In Vivo Direct Evidence of Free Radical Generation in Diesel Exhaust Particles-Induced Lung Injury in Rats

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Diesel exhaust particles (DEPs) from diesel engine-powered automobiles cause serious health effects, especially in the lungs, which has been linked to lung cancer, pulmonary fibrosis, chronic alveolotis, bronchitis, and airway inflammation with hyperresponsiveness. Polycyclic aromatic hydrocarbons and reactive oxygen species derived from DEPs are implicated in the pulmonary toxicities. However, the underlying mechanism is poorly understood. This study was undertaken to test the hypothesis that lung exposure to DEPs causes in vivo free radical production which could be directly detected by electron spin resonance (ESR) with the spin trap a-(4-pyridyl-1-oxide)-*N*-*t*-butylnitrone (POBN). Seven days after intratracheal instillation of DEPs (25 mg/kg), radical adduct of POBN was detected by ESR from lipid extract of lungs. The hyperfine coupling constants for the radical adduct were $a^N = 14.81$ G, $a^H_\beta = 2.53$ G. They are consistent with those of a carbon-centered radical adduct, suggesting the signals detected here are derived from enhanced lipid peroxidation. No radical adducts were detected at 24 h and 72 h after DEPs instillation, indicating that free radical generation by DEPs is time-dependent. Histopathlogical analysis of the cellular profile of the broncho-alveolar lavage fluid (BALF) revealed a marked increase in the number of macrophages in the DEPs group as compared to the control. To assess whether macrophages were involved in the mechanism of free radical generation, rats were pretreated with gadolinium chloride, a blocker of macrophages activation. Gadolinium chloride pretreatment significantly eliminated the number of macrophages in BALF. However, the ESR spectrum in the lung exposed to DEPs was not affected. In addition, protein content in BALF was not changed by gadolinium chloride pretreatment. The results obtained here provide for the first time in vivo evidence of free radical generation in the lung by DEPs.