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Lack of formic acid production in rat hepatocytes and human renal proximal tubule cells exposed to chloral hydrate or trichloroacetic acid

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Abstract

The industrial solvent trichloroethylene (TCE) and its major metabolites have been shown to cause formic aciduria in male rats. We have examined whether chloral hydrate (CH) and trichloroacetic acid (TCA), known metabolites of TCE, produce an increase in formic acid *in vitro* in cultures of rat hepatocytes or human renal proximal tubule cells (HRPTC). The metabolism and cytotoxicity of CH was also examined to establish that the cells were metabolically active and not compromised by toxicity. Rat hepatocytes and HRPTC were cultured in serum-free medium and then treated with 0.3–3 mM CH for 3 days or 0.03–3 mM CH for 10 days, respectively and formic acid production, metabolism to trichloroethanol (TCE-OH) and TCA and cytotoxicity determined. No increase in formic acid production in rat hepatocytes or HRPTC exposed to CH was observed over and above that due to chemical degradation, neither was formic acid production observed in rat hepatocytes exposed to TCA. HRPTC metabolized CH to TCE-OH and TCA with a 12-fold greater capacity to form TCE-OH versus TCA. Rat hepatocytes exhibited a 1.6-fold and three-fold greater capacity than HRPTC to form TCE-OH and TCA, respectively. CH and TCA were not cytotoxic to rat hepatocytes at concentrations up to 3 mM/day for 3 days. With HRPTC, one sample showed no cytotoxicity to CH at concentrations up to 3 mM/day for 10 days, while in another cytotoxicity was seen at 1 mM/day for 3 days. In summary, increased formic acid production was not observed in rat hepatocytes or HRPTC exposed to TCE metabolites, suggesting that the *in vivo* response cannot be modelled *in vitro*. CH was toxic to HRPTC at millimolar concentrations/day over 10 days, while glutathione derived metabolites of TCE were toxic at micromolar concentrations/day over 10 days [Lock, E.A., Reed, C.J., 2006. Trichloroethylene: mechanisms of renal toxicity and renal cancer and relevance to risk assessment. Toxicol. Sci. 19, 313–331] supporting the view that glutathione derived metabolites are likely to be responsible for nephrotoxicity.

Keywords: Trichloroethylene; Chloral hydrate; Trichloroethanol; Trichloroacetic acid; Formic acid; Human renal proximal tubule cells; Rat hepatocytes

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1. Introduction

The industrial solvent 1,1,2-trichloroethylene (TCE) has been reported to interfere with folic acid metabolism in male rats leading to excretion of large quantities of formic acid in urine (Green et al., 1998; Dow and Green, 2000). Formic aciduria has been suggested to account for the renal injury observed following chronic exposure in the rat. Metabolites of TCE, trichloroethanol (TCE-OH), trichloroacetic acid (TCA) and to a lesser extent CH also cause formic aciduria in the rat (Dow and Green, 2000). While workers occupationally exposed to TCE excrete formic acid in their urine, the amount is small and unlike that observed in the male rat (Green et al., 2004).

It is well established that TCE undergoes metabolism by hepatic cytochrome P450 in experimental animals and man to produce chloral hydrate (CH) which is then further metabolized to TCE-OH and TCA (Davidson and Beliles, 1991; Elfarra et al., 1998; Lash et al., 2000; Bloemen et al., 2001; Lock and Reed, 2006). The target organs for toxicity and carcinogenicity following exposure of rats or mice to TCE are the liver, kidney and lung (Bull, 2000; Green, 2000; Lock and Reed, 2006). TCE has been reported to undergo metabolism to CH in rat liver (see references above) and rat kidney (Cummings et al., 2001) primarily by cytochrome P450 2E1, although other cytochromes P450 such as 2B1 and 2C11 can also convert TCE to CH. Human kidney contains little or no cytochrome P450 2E1 (Amet et al., 1997), hence studies with human renal microsomes failed to detect CH formation in three out of four samples, and in the fourth only a very small amount was observed at the highest substrate concentration (Cummings and Lash, 2000). Thus, exposure of human kidneys to oxidative metabolites of TCE is largely due to extra-renal metabolism.

The aim of this current work was to: (1) ascertain if rat hepatocytes exposed to CH or TCA in culture produce formic acid, like that observed in the whole animal; (2) determine that rat hepatocytes under our culture conditions can metabolise CH to TCE-OH and TCA; (3) examine the cytotoxicity of CH and TCA to rat hepatocytes in culture; (4) compare the findings in rat hepatocytes with those in human renal proximal tubule cells (HRPTC).

2. Materials and methods

2.1. Materials

Collagen, dexamethasone, gentamicin sulfate, insulin, chloral hydrate (CH), trichloroethanol (TCE-OH), trichloroacetic acid (TCA) and William's Medium E were obtained from Sigma Chemical Company (St. Louis, MO). Collagenase Type 2 was obtained from Worthington Biochemicals (Freehold, NJ); Hank's balanced salt solutions and penicillin–streptomycin were purchased from Gibco (Grand Island, NY). Fetal bovine serum (FBS) was purchased from Atlas Biologicals (Fort Collins, CO). Sodium 3-trimethylsilylpropionate (TMSP-2,2,3,3-d_4) [D, 98%] was obtained from Cambridge Isotope Laboratories (Andover, MA).

2.2. Animals

Adult male Sprague–Dawley rats (100–150 g) were purchased from Harlan Lab Animals (Indianapolis, IN). They were housed in AAALAC-accredited animal facilities and maintained on a 12-h light:12-h dark cycle with free access to food and water for at least 1 week prior to use.

2.3. Hepatocyte isolation, culture and treatment

Hepatocytes were isolated from rats by collagenase perfusion as previously described (McMillan and Jollow, 1992). Hepatocytes were separated from other liver cell types by centrifugation at $50 \times g$ for 2 min at 4 °C. Viability of the hepatocytes was determined by trypan blue exclusion; preparations with viability >90% were used. The cells were plated on collagen-coated six-well tissue culture plates at confluency (1.25×10^6 cells/well) in William's Medium E containing 2 mM l-glutamine, 34 ng/ml insulin and 0.1 mg/ml gentamicin sulfate, and 10% FBS. After a 2 h attachment period, medium and unattached cells were removed and the medium was replaced with serum-free, insulin-free William's Medium E containing 1 μM dexamethasone, 2 mM l-glutamine, and 0.1 mg/ml gentamicin sulfate. Twenty-four hours later, hepatocytes were treated with CH or TCA (0.3–3.0 mM). Stock solutions of TCA and CH were prepared in serum-free culture medium and neutralised with NaOH. A small volume of the neutralised stock solutions was added to the cell cultures to achieve the desired concentration. Twenty-four hours after exposure (Day 1) the medium was removed and stored at −70 °C for analysis of CH metabolites and formic acid. The cells were then exposed to CH or TCA in fresh medium for a further 24 h and the medium removed (Day 2), and the process was then repeated (Day 3). After the last dose the medium was removed and stored for subsequent analysis, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) activity was determined in the cells as described in Section 2.4.

2.4. Renal proximal tubule cell isolation, culture and treatment

HRPTC were isolated from whole kidneys procured by International Bioresearch Solutions (Tucson, AZ). MUSC Institutional Review Board approved this protocol. Kidneys were obtained from two donors with the following char-