Differential Transcriptome Responses Reveal That Cache Valley PM2.5 Triggers ER Stress and the Unfolded Protein Response in Human Lung Cells

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Background & Introduction

- Fine particulate matter air pollution with a mean diameter of ≤2.5μm ($PM_{2.5}$), is a known and potent public health threat
- Cache Valley often experiences some of the highest $PM_{2.5}$ (CVPM) concentrations in the United States
- Goal of research: Determine potency of CVPM relative to other $PM_{2.5}$ types

Hypothesis

The mechanism of cellular dysregulation and toxicity of CVPM is due, at least in part, to induction of ER stress and the unfolded protein response (UPR), common events in many disease states.

Materials & Methods

CVPM Collection and Cell Treatment:

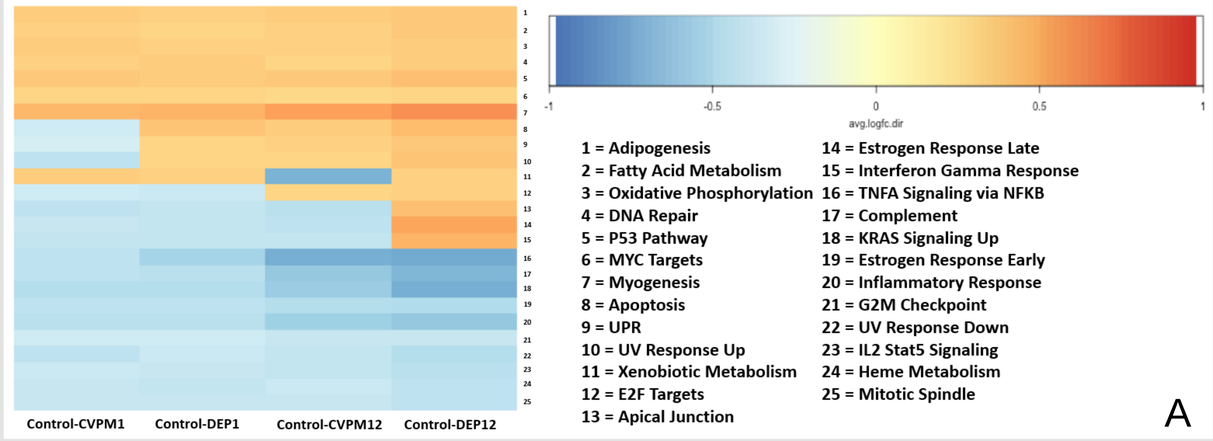
- Collected via Tisch impaction system during inversion events.
- CVPM removed from SS discs and transferred to cell culture media.
- Cultured BEAS-2B human lung cells (80% confluent) treated with CVPM or diesel exhaust particles (DEP; as positive control) for 24 hours.

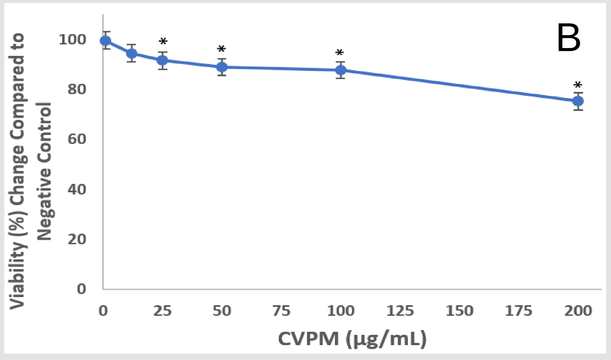
RNA-Sequencing

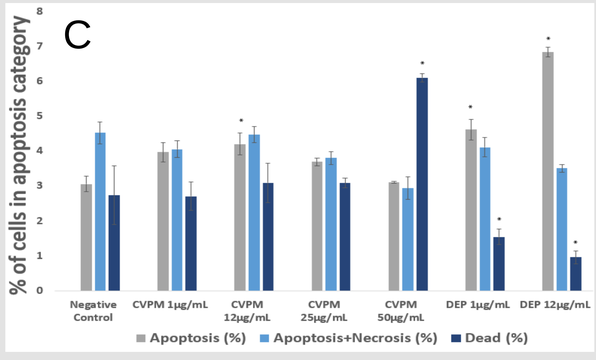
- RNA isolated and sequenced (Illumina NextSeq); reads mapped to human genome (HISAT2 aligner).
- Differentially expressed genes determined with EdgeR and Limma + voom.
- Ensemble gene set enrichment analysis (EGSEA) used for downstream pathway analysis.

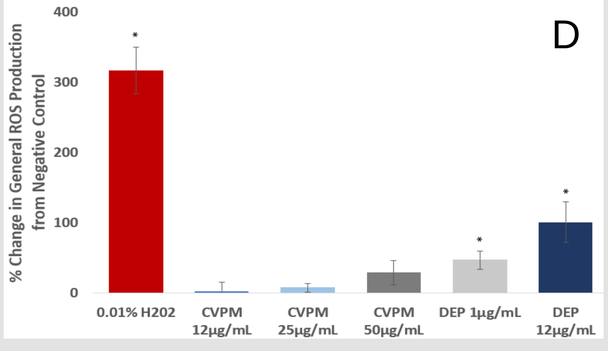
Confirmatory Bioassays

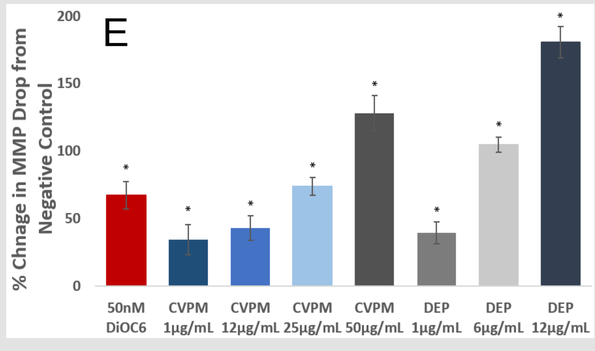
- PM cytotoxicity was by CCK8 Cell Viability Kit;
- Cellular apoptosis was quantified by Annexin-V-FLOUS Staining Kit;
- Reactive oxygen species (ROS) was determined by iQue Screener Flow Cytometer (Sartorius)
- Changes in mitochondrial membrane potential (MMP), and intracellular calcium imbalances was by iQue Screener Flow Cytometer.

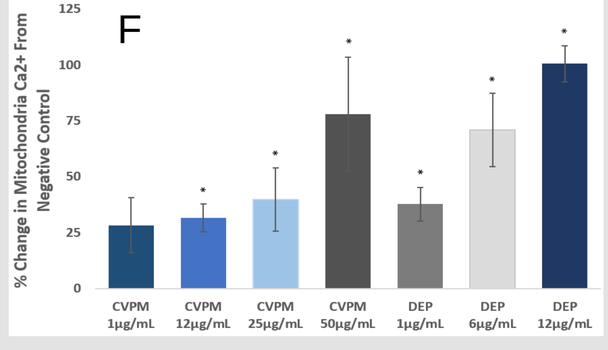


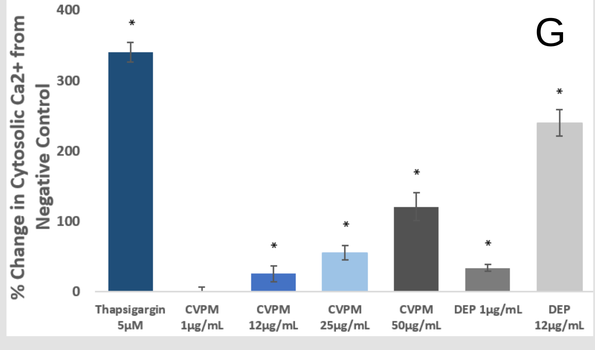












Results

For all studies, conditions marked with * are significantly different from the negative control ($p<0.05$; JMP version 14).

Figure A. EGSEA summary heatmap results of top 25 significantly affected pathways (FDR = 0.05). The UPR pathway was upregulated in all test conditions except CVPM 1μg/mL.

Figure B. CCK8 cytotoxicity results. There was a dose-related decrease in cell viability with increasing CVPM concentrations.

Figure C. Annexin-V-FLOUS apoptosis results. Apoptosis (%) was significantly ($p<0.05$) increased for CVPM 1μg/mL and dead (%) was significantly increased for CVPM 50μg/mL, suggesting increasing CVPM concentrations leads to increased cell death rather than increased apoptosis alone.

Figure D. General ROS production results. CVPM treatment up to 50μg/mL did not significantly increase general ROS production. DEP was more potent than CVPM.

Figure E. Evaluation of MMP drop results. There was a significant drop in MMP at all tested CVPM concentrations. DEP was more potent than CVPM.

Figures F & G. Alterations in mitochondrial and cytosolic calcium results. CVPM treatment caused a significant change in both mitochondrial and cytosolic calcium concentrations at all tested CVPM concentrations, except CVPM 1μg/mL. DEP was more potent than CVPM.

Conclusions & Next Steps

Taken together, these results support our hypothesis that a principal toxic mechanism of CVPM pollution involves ER stress and the UPR, which are known also to be associated to diseases such as asthma, cardiovascular disease, neurodegenerative disease, ischemic stroke, and chronic obstructive pulmonary disease (COPD).

Next steps include:

- Confirmation of RNA-Seq results via qPCR
- Additional bioassay development related to the UPR, including further investigation of CVPM-associated effects on mitochondrial health and ROS production.

Acknowledgements

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