

In vivo direct evidence of free radical formation in rat pneumonia model induced by *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa (*P. aeruginosa*) is invasive and toxigenic, produces infections in patients with abnormal host defenses, and is an important nosocomial pathogen. Pneumonia caused by *P. aeruginosa* is very severe, results in necrotizing pneumonia. Intratracheal instillation of *P. aeruginosa* is well known as a model of pneumonia and lung injury. Recently, free radicals are a special focus as the final causative molecules in the pathogenesis of lung injury caused by *P. aeruginosa* infection. Although there is no direct evidence of free radical generation in lung injury caused by *P. aeruginosa in vivo*.

Therefore, using electron spin resonance (ESR) and the spin-trapping technique with α -(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (POBN), we investigated *in vivo* free radical production by rats treated with intratracheal instillation of *P. aeruginosa*. ESR spectroscopy of lipid extract from lungs infected with *P. aeruginosa* for 24 hours gave a spectrum consistent with that of a carbon-centered radical adduct ($a^N = 14.86 \pm 0.03$ G and $a_b^H = 2.48 \pm 0.09$ G). Previous investigations have tentatively assigned this radical adduct as a product of *in vivo* lipid peroxidation. The intensity of ESR spectra were significantly increased by the infection of *P. aeruginosa* (Intensity (cm); *P. aeruginosa* 2.07 ± 0.87 , Control 0.65 ± 0.14 , $p < 0.0001$).

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 and broncho-alveolar lavage. In conclusion, rats treated with intratracheal instillation of *P. aeruginosa* generate free radicals in the lung as demonstrated by ESR spectroscopy.