Differential Transcriptome Responses Reveal that Cache Valley $PM_{2.5}$ Triggers ER Stress and the Unfolded Protein Response in Human Lung Cells

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Ambient particulate matter $PM_{2.5}$ poses a serious public health risk and is associated with increases in all-cause mortality, cardiopulmonary and cardiovascular disease, stroke, diabetes, cancer, and Alzheimer's disease. The normally picturesque Cache Valley of Northern Utah frequently experiences some of the highest $PM_{2.5}$ concentrations in the United States. However, the exact mechanism(s) of Cache Valley's $PM_{2.5}$ (CVPM) toxicity have yet to be fully characterized. We recently demonstrated that CVPM exposure is associated with the inflammatory response, endoplasmic reticulum (ER) stress, and the unfolded protein response (UPR), a highly conserved stress-response mechanism. The purpose of this study was to use whole transcriptome sequencing and network analysis test to confirm the involvement of these mechanisms in cultured human lung (BEAS-2B) cells exposed to CVPM at 1 and 12 μg/mL for 24 hours. All experiments were conducted in parallel using diesel exhaust particles (DEP) as a positive control. RNA sequencing with Ensemble Gene Set Enrichment Analysis (EGSEA) confirmed CVPM exposure had a significant (FDR adjusted p=0.05) effect on the UPR pathway, specifically upregulating the expression of *ATF4*, *ATF6*, *XBP1*, and *SERP1* genes. Significantly affected UPR associated pathways included the inflammatory response, DNA repair, P53 repair, xenobiotic metabolism, and apoptosis. Flow cytometry revealed that CVPM (12 μg/mL; 24 hours) caused significant cellular effects related to UPR activation, including reductions in mitochondrial membrane potential and alterations in intracellular Ca^{2+} homeostasis, as evidenced by a significant influx of Ca^{2+} in the cytosol and mitochondria, likely from the ER network. CVPM also initiated the release of cytochrome c oxidase from the mitochondria to the cytosol, and early indicator of apoptosis. CVPM-associated apoptosis (CVPM 50 μg/mL; 24 hours) and cytotoxicity (CVPM 200μg/mL; 24 hours) was observed. Across most experiments, 1μg/mL DEP elicited similar results to CVPM at 12μg/mL, suggesting that CVPM is less potent than DEP. 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). This research supported by the Marriner S. Eccles Foundation, Cytiva, and Utah State University.