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Short communication

Mutagenicity of the cysteine *S*-conjugate sulfoxides of trichloroethylene and tetrachloroethylene in the Ames test

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ABSTRACT

The nephrotoxicity and nephrocarcinogenicity of trichloroethylene (TCE) and tetrachloroethylene (PCE) are believed to be mediated primarily through the cysteine *S*-conjugate β-lyase-dependent bioactivation of the corresponding cysteine *S*-conjugate metabolites *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC) and *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVC), respectively. DCVC and TCVC have previously been demonstrated to be mutagenic by the Ames Salmonella mutagenicity assay, and reduction in mutagenicity was observed upon treatment with the β-lyase inhibitor aminooxyacetic acid (AOAA). Because DCVC and TCVC can also be bioactivated through sulfoxidation to yield the potent nephrotoxicants *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide (DCVCS) and *S*-(1,2,2-trichlorovinyl)-L-cysteine sulfoxide (TCVCS), respectively, the mutagenic potential of these two sulfoxides was investigated using the Ames *Salmonella typhimurium* TA100 mutagenicity assay. The results show both DCVCS and TCVCS were mutagenic, and TCVCS exhibited 3-fold higher mutagenicity than DCVCS. However, DCVCS and TCVCS mutagenic activity was approximately 700-fold and 30-fold lower than DCVC and TCVC, respectively. DCVC and DCVCS appeared to induce toxicity in TA100, as evidenced by increased microcolony formation and decreased mutant frequency above threshold concentrations. TCVC and TCVCS were not toxic in TA100. The toxic effects of DCVC limited the sensitivity of TA100 to DCVC mutagenic effects and rendered it difficult to investigate the effects of AOAA on DCVC mutagenic activity. Collectively, these results suggest that DCVCS and TCVCS exerted a definite but weak mutagenicity in the TA100 strain. Therefore, despite their potent nephrotoxicity, DCVCS and TCVCS are not likely to play a major role in DCVC or TCVC mutagenicity in this strain.

1. Introduction

Trichloroethylene (TCE) and tetrachloroethylene (PCE; also known as perchloroethylene and abbreviated Perc or Tetra), halogenated hydrocarbons used as organic solvents, are common groundwater contaminants. Long term exposures to TCE (Brüning et al., 1996, 1998, 1999; Vermeulen et al., 2012) and PCE are known to cause nephrotoxicity (Lash and Parker, 2001) and each is classified as "reasonably anticipated to be a human carcinogen" by the National Toxicology Program (National Toxicology Program, 2011). There is an association between TCE exposure and the development of renal cancer in humans. Exposure to PCE has been associated with the development of esophageal cancer, cervical cancer, and non-Hodgkin's lymphoma. TCE and PCE are known to be renal carcinogens in rats.

The nephrotoxicity and nephrocarcinogenicity of TCE and PCE are attributed to metabolites formed by the metabolic pathway that is initiated by GSH *S*-transferases (Dekant et al., 1986, 1987, 1994; Lash et al., 1998, 2000; Lash and Parker, 2001). In this pathway, TCE and PCE are initially conjugated to GSH in the liver. The GSH conjugates are then processed further into cysteine *S*-conjugates (Fig. 1), *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC; derived from TCE) and *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVC; derived from PCE) in the kidney, bile duct epithelium, intestinal lumen or bile canalicular membrane of hepatocytes. DCVC and TCVC can enter the circulation and translocate to the kidneys. The mercapturic acids of DCVC and TCVC, *N*-acetyl DCVC and *N*-acetyl TCVC, are formed by *N*-acetylation in the kidney or liver and have been detected in the urine of humans exposed to TCE (Bernauer et al., 1996) or PCE (Birner et al., 1996; Völkel et al., 1998), respectively.

Abbreviations: β-lyase, cysteine *S*-conjugate β-lyase; AOAA, aminooxyacetic acid; DCVC, *S*-(1,2-dichlorovinyl)-L-cysteine; DCVCS, *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide; FMO, flavin-containing monooxygenase; GSH, glutathione; PCE, tetrachloroethylene; TA100, *Salmonella typhimurium* TA100 tester strain; TA2638, *Salmonella typhimurium* TA2638 tester strain; TCE, trichloroethylene; TCVC, *S*-(1,2,2-trichlorovinyl)-L-cysteine; TCVCS, *S*-(1,2,2-trichlorovinyl)-L-cysteine sulfoxide.

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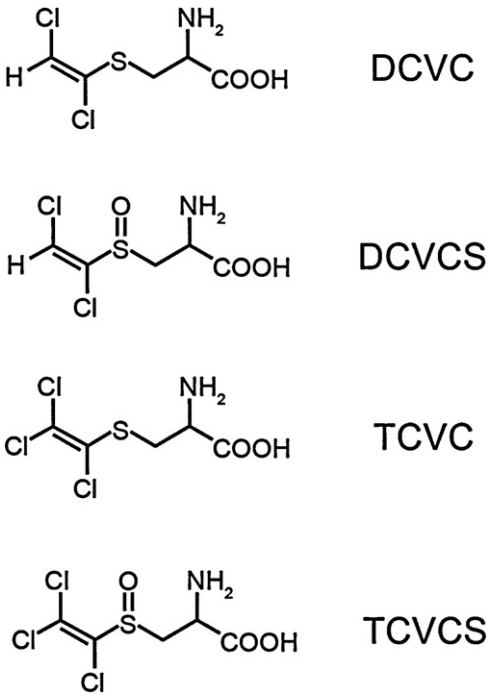

Fig. 1. Structures of *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC), *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide (DCVCS), *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVC), and *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVCS).

Two bioactivation mechanisms have been elucidated for DCVC and TCVC. Both compounds can undergo β-elimination by cysteine *S*-conjugate β-lyases (β-lyase) to form reactive electrophilic sulfur species (Elfarra et al., 1986; Lash and Anders, 1986; Dekant et al., 1988, 1994; Vamvakas et al., 1989a,b; Pähler et al., 1999a). Alternatively, DCVC and TCVC can be oxidized to form the Michael acceptor reactive sulfoxides, *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide (DCVCS; derived from TCE) and *S*-(1,2,2-trichlorovinyl)-L-cysteine sulfoxide (TCVCS; derived from PCE) (Fig. 1). DCVC can be oxidized by flavin-containing monooxygenases (FMOs) (Ripp et al., 1997; Krause et al., 2003) and TCVC can be oxidized by FMOs or cytochrome P450s (Ripp et al., 1997). DCVCS (Lash et al., 1994) and TCVCS (Elfarra and Krause, 2007) have both been demonstrated to be more nephrotoxic than the precursor cysteine *S*-conjugates, likely due to their ability to act as direct-acting toxicants through covalent reactions with sulfhydryl-containing molecules. Recently, DCVCS–hemoglobin adducts and cross-links have been detected in rats given DCVC, providing evidence for in vivo metabolism of DCVC to DCVCS (Barshteyn and Elfarra, 2009).

In addition to their nephrotoxicity, DCVC and TCVC have been demonstrated to be mutagenic by the Ames Salmonella mutagenicity assay (Vamvakas et al., 1988; Dekant et al., 1986). DCVC and TCVC were suggested to induce base-pair substitution mutations based upon the potent effect observed in the base-pair substitution strain TA100 and the low effects in the frame-shift strain TA98 (Dekant et al., 1986). The addition of aminooxyacetic acid (AOAA), a potent inhibitor of β-lyase, reduced the mutagenicity of DCVC and TCVC, implicating the β-lyase derived metabolites in the mutagenicity of these compounds. However, the ability of DCVCS and TCVCS to also contribute to the mutagenicity of DCVC and TCVC remains unknown. Therefore, the present study sought to investigate the mutagenicity of DCVCS and TCVCS, and compare the mutagenic potency of these compounds to the mutagenic potency of DCVC and TCVC.

2. Materials and methods

2.1. Materials

DCVC, DCVCS, TCVC, and TCVCS were synthesized as previously described (Sausen and Elfarra, 1991; Ripp et al., 1997). Purity of the synthesized chemicals was demonstrated to be >95% by HPLC. *Salmonella typhimurium* strain TA100 was obtained from Bioreliance (Rockville, MD).

2.2. Assessing DCVCS and TCVCS mutagenicity by the Ames Salmonella mutagenicity assay

The mutagenicity of DCVCS and TCVCS was characterized and compared to their precursor cysteine *S*-conjugates, DCVC and TCVC, utilizing the *S. typhimurium* tester strain TA100 in the Ames salmonella mutagenicity assay. This strain was chosen for the present study because it has been previously used to characterize the mutagenicity of DCVC, TCVC, and the related cysteine *S*-conjugates of hexachloro-1,3-butadiene (Dekant et al., 1986; Vamvakas et al., 1988). In addition, crotonaldehyde and acrolein, which like DCVCS and TCVCS are Michael acceptors, were mutagenic in TA100 (Jha et al., 2007; Parent et al., 1996), suggesting that Michael acceptors may induce mutagenicity through base-pair substitutions. Furthermore, DCVC and TCVC were demonstrated to be mutagenic in this strain without needing the addition of rat kidney or liver microsomes. The assay was conducted following previously described protocols (Mortelmans and Zeiger, 2000). Briefly, a culture of TA100 was grown overnight in nutrient broth containing 24 μg/mL ampicilin to (1–2) × 10^9 CFU/mL (OD_{540} 0.1–0.2). An aliquot of the overnight culture (0.05–0.1 mL; (1–2) × 10^8 cells/plate final concentration) was added to a test tube containing 0.1 mM sodium phosphate buffer pH 7.4 (0.5 mL) and test chemical dissolved in buffer (50 μL). The final concentration ranges for the test chemicals were: DCVC, 0–25 nmol/plate; DCVCS, 0–4000 nmol/plate; TCVC, 0–150 nmol/plate; and TCVCS, 0–500 nmol/plate. Sodium azide (1.33 μg/plate) was used as a positive control. Samples were incubated for 20 min at 37 °C. Molten top agar (2 mL; 0.6% Agar, 0.5% NaCl, 0.05 mM biotin, 0.05 mM histidine maintained at 43–48 °C) was added to each tube. Samples were mixed and transferred to plates (1.5% agar, 2% glucose in Vogel Bonner medium E). After top agar hardened, plates were inverted and incubated at 37 °C for 48 h, after which colonies were counted. The spontaneous mutant frequency ranged from 90 to 150 revertants per plate, which is within established acceptable control value ranges for TA100 (Mortelmans and Zeiger, 2000). All test chemical concentrations were assayed in triplicate and differences between analogous plates did not exceed 30%. Mutagenic activity was expressed as revertants per nmol of chemical and calculated using the linear portion of the plot of revertants versus nmol of test chemical per plate. Points where toxicity was observed were not included, and as a result, the linear range was presumed to suggest no significant toxicity.

In addition, the effects of AOAA, a potent inhibitor of β-lyase, on DCVCS mutagenicity was investigated. In these experiments, AOAA (1 mM final concentration) was added to diluted cultures of TA100 ((1–2) × 10^8 cells/plate) along with DCVC (0–25 mM final concentration), and the mutagenicity assay was carried out as described above. Statistical analysis was performed using the Wilcoxon rank sum test (Mstat, http://www.mcardle.wisc.edu/mstat/) to compare mutagenic activity data from TA100 exposed to DCVC alone or DCVC and AOAA. Results were considered significant if $p < 0.05$.

3. Results

3.1. Assessing DCVCS and TCVCS mutagenicity by the Ames Salmonella mutagenicity assay

DCVCS (Fig. 2B) and TCVCS (Fig. 3B) were definite but weak mutagens in the TA100 tester strain in the Ames assay. The two sulfoxides exhibited different mutagenic activity; TCVCS mutagenic activity (0.32 revertants/nmol) was 3-fold higher than DCVCS (0.1 revertants/nmol). DCVC (76 revertants/nmol) and TCVC (11 revertants/nmol) had higher mutagenic activity compared to their corresponding sulfoxides (Figs. 2A and 3A). In addition, DCVC and DCVCS appeared to induce toxicity as indicated by the increased detection of microcolonies and decreasing numbers of revertants above certain threshold concentrations (DCVC > 2.5 nmol/plate; DCVCS > 1000 nmol/plate) (Fig. 2). However, TCVC and TCVCS did not appear to induce toxicity in TA100; no microcolony formation or decreasing total number of revertants was observed as TCVC or TCVCS concentration was increased.

Previously, it was demonstrated that in other tester strains (i.e. TA2638), DCVC mutagenicity was reduced in the presence of the β-lyase inhibitor AOAA (Dekant et al., 1986). DCVC is known to be