Effects of Ozone Exposure on Macrophage Glycolysis and Mitochondrial Integrity and Homeostasis in RAW 264.7 Macrophages

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Ozone is a cytotoxic oxidative air pollutant known to induce lung epithelial injury, leading to the recruitment of macrophages to the lungs. Evidence suggests that ozone negatively affects mitochondrial function in macrophages, leading to decreased ATP production through oxidative phosphorylation. We hypothesized that ozone exposure upregulates glycolysis in macrophages as a compensatory mechanism in response to ozone-induced mitochondrial dysfunction. RAW macrophages were exposed to ozone (300 ppb) or air for 30 min; RNA was extracted 0.5 h, 1 h, 2 h, or 4 h post-exposure. Gene expression of rate-limiting glycolytic enzymes (Hk1, Pfkm, Pklr) and mitochondrial homeostasis, fusion, and fission proteins (Hspa9, Opa1, Dmn1l) were assessed by qPCR. Hspa9, Opa1, and Dmn1l expression was reduced 1 h following ozone exposure compared to air controls and returned to baseline after 2 h. In contrast, the expression of glycolytic genes Hk1 and Pfkm was upregulated 1 h post-exposure, followed by a decrease to control levels at 4 h. Unexpectedly, Pklr expression increased 0.5 h post-exposure, but returned to baseline at 1 h. These findings suggest that glycolysis increases in response to mitochondrial dysregulation caused by ozone exposure. Given its role in modulating metabolic proteins and driving cells towards recovery or apoptosis after oxidative stress, the integrated stress response (ISR) may contribute to this effect. Future studies will use pathway inhibitors to examine the role of the ISR in regulating mitochondrial bioenergetics and morphology. Supported by NIH R25ES020721, ES004738, ES005022, and the American Society for Pharmacology and Experimental Therapeutics.