Role of NADPH Oxidases in Organic Dust Induction of Inflammatory Gene Expression in Lung Epithelial Cells

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RATIONALE: Exposure to organic dust is a risk factor for the development of respiratory symptoms and respiratory diseases. We found previously [Natarajan et al Respir Res 2016 Oct 22;17(1):137] that induction of inflammatory gene expression poultry dust extract in bronchial epithelial cells is dependent on increased generation of reactive oxygen species levels (ROS). However, it is not known whether ROS derived from NADPH oxidase (NOX) family of enzymes, xanthine oxidase or mitochondria are involved in the induction. METHODS: Aqueous extracts prepared from poultry farm dust were used to study the effects of organic dust. The involvement of NOX enzymes and xanthine oxidase was investigated by determining the effects of chemical inhibitors on the induction of interleukin (IL)-6, IL-8, IL-1β, ICAM-1, PTGS2/COX-2 expression in Beas2B and normal human bronchial epithelial cells. siRNA Knockdown of NOX enzymes was also employed to understand their involvement in the induction. The effects of MitoTempo, a mitochondrial antioxidant on inflammatory gene induction were determined to understand the role of mitochondria-derived ROS. ROS generation in cells was analyzed by DCFDA labeling and mitochondrial ROS was analyzed by labeling with DCFDA and MitoTrackerRed FM. RESULTS: We found that pan-Nox inhibitors, DPI and VAS2870 suppressed induction of interleukin (IL)-6, IL-8, IL-1β, ICAM-1, PTGS2/COX-2 protein levels in Beas2B cells. Inhibitions of protein levels were associated with inhibition of mRNA levels except in the case of ICAM-1 in DPI treated cells. GKT137831 which has been reported to inhibit NOX-1 and NOX-4 suppressed induction of interleukin (IL)-8, IL-1β, ICAM-1, PTGS2/COX-2 protein levels, but had no effect on the induction of mRNA levels. siRNA knockdown reduced NOX-2 protein levels by 50% and resulted in the suppression of induction of IL-6, IL-8, IL-1β and ICAM-1 protein levels by approximately 50%. As in the case of Beas2B cells, DPI, VAS2870 and GKT137831 suppressed induction of IL-8, IL-1β, ICAM-1, PTGS2/COX-2 protein levels in normal human bronchial epithelial cells. Inhibition of inflammatory mediator expression by DPI and VAS2870 was associated with inhibition of ROS generation. Xanthine oxidase inhibitor, Febuxostat, had no effect on IL-1β and ICAM-1 protein levels in Beas2B cells. Mitochondrial antioxidant, MitoTempo (20 μM) suppressed induction of IL-1β and ICAM-1 protein levels by 30 - 40%, but had no effect on IL-6 and IL-8 levels. CONCLUSION: Our results indicated that NOX-derived and to a lesser extent mitochondria-derived ROS are important for the induction of inflammatory gene expression by organic dust extracts.

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