Toxicology and Applied Pharmacology 247 (2010) 28–35

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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

Species differences in methanol and formic acid pharmacokinetics in mice, rabbits and primates☆

J. Nicole Sweeting [a,1], Michelle Siu [a,1], Gordon P. McCallum [a,1], Lutfiya Miller [b], Peter G. Wells [a,b,*]

[a] Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada
[b] Department of Pharmacology and Toxicology, University of Toronto, Toronto, Ontario, Canada

ARTICLE INFO

Article history:
Received 22 February 2010
Revised 12 May 2010
Accepted 15 May 2010
Available online 25 May 2010

Keywords:
Methanol
Formic acid
Pharmacokinetics
Species differences
Rabbits
Mice
Primates

ABSTRACT

Methanol (MeOH) is metabolized primarily by alcohol dehydrogenase in humans, but by catalase in rodents, with species variations in the pharmacokinetics of its formic acid (FA) metabolite. The teratogenic potential of MeOH in humans is unknown, and its teratogenicity in rodents may not accurately reflect human developmental risk due to differential species metabolism, as for some other teratogens. To determine if human MeOH metabolism might be better reflected in rabbits than rodents, the plasma pharmacokinetics of MeOH and FA were compared in male CD-1 mice, New Zealand white rabbits and cynomolgus monkeys over time (24, 48 and 6 h, respectively) following a single intraperitoneal injection of 0.5 or 2 g/kg MeOH or its saline vehicle. Following the high dose, MeOH exhibited saturated elimination kinetics in all 3 species, with similar peak concentrations and a 2.5-fold higher clearance in mice than rabbits. FA accumulation within 6 h in primates was 5-fold and 43-fold higher than in rabbits and mice respectively, with accumulation being 10-fold higher in rabbits than mice. Over 48 h, FA accumulation was nearly 5-fold higher in rabbits than mice. Low-dose MeOH in mice and rabbits resulted in similarly saturated MeOH elimination in both species, but with approximately 2-fold higher clearance rates in mice. FA accumulation was 3.8-fold higher in rabbits than mice. Rabbits more closely than mice reflected primates for *in vivo* MeOH metabolism, and particularly FA accumulation, suggesting that developmental studies in rabbits may be useful for assessing potential human teratological risk.

Introduction

Human exposure to methanol (MeOH) can result from its use as an industrial solvent, through accidental ingestion and as a potential alternate fuel source (Harris et al., 2004). Most toxicological studies use rodents as models of human MeOH toxicity, but there are key species differences in MeOH metabolism. Primates, including humans, metabolize MeOH to formaldehyde using the enzyme alcohol dehydrogenase (ADH), whereas rodents use catalase (Cederbaum and Qureshi, 1982) (Fig. 1). Humans and rodents metabolize formaldehyde to formic acid (FA) by formaldehyde dehydrogenase (ADH III). FA is subsequently converted to the non-toxic metabolites carbon dioxide and water by a folate-dependent dehydrogenase (Johlin et al., 1987). Humans have limited folate, resulting in FA accumulation following higher MeOH exposures (Perkins et al., 1995). Conversely, folate is not limited in rodents, which metabolize FA via both catalase- and folate-dependent pathways, thereby preventing FA accumulation (Clary, 2003; Harris et al., 2004). Aside from its role in rodent MeOH metabolism, catalase in all species provides cytoprotection against reactive oxygen species (ROS) by detoxifying hydrogen peroxide (Halliwell and Gutteridge, 2007; Wells et al., 2009), which complicates the interpretation of rodent data for MeOH toxicity.

Human MeOH overdose causes acute ocular toxicity, CNS depression and death, apparently due to FA accumulation and subsequent metabolic acidosis (Lanigan, 2001; Wallage and Watterson, 2008). Rodents are resistant to this acute toxicity, presumably due to the absence of FA accumulation. Conversely, rodents can be susceptible to delayed adverse consequences of MeOH exposure, such as fetal neural tube defects and cleft palates following *in utero* exposure (Bolon et al., 1994; Rogers and Mole, 1997; Rogers et al., 2004), and possibly cancer in adult rats (Soffritti et al., 2007), although the latter remains controversial (Cruzan, 2009; Schoeb et al., 2009). Mechanisms of acute MeOH toxicity likely differ from those underlying its delayed toxicities. Although the mechanisms underlying delayed MeOH toxicities are

Abbreviations: ADH, alcohol dehydrogenase; ADH III, formaldehyde dehydrogenase; AO, alcohol oxidase; AUC, area under the plasma concentration–time curve; Cl, clearance; FA, formic acid; FDH, formate dehydrogenase; FID, flame ionization detector; GC, gas chromatography; MeOH, methanol; NZW, New Zealand white; ROS, reactive oxygen species; SOD, superoxide dismutase.

☆ Preliminary reports of this research were presented at the 2007, 2008 and 2009 annual meetings of the Teratology Society (Birth Defects Res. Part A: Clinical and Molecular Teratology 79(5): 418 (no. P25) and 419 (no. P26), 2007; Birth Defects Res. Part A: 82(5): 373 (no. P48) and 377 (no. P56) and, Birth Defects Res. Part A: 85: 453 (no. P74), 2009).

* Corresponding author. Division of Biomolecular Sciences, Faculty of Pharmacy, University of Toronto, 144 College St., Toronto, ON, Canada M5S 3M2. Fax: +1 416 267 7797.
E-mail address: pg.wells@utoronto.ca (P.G. Wells).

[1] These authors contributed equally.

0041-008X/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.taap.2010.05.009

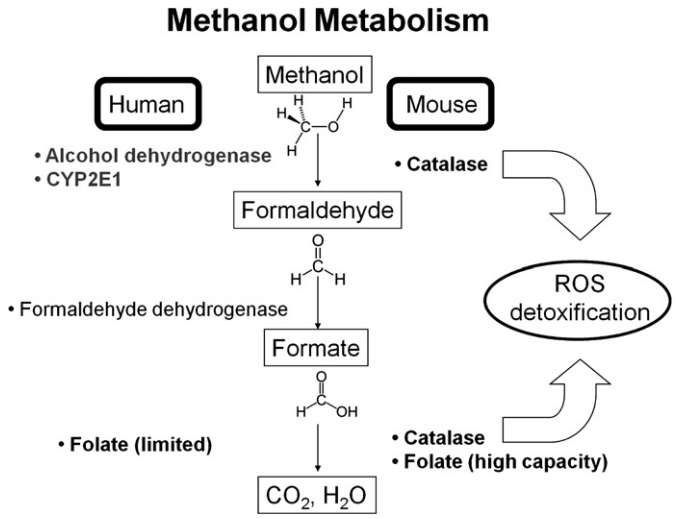

Fig. 1. Species differences in the enzymes catalyzing the metabolism of methanol (MeOH) to formaldehyde and formic acid (FA) in mice and primates, including humans. In addition to its role in MeOH metabolism in mice, catalase is used by all species in the detoxification of reactive oxygen species (ROS).

unclear, a role for ROS has been implicated in a rat embryo culture study, where depletion of the antioxidative peptide glutathione (GSH) increased the embryopathic effect of MeOH and its metabolites (Harris et al., 2004). The proximal toxicant of MeOH developmental toxicity is unknown, although in rat embryo culture exogenous administration of formaldehyde is substantially more embryopathic than MeOH and FA (Harris et al., 2004; Hansen et al., 2005). Direct *in vitro* exposure to formaldehyde, which is highly reactive and transient, may not accurately reflect *in utero* exposure via its production from MeOH metabolism, but a role for formaldehyde in MeOH developmental toxicity remains plausible. ROS also have been implicated in adult MeOH toxicity, including *in vivo* rat studies reporting that MeOH increased lipid peroxidation in lymphoid organs and the brain, as well as decreasing GSH and activity of the antioxidative enzyme superoxide dismutase (SOD) (Farbiszewski et al., 2000; Parthasarathy et al., 2006).

The species differences between rodents and humans in MeOH metabolism and susceptibility to at least acute toxicity suggest that rodents may not constitute the most predictive model of human risk for delayed adverse effects of MeOH. Conversely, the rabbit is an attractive non-primate alternative for several reasons. Rabbits, but not mice, are susceptible to enhanced embryonic oxidative DNA damage and teratogenic effects of thalidomide, a known ROS-initiating drug, which may prove relevant to the delayed effects of MeOH (Fratta et al., 1965; Parman et al., 1999). Additionally, rabbits but not rats are insensitive to ethylene glycol teratogenicity, where the toxicokinetic profile in rabbits more closely reflects that in humans (Carney et al., 2008). Finally, there is *in vitro* evidence in liver homogenates to suggest that ADH activity in alcohol metabolism is more similar in rabbits than mice to that in humans (Otani, 1978).

To determine if primate metabolism of MeOH, and particularly FA accumulation, was reflected more accurately in rabbits than rodents, we examined the *in vivo* plasma pharmacokinetics of MeOH and FA in cynomolgus monkeys, rabbits and mice following single doses of MeOH either below or above the reported saturation level for catalase (600 mg/kg) (NEDO, 1986, 1987). Males were used to avoid potential confounding effects of hormonal differences on MeOH metabolism between animals and species. Our results provide the first *in vivo* evidence that rabbits more closely than mice reflect primate MeOH metabolism, particularly in regard to FA accumulation. Developmental studies are needed to determine if rabbits similarly predict more accurately than mice the human risk for delayed adverse MeOH effects.

Methods

Chemicals. HPLC grade MeOH was purchased from EMD Sereno Canada, Inc. (Mississauga, ON). Saline (0.9%, sterile) was purchased from Baxter Corporation (Mississauga, ON). Isoflurane was purchased from Abbott Laboratories Ltd. (Saint-Laurant, QC). Compressed oxygen (99%) was purchased from BOC Gases (Mississauga, ON). Alcohol oxidase (AO; A6941) and formate dehydrogenase (FDH; F8649) from *Candida boidinii*, diaphorase from *Clostridium kluyveri* (D5540), sodium formate, and β-nicotinamide adenine dinucleotide (β-NAD^+; N1511) were purchased from Sigma-Aldrich (St. Louis, MO). Formaldehyde dehydrogenase from *Pseudomonas putida* was obtained from MP Biomedicals (Solon, OH). *p*-iodonitrotetrazolium violet (INT) was purchased from Alfa Aesar (Ward Hill, MA).

Animals and diet. All animal protocols used were approved by the institutional animal care committee in conformance with the guidelines established by the Canadian Council on Animal Care.

Mice. Male CD-1 mice were purchased from Charles River Laboratories (Saint-Constant, QC) and were 2–5 months old and approximately 33–52 g at the time of study. Mice were housed in vented cages from Allentown, Inc. (Allentown, NJ) with ground corn cob bedding (Bed-O' Cobs Laboratory Animal Bedding, The Andersons Industrial Products Group, Maumee, OH). Animal rooms were climate- and light-controlled (20 °C, 50% humidity, 14-hour light–10-hour dark cycle). Mice were fed rodent chow (Harlan Labs: 2018, Harlan Teklad, Montreal, QC) and provided with water *ad libitum*. All mice were transported from the animal facility to the main laboratory for dosing and euthanizing, but were allowed 1 h for acclimatization prior to the commencement of each study.

Rabbits. Male New Zealand white (NZW) rabbits were purchased from Charles River Laboratories. At the time of experiments, rabbits were between the ages of 5 and 12 months, with a weight range of 3.25–4.75 kg. Rabbits were housed in plastic cages (Allentown, Inc.) in rooms maintained at 20 °C and 60% humidity, with an automated 12-hour light–dark cycle. Rabbits were fed a diet of standard high-fibre rabbit chow (Lab Diet: 5236 Hi-Fibre, PMI Nutrition International LLC, Brentwood, MO), and provided with water *ad libitum*. Three days prior to the commencement of each study, rabbits did not receive any vegetable supplementation to their diet to avoid exposure to exogenous sources of antioxidants.

Primates. Studies were conducted with male cynomolgus monkeys (*Macaca fascicularis*) at Charles River Laboratories (Sparks, NV). At the time of experiments, monkeys were between the ages of 3.4 and 5.7 years, with a weight range of 2.8–4.8 kg. Monkeys were acclimatized to individual stainless-steel cages two weeks prior to commencement of the study in rooms maintained between 18 and 29 °C, with an automated 12-hour light–dark cycle. Monkeys were fed a certified primate chow diet (# 5048) from Purina Mills (St. Louis, MO) supplemented with fruit or vegetables 2–3 times weekly, and provided with water *ad libitum*.

Dosing and blood collection. Mice were administered either a single low dose (0.5 g/kg bw) or high dose (2 g/kg bw) of MeOH (20% [w/v] in sterile saline) or a saline vehicle control. MeOH was administered via intraperitoneal (ip) injection using a 26 gauge (G) 3/8 needle. Prior to blood collection, mice were anaesthetized by placing a 15 mL conical tube containing isoflurane-soaked gauze over the snout of the animal until it was unresponsive. Blood samples (approximately 1 mL) were collected in heparinised vacutainers (lithium heparin 68 USP units per tube, Becton, Dickinson and Company, Oakville, ON) either directly from the atrium or by puncturing the portal vein or inferior vena cava, using a 20 G 1 1/2 needle. For mice administered a