodium formate; NF-κB, nuclear factor transcription factor kappa-B; NOX, NADPH oxidase; NTD, neural tube defect; NZW, New Zealand White rabbits; PBN, *alpha*-phenyl-N-*tert*-butylnitrone; RFU, relative fluorescence units; ROS, reactive oxygen species.

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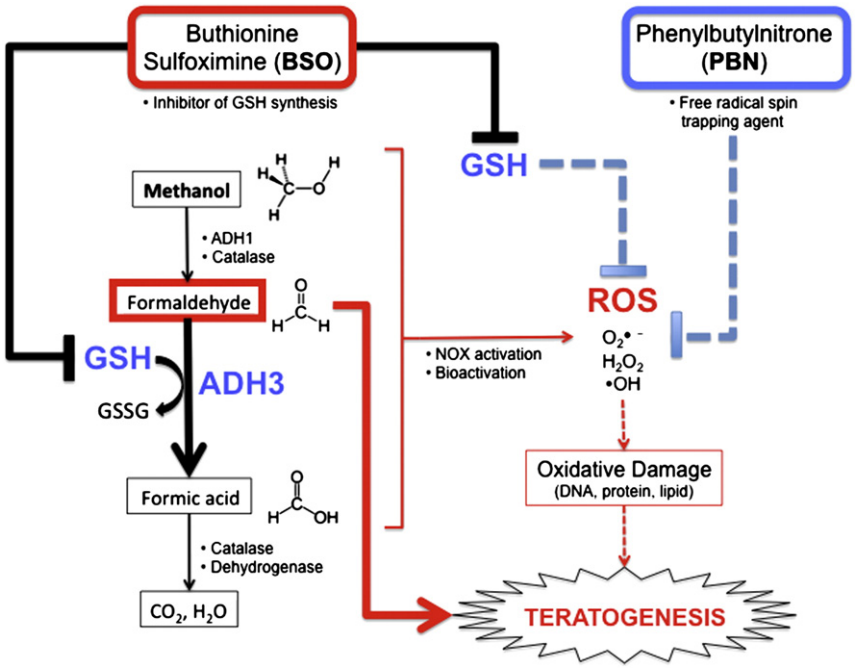

Fig. 1. Putative mechanisms of methanol (MeOH) developmental toxicity in rodents. Published evidence from in vivo and embryo culture studies in rats and mice suggests that MeOH and/or its metabolites may enhance the formation of reactive oxygen species (ROS) by inducing ROS-producing NADPH oxidases (NOXs), and potentially via the enzymatic bioactivation of MeOH or its metabolites. ROS can initiate teratogenesis by altering signal transduction or by oxidatively damaging cellular macromolecules. ROS and drug free radical intermediates can be detoxified by *alpha*-phenyl-N-*tert*-butylnitrone (PBN), a free radical spin trapping agent. Glutathione (GSH) detoxifies ROS and hydroperoxides directly as an antioxidant and/or by serving as a cofactor for protective GSH peroxidase. Alternatively, GSH is a cofactor for formaldehyde dehydrogenase (ADH3), which detoxifies the embryopathic formaldehyde metabolite of MeOH. GSH can be depleted by L-buthionine-(S,R)-sulfoximine (BSO), which inhibits GSH synthesis. Herein, maternal BSO pretreatment enhanced some new MeOH-initiated anomalies (micrognathia, agnathia, short snout, fused digits, cleft lip, low set ears), albeit not the most common birth defects caused by MeOH in C57BL/6J mice (ophthalmic defects, cleft palate). However, MeOH did not cause embryonic ROS formation or DNA oxidation, nor was PBN protective against any MeOH-initiated anomalies. These results suggest that in this in vivo model the proximate teratogenic species for the new anomalies is formaldehyde rather than MeOH, FA or ROS, and that GSH may be protective by serving as a cofactor for ADH3 rather than via ROS detoxification.

Rodents are, however, sensitive to the developmental toxicity of MeOH in vivo and in embryo culture (Bolon et al., 1994; Hansen et al., 2005; Harris et al., 2003, 2004; Miller and Wells, 2011; Miller et al., 2013a, 2013b; Rogers and Mole, 1997; Rogers et al., 2004; Siu et al., 2013; Sweeting et al., 2011). The effects of in utero exposure to MeOH have been described in several animal models. Strain-specific malformations in mice, with exposure on gestational day (GD) 7 as the most susceptible period, include cephalic neural tube defects (NTDs), primarily seen in CD-1 mice, and ophthalmic, skeletal and craniofacial abnormalities, seen in C57BL/6J mice (Bolon et al., 1994; Rogers and Mole, 1997; Rogers et al., 2004). In addition, in a previous study comparing the plasma disposition of MeOH and FA and the teratogenic outcomes of MeOH exposure in C57BL/6J and C3H mouse strains, and in New Zealand White (NZW) rabbits, we found a strain of mouse (C3H) and species (NZW rabbit) resistant to MeOH teratogenicity (Sweeting et al., 2011). These strain- and species-dependent effects could not be explained by differences in the pharmacokinetic profile of MeOH or FA. From the few investigations aiming to elucidate the underlying mechanism of MeOH-induced developmental toxicity, a role for reactive oxygen species (ROS) and oxidative stress has been suggested (Fig. 1) (Harris et al., 2004). MeOH has been reported to cause ROS-induced macromolecular damage and changes in ROS-related enzymes in adult tissues (Parthasarathy et al., 2006; Skrzydlewska et al., 2000), and embryopathies in rat embryos following the depletion of the antioxidative peptide, glutathione (GSH) (Harris et al., 2004). As previously mentioned, the enzyme catalase also exhibits antioxidative activity that can protect the developing fetus from damage caused by hydrogen peroxide (H_2O_2), a type of ROS that is formed physiologically during development and potentially enhanced by some xenobiotics (Wells et al., 2009). However, we recently found that mice genetically altered to express either high or low catalase activity showed no differences in teratological outcome following in utero MeOH exposure, implying a lesser or obscured role for catalase in its antioxidative capacity, and possibly ROS, in these in vivo mouse models (Siu et al., 2013). In contrast, studies in an in vitro embryo culture model using the same strains of mice found that enhanced catalase protected against embryopathies caused by MeOH and the ROS-initiating teratogen phenytoin, whereas low catalase expression enhanced the embryopathic effects (Abramov and Wells, 2011a, 2011b; Miller and Wells, 2011), suggesting both an embryopathic role for ROS and a protective role for catalase. The basis for the contrasting evidence for ROS involvement in MeOH embryopathies in vivo vs. in embryo culture has yet to be determined, and the molecular mechanisms of in vivo MeOH teratogenesis remain unclear.

To further investigate the potential role of ROS in in vivo MeOH developmental toxicity, we measured embryonic ROS production and ROS-mediated oxidatively damaged DNA, and examined the effect on MeOH teratogenesis by pretreatment with the free radical spin trapping agent *alpha*-phenyl-N-*tert*-butylnitrone (PBN), and the GSH depletor L-buthionine-(S,R)-sulfoximine (BSO). There was no evidence for the involvement of embryonic oxidative stress in MeOH teratogenesis in our in vivo mouse model, but an enhancement in some birth defects by the GSH depletor BSO suggests that formaldehyde may be a proximate teratogen, and GSH protects the embryo in this model not via ROS detoxification, but by serving as a cofactor for formaldehyde dehydrogenase (Fig. 1).

Methods

Chemicals

HPLC grade MeOH was purchased from EMD Sereno Canada, Inc. (Mississauga, ON). Ethanol (EtOH) was purchased from Commercial Alcohols (Ontario). Saline (0.9%, sterile) was purchased from Baxter Corporation (Mississauga, ON). Carnoy's solution was prepared in the laboratory in a 60:30:10 solution of 100% ethanol:chloroform:glacial