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ANTIOXIDANT-SURFACTANT LIPOSOMES MITIGATE HYPEROXIC LUNG INJURY IN PRETERM RABBITS.

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Antioxidant-surfactant liposomes, made by encapsulating superoxide dismutase (SOD) and catalase in surfactant lipids, increase alveolar type II cell antioxidant activity and protect against oxidant stress (Am J Physiol 1993; 265: L330-339). We examined whether intratracheal instillation of these liposomes reduces hyperoxic lung injury in 28-day gestational age rabbits. New Zealand White rabbits were delivered by cesarean section and pups were divided into 3 groups. Twenty pups received 0.1 ml/15 g body weight of antioxidant-surfactant liposomes by intratracheal injection and hyperoxia (≥95% oxygen) for 24 h, fifteen pups received an equal amount of surfactant liposomes without antioxidants and hyperoxia for 24 h, and 18 pups were sacrificed at birth. After sacrifice, lung homogenates were investigated for SOD and catalase activity and protein content. Lung SOD and catalase activity in pups treated with antioxidant-surfactant liposomes was 2.1 ± 0.5 and 25.9 ± 7.7 U/mg protein, in pups treated with liposomes without antioxidants 1.5 ± 0.4 and 12.6 ± 5.5 U/mg protein, and in pups sacrificed at birth 2.0 ± 0.4 and 23.9 ± 8.4 U/mg protein. These data suggest that intratracheal instillation of antioxidant-surfactant liposomes at birth maintains or boosts antioxidant capacity during hyperoxia in preterm rabbits.

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ROLE OF ENDOGENOUS CYTOKINES IN ENDOTOXIN-INDUCED OXYGEN TOLERANCE.

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Endotoxin (LPS) and the cytokines, tumor necrosis factor (TNF) and interleukin-1 (IL-1), are known to protect adult rats against O_2 toxicity. Whether the protective effect of LPS is mediated through its direct effect on lung cells or through cytokines is not clear. In this study, we demonstrated that: 1) Tracheal insufflation of LPS (5 µg/rat, 14-20 µg/kg) markedly attenuated O_2-induced pulmonary injury and prolonged the survival of rats exposed to 100% O_2. 2) LPS-induced O_2 tolerance was associated with a selective enhancement of pulmonary Mn superoxide dismutase (MnSOD), but not CuZnSOD, mRNA. 3) Depletion of alveolar macrophages (AM) by 84%, which reduced LPS-induced release of TNF by 86%, did not prevent LPS-induced O_2 tolerance. 4) Co-insufflation of LPS with anti-TNF antibody (1.65 mg/rat), which completely neutralized TNF activity induced by LPS, with or without 400 µg IL-1 receptor antagonist (IL-1ra), also did not affect LPS-induced O_2 tolerance. These data suggest that LPS-induced protection against O_2 toxicity is likely due to its direct effect on lung cells.

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POTENTIAL ROLE OF FREE RADICALS IN ACUTE SILICOSIS.

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Acute silicosis is a fatal lung disease often associated with sandblasting, drilling, tunnelling, and silica flour mill operations. We have shown recently that fracturing of silica generates free radicals on its cleavage planes, and that freshly fractured silica is more cytotoxic in vitro than aged silica. The objective of this study was to determine if freshly fractured silica is biologically more active in vivo than aged silica in inducing lung injury in an animal model. Male pathogen-free Fischer 344 rats were exposed to freshly fractured or aged silica (20mg/m³, 5 hr/day for 10 days). Silica dust was fractured by jet milling. The aged dust was stored for 60 days before use. Analysis of surface radicals on silica samples confirmed the increased concentration of radicals in fresh vs aged silica. Compared to aged silica, freshly fractured silica resulted in dramatically greater increases in cellular infiltrates, lactate dehydrogenase, β-N-acetyl glucosaminidase and decreases in anti-oxidant enzymes superoxide dismutase, glutathione peroxidase, and catalase in alveolar macrophages (AM). AM induced enhanced oxygen radical generation, and pulmonary tissue showed increased lipid peroxidation in fresh vs aged silica-exposed animals. These results indicate that exposure to freshly fractured silica causes impairment of antioxidant defenses leading to acute silicosis.

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PLASMA REACTIONS WITH HYDROGEN PEROXIDE AND HYPOCHLOROUS ACID.

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Activated phagocytes, which generate superoxide (O_2^-), hydrogen peroxide(H_2O_2) and hypochlorous acid(HOCl), deplete plasma antioxidants and induce lipid peroxidation. Both O_2^-, H_2O_2 do not induce plasma lipid peroxidation and HOCl generates only trace amounts of lipid hydroperoxides at concentrations ≥ 1 mM. We investigated the synergistic ability of H_2O_2 and HOCl to induce oxidative damage in human plasma. H_2O_2 and NaOCl were added separately to plasma at 37°C and incubated for 20 min. Various antioxidants, lipid oxidation and protein modification products were measured. H_2O_2 and HOCl depleted ascorbate and total-SH, although HOCl was more potent. H_2O_2 and HOCl effects were additive but not synergistic in the depletion of ascorbate and total-SH groups. H_2O_2(2 mM) did not induce plasma lipid hydroperoxide formation, even in the presence of sodium azide(NaN_3), a catalase inhibitor. HOCl(2 mM) induced a trace amount of lipid hydroperoxide formation(-50 nM). Combined addition of oxidants(2 mM each) increased lipid hydroperoxide formation(-100 nM) which was augmented further(4-5x) with NaN_3, depleted ubiquinol, but left α-tocopherol unaffected. Lipid hydroperoxides generated by H_2O_2 /HOCl were not inhibited by desferrioxamine or histidine, excluding Fe(II) or singlet oxygen in this oxidative process. It was concluded that H_2O_2 /HOCl generate a stronger oxidant, possibly hydroxyl radical, which may initiate lipid peroxidation.

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HYDROPEROXIDE MEDIATED MODULATION OF THE ALVEOLAR MACROPHAGE RESPIRATORY BURST BY INTRACELLULAR CALCIUM IS INDEPENDENT OF THAPSIGARGIN EFFECTS.

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Exposure of alveolar macrophages (AM) to sublethal oxidant stress has previously been shown to produce a dual effect upon the AM respiratory burst. A stimulating effect is observed at <25 µM [tBOOH] and an inhibition of the burst is observed at >50 µM [tBOOH]. These effects have been correlated with a transient versus a sustained rise in intracellular calcium, $[Ca^{2+}]_i$, respectively. We investigated the potential role of $[Ca^{2+}]_i$ in mediating these effects using thapsigargin (TG), which elicits $[Ca^{2+}]_i$ release from endomembranous stores and blocks Ca-ATPase. When TG was given prior to tBOOH, the initial release of $[Ca^{2+}]_i$ remained the same, but the latter phase of $[Ca^{2+}]_i$ release was diminished. Furthermore, TG exposure of AM after incubation with tBOOH resulted in release of the TG calcium pool. When AM were treated with TG there was a decrease in the ADP stimulated respiratory burst. Yet, this decrease had no effect on the dual role tBOOH produced upon the burst. Thus, we concluded that the tBOOH effect upon the burst and intracellular calcium release was not related to the TG sensitive $[Ca^{2+}]_i$ store.

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RATE OF DIFFUSION OF HYDROGEN PEROXIDE INTO ERYTHROCYTES

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Hydrogen peroxide is a potentially toxic metabolite produced by many cells including neutrophils and macrophages. Degradation of H_2O_2 is accelerated by catalase and a variety of peroxidases which are located primarily within cells. The rate at which H_2O_2 crosses cell membranes is generally assumed to be very rapid but has not been directly measured. In the present study, advantage was taken of the fact that any H_2O_2 which enters red cells should be rapidly degraded by intracellular enzymes, thereby minimizing back-diffusion into the extracellular medium. Fresh rat red cells were diluted from 1:5000 to 1:20,000 in saline containing from 0.2 to 2.0 mM of H_2O_2 at 25°C and after 1 to 10 minutes exposure they were centrifuged at 13,000xg for less than 10 seconds. Concentrations of H_2O_2 fell monoexponentially suggesting that intracellular concentrations of H_2O_2 remained negligible and that the rate of decrease could be used to define the permeability of the red cell membrane to H_2O_2. Comparable dilutions of hemolyzed cells indicated that intacellular metabolism of H_2O_2 must occur much more rapidly than diffusion of H_2O_2 into the cells and corrections were made for catalysis which was produced by hemolysis during incubation. Assuming that the surface area of each red cell is 163µm², then P was calculated to be 7.52 x 10^{-4} cm/s or about 15% that of labeled water. (NIH grant HL18606).

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