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Occupational exposures at a polyvinyl chloride production facility are associated with significant changes to the plasma metabolome

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ABSTRACT

Background: Occupational vinyl chloride (VC) exposures have been associated with toxicant-associated steatohepatitis and liver cancer. Metabolomics has been used to clarify mode of action in drug-induced liver injury but has not been performed following VC exposures.
Methods: Plasma samples from 17 highly exposed VC workers without liver cancer and 27 unexposed healthy volunteers were obtained for metabolite extraction and GC/MS and LC/MS^2 analysis. Following ion identification/quantification, Ingenuity pathway analysis was performed.
Results: 613 unique named metabolites were identified. Of these, 189 metabolites were increased in the VC exposure group while 94 metabolites were decreased. Random Forest analysis indicated that the metabolite signature could separate the groups with 94% accuracy. VC exposures were associated with increased long chain (including arachidonic acid) and essential (including linoleic acid) fatty acids. Occupational exposure increased lipid peroxidation products including monohydroxy fatty acids (including 13-HODE); fatty acid dicarboxylates; and oxidized arachidonic acid products (including 5,9, and 15-HETE). Carnitine and carnitine esters were decreased, suggesting peroxisomal/mitochondrial dysfunction and alternate modes of lipid oxidation. Differentially regulated metabolites were shown to interact with extracellular-signal-regulated kinase 1/2 (ERK1/2), Akt, AMP-activated protein kinase (AMPK), and the N-Methyl-D-aspartate (NMDA) receptor. The top canonical pathways affected by occupational exposure included tRNA charging, nucleotide degradation, amino acid synthesis/degradation and urea cycle. Methionine and homocysteine was increased with decreased cysteine, suggesting altered 1-carbon metabolism.
Conclusions: Occupational exposure generated a distinct plasma metabolome with markedly altered lipid and amino acid metabolites. ERK1/2, Akt, AMPK, and NMDA were identified as protein targets for vinyl chloride toxicity.

1. Introduction

Vinyl chloride (VC) is an odorless, colorless gas polymerized to produce the ubiquitous plastic polyvinyl chloride (PVC). VC is associated with several pathologies. VC exposure is generally via inhalation in an occupational setting. Over 80,000 chemical workers have been exposed to vinyl chloride. VC may enter the environment via airborne industrial emissions, but may also leech into ground water as a solvent degradation product thus exposing surrounding populations (Kielhorn et al., 2000; Anon., 2015). The Agency for Toxic Substances & Diseases Registry lists VC as #4 on its Substance Priority List, a list prioritized by a combination of a substance's frequency, toxicity, and potential for human exposure at National Priorities List sites (Anon., n.d.). Occupational VC exposure was first associated with the development of hepatic hemangiosarcoma at a Louisville, KY B.F. Goodrich Plant in 1974

Abbreviations: 4-HNE, 4-hydroxynonenal; AA, arachidonic acid; Akt, protein kinase B; AMPK, AMP-activated protein kinase; ASH, alcoholic steatohepatitis; BMI, body mass index; CK18, cytokeratin-18; CERM, cumulative exposure risk month; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ERK, extracellular-signal-regulated kinase; GC-MS, gas chromatography-mass spectrometry; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyicosatetraenoic; HODE, hydroxyoctadecadienoic; HMDB, human metabolome database; LA, linoleic acid; LC, liquid chromatography; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; OXLAM, oxidized linoleic acid metabolite; PUFA, poly-unsaturated fatty acid; PVC, polyvinyl chloride; TASH, toxicant-associated steatohepatitis; VC, vinyl chloride.

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(Makk et al., 1974). In response to this outbreak, a large occupational hepatology database and specimen bank was formed at the University of Louisville as part of a cancer surveillance project. More recently, we reported an 80% prevalence of toxicant-associated steatohepatitis (TASH) in highly exposed VC workers from this plant (Cave et al., 2010). Although those workers had much higher cumulative exposures than allowed under the Occupational Safety and Health Administration Vinyl Chloride Standard, a more recent ultrasound study of 347 asymptomatic current PVC workers reported a 38.9% prevalence of steatosis with a normal mean body mass index (Hsiao et al., 2004). This suggests TASH is a relevant health issue which may also occur in individuals with much lower level exposures to vinyl chloride monomer. The mechanisms leading to the disruption of hepatic lipid metabolism and TASH have recently been reviewed (Wahlang et al., 2013; Joshi-Barve et al., 2015). TASH appears mechanistically different from obesity or alcohol induced steatohepatitis. The metabolic disruption resulting from known VC exposure has never been characterized.

Metabolomics is the study of low molecular weight molecules (i.e., metabolites) found within cells and biological systems. With the advantages of high sensitivity and accuracy, wide dynamic range, and the ability to identify metabolites from complex samples, high-resolution mass spectrometry is an attractive option for metabolomics research. Metabolomics has been utilized for the evaluation of mechanistic disease processes and biomarker development of non-alcoholic fatty liver disease, non-alcoholic steatohepatitis (Kalhan et al., 2011; Puri et al., 2009), alcoholic steatohepatitis (Raszeja-Wyszomirska et al., 2012), and drug-induced liver injury (O'Connell and Watkins, 2010), but has not been performed for industrial chemical exposure, which is the purpose of this study. The goals of the present study are to describe the alterations of the plasma metabolome following chemical exposure at a polyvinyl chloride production facility compared to healthy controls. The changes in these metabolites could contribute to adverse health outcomes in chemical workers.

2. Methods

2.1. Subjects

De-identified data and plasma samples were obtained for highly exposed vinyl chloride workers ($n = 17$) from the University of Louisville Occupational Toxicology Specimen Bank. Samples were stored at −80 °C in polypropylene microfuge tubes. They were thawed, aliquoted, and sent on dry ice to Metabolon for analysis. There were no other freeze-thaw cycles. These workers were employed at a single B.F. Goodrich plant in Louisville, KY, and were selected (inclusion criteria) based on exceptionally high cumulative VC exposures prior to implementation of the Occupational Safety and Health Administration (OSHA) Vinyl Chloride Standard in 1975; however, workers were also simultaneously exposed to a variety of other chemicals (Supplemental Table) in addition to VC. These workers came from the same cohort of "poly cleaners" that developed hepatic hemangiosarcoma ($n = 26$ to date) and toxicant-associated steatohepatitis (TASH)(Cave et al., 2010). These 17 workers never developed hepatic hemangiosarcoma during a prolonged follow-up period - now approximately 40 years in duration. Their TASH status was unknown. Cumulative VC exposures were quantified by the previously described cumulative exposure rank month (CERM) (Greenberg and Tamburro, 1981). Subjects were selected so that their CERMS matched as closely as possible to workers with hemangiosarcoma to allow for future comparison. By doing so, we hoped to gain mechanistic metabolic information about high-level exposures previously linked to liver disease. Unfortunately, healthy unexposed controls with a similar storage time were not available from the biorepository; therefore, 27 unexposed volunteers without a history of liver disease matched by age and gender as closely as possible to the chemical exposed group were recruited as controls. In order to obtain a relatively homogenous population (apart from exposure) for metabolomics analysis, the following exclusion criteria were used for all groups: age > 65 years old, body mass index (BMI) > 35 kg/m^2, alcohol consumption >30 g per day, and history of malignancy. The protocol was approved by the University of Louisville Institutional Review Board and informed consent was obtained. Potential differences in demographics and exposure variables were determined by student's *t*-test using GraphPad Prism 6 (La Jolla, CA). Statistical significance was set at $p \leq 0.05$.

2.2. Metabolomics

Metabolon, Inc. (Durham, NC) performed gas chromatography/mass-spectrometry (GC/MS) (Thermo-Finnigan Trace DSQ fast-scanning single-quadruple mass spectrometer) and liquid chromatography-tandem mass spectrometry LC/MS/MS (Waters ACQUITY UPLC, Thermo-Finnigan LTQ mass spectrometer) following metabolite extraction. Software was used to match ions to a library of standards for metabolite identification and for metabolite quantitation by peak area integration. Statistical significance was determined using Welch's *t*-tests, and $p \leq 0.05$ was considered statistically significant.

2.3. Liver injury assessment

Plasma cytokeratin 18 (CK-18) whole (M65®) and caspase-cleaved fragments (M30 Apoptosense®) were measured by ELISA (diaPharma, Columbus, OH). Clinical chemistry analysis was performed at the University of Louisville Hospital laboratory. Values were measured by clinical chemistry analyzer. Statistical analysis was performed by student's t-test using GraphPad Prism. Statistical significance was set as $p \leq 0.05$. Results are reported ad means ± standard deviation.

2.4. Ingenuity pathway analysis

We investigated interactions between metabolites using the Ingenuity Pathway Analysis (IPA) software (QIAGEN; Redwood City, CA) and found 354 of our 613 metabolites could be mapped using the Human Metabolomic Database (HMDB).

3. Results

3.1. Demographics

The mean age of the exposed group (50 ± 6.3, $n = 17$) was significantly higher ($p = 0.0002$) than the mean age of the control group (38 ± 12, $n = 26$). All members of the exposed group were Caucasian males and this is reflective of plant hiring practices at the time. At the time of metabolomics analysis, sample storage age in years was significantly longer for exposed vs. unexposed group (35 ± 5.1 vs. 0.18 ± 0.0 years, $p < 0.0001$). Mean BMI (27 ± 3.4 vs. 26 ± 4.3 kg/m^2, $p = 0.46$) was similar between VC-exposed and control groups. Alcohol consumption (5.0 ± 5.9 vs. 2.2 ± 2.5 drinks per week, $p = 0.0342$) was significantly higher in exposed group; however, their alcohol consumption was still less than one drink per day on average. There were 6 smokers in VC-exposed group and 1 smoker in the control group. VC exposures were determined by cumulative exposure rank month (CERM). The mean CERM was 1156 ± 420 and this corresponds to a mean duration of employment and high-level VC exposures (often exceeding 1000 PPM) of 25.50 ± 5.688 years. The demographic data are presented in Table 1. Results are reported as mean ± standard deviation.

3.2. Liver injury assessment

Routine liver chemistry results are demonstrated in Table 2. There were no significant differences between the groups in albumin (4.6 ± 2.3 vs. 4.5 ± 3.8 g/dL Reference: 3.5–5.0), total bilirubin (0.58 ± 0.25 vs. 0.57 ± 0.72 mg/dL Reference: 0.2–1.0), and ALT (23 ± 19 vs. 22 ±