In vivo radical formation by NADPH oxidase in lung induced by lipopolysaccharide - a model for ARDS

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Intratracheal instillation of lipopolysaccharide (LPS) activates alveolar macrophages and infiltration of neutrophils causing lung injury / acute respiratory distress syndrome (ARDS) . Free radicals are a special focus as the final causative molecules in the pathogenesis of lung injury caused by LPS. While *in vitro* investigation has demonstrated radical generation after exposure of cells to LPS, *in vivo* evidence is lacking.

Therefore, using electron spin resonance (ESR) and the spin trap ? -(4-pyridyl-1-oxide)-*N-tert*-butylnitrone (POBN), we investigated *in vivo* free radical production by rats treated with intratracheal instillation of LPS. ESR spectroscopy of lipid extract from lungs exposed to LPS for six h gave a spectrum consistent with that of a POBN/carbon-centered radical adduct (a^{N} = 14.94 ± 0.07 G and a_b^{H} = 2.42 ± 0.06 G) tentatively assigned as a product of lipid peroxidation. To further investigate the mechanism of LPS-initiated free radical generation, rats were pretreated with the phagocytic toxicant $GdCl_3$, which significantly decreased the production of radical adducts with a corresponding decrease in neutrophil infiltration as indicated by histopathological studies. NADPH oxidase knockout mice totally blocked this phagocyte-mediated ESR detectable radical production in this model of acute lung injury. In conclusion, rats treated with intratracheal instillation of LPS generate lipid-derived free radicals via the activation of NADPH oxidase.