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INCREASED PULMONARY RESIDUAL VOLUME (IPRV) GENERATED BY INTRAVENOUS PERFLUOROCHEMICAL (PFC) EMULSIONS IN SWINE: LACK OF HEMODYNAMIC EFFECTS AND POTENTIAL TREATMENT REGIMENS.
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IPRV, (increased lung volume due to stable bubble formation and subsequent gas trapping), is a reversible side effect associated with i.v. infusion of PFC emulsions in certain species. No signs of pulmonary edema or other histological alterations have been observed with IPRV other than the appearance of vacuolated macrophages (characteristic of the normal PFC clearance mechanism). Swine were infused i.v. with 4.05 g emulsified PFC/kg or saline (4.5 mL/kg) and divided into 2 study groups. Three days after infusion, animals in group 1 (Saline: n=6; PFC: n=6) were anesthetized, mechanically ventilated, and instrumented to measure cardiac output, heart rate, pulmonary artery and wedge, left ventricular, and mean arterial pressures and arterial blood gases. Right lung volume (RLV) and left lung dry and wet weights (LLWW), extravascular lung water (EVLW) and wet-to-dry tissue ratios (W/D) were also determined (Saline: n=4, PFC: n=5). Animals in group 2, (Saline+Ethanol: n=4; PFC+Ethanol: n=4) were sedated and treated with 20 min of ethanol vapor breathing (nebulized into endotracheal tube), 3 days after infusion, and lung parameters were determined. Values are expressed as % change from respective control groups. (* $p < 0.05$ vs. own control)

GROUP	RLV	LLWW	W/D	EVLW
1) PFC	100.0*	49.7*	9.8	64.7*
2) PFC+Ethanol	19.6	0.6	4.0	2.9

In group 1, no significant differences in pulmonary or systemic hemodynamic parameters were observed. The changes in PaO_2 (14% decrease) and pulmonary vascular resistance (42% increase) in PFC animals could be lessened by increasing the FiO_2. In group 2, increases in lung weight and size were markedly attenuated by ethanol vapor breathing. These results indicate that PFC-induced IPRV does not significantly alter pulmonary or systemic hemodynamics in swine. Modest changes in PaO_2 and PVR are alleviated with oxygen breathing and changes in lung weight and size are eliminated with ethanol vapor breathing.

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PULMONARY EFFECTS OF INTRAVENOUS PERFLUOROCHEMICAL (PFC) EMULSIONS: REVERSIBILITY, SPECIES SPECIFICITY, AND PFC DEPENDENCE.
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PFC emulsions administered intravenously have been shown to cause increased pulmonary residual volume (IPRV) in rabbits. The proposed mechanism of IPRV is through alveolar gas (air) trapping and is primarily dependent upon the PFC vapor pressure. These studies compared the effects of two different PFC emulsions to induce IPRV and evaluated the reversibility of this effect following a single intravenous injection in rabbits and rats. In the rabbit study, animals received either AF0104 (90% w/v perflubron emulsion) at 2.7 or 8.1 g PFC/kg or *Fluosol*® (20% w/v PFC emulsion) at 3.0 g PFC/kg. Lung weight, volume (fluid displacement), and pathology were evaluated 3 and 22 days postdosing. Four of the 6 rabbits treated with *Fluosol* died or were euthanized in poor health prior to necropsy on Day 22 whereas only one rabbit in the high dose AF0104 group died prior to study termination. AF0104 at both doses and *Fluosol* caused approximately 1.5 to 3 fold increases in *ex vivo* lung volume and weight, and uncollapsed lungs were observed both macro- and microscopically. Vacuolated mononuclear cells, reflecting the presence of PFC in the lung tissue and alveolar distention were present; no edema or other lesions were observed. Reversibility of lung effects was apparent by Day 22 in rabbits receiving 2.7 g/kg AF0104 but not *Fluosol* at a comparable PFC dose. In rats, AF0104 or *Fluosol* were administered as a single dose of 9.0 g PFC/kg. Lung volumes were measured 14 and 49 day postdosing. A 2 to 3 fold increase in *ex vivo* lung volume was measured 14 days postdosing. This effect was transient in rats treated with AF0104 in that lung volume was no different from control when measured 49 days postdosing. However, increased lung volumes persisted in the *Fluosol*-treated rats. Despite the use of these higher doses in rats, no mortality was seen and the sensitivity to the pulmonary effects of intravenous PFC emulsions was less in rats than in rabbits. These studies indicate that the pulmonary changes produced by AF0104 in rabbits and rats are reversible and less severe compared to the changes induced by a dose of *Fluosol* containing an equivalent amount of PFC.

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INFLUENCE OF PERFLUOROCHEMICAL (PFC) VAPOR PRESSURE AND PFC EMULSION PARTICLE SIZE ON INCREASED PULMONARY RESIDUAL VOLUME IN RABBITS.
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High capacity to dissolve gases allow PFCs to be used as temporary oxygen carriers. To achieve i.v. compatibility, PFCs are emulsified under energy to form particulates in the submicron range. Emulsions using high vapor pressure (>9 torr at 37°C) PFCs are associated with species-specific increased pulmonary residual volume (IPRV). IPRV is observed in rabbit lungs which fail to collapse at necropsy. No pulmonary signs suggesting IPRV are observed in humans. We studied the relationship between PFC vapor pressure (VP) and PFC emulsion particle size on IPRV in rabbits. All PFC emulsions tested were 90% w/v PFC. Emulsion VP was adjusted (0.1-10.5 torr) by quantitatively varying mixtures of perflubron, a brominated PFC, and various non brominated PFCs of different VPs. VPs ranged from 0.1-3.0 in non brominated PFC emulsions and from 8.3-10.5 in perflubron-based emulsions. Mean particle size was adjusted (0.05-0.51 μm) by varying emulsifying agents and processing conditions. PFC emulsions were infused into the ear vein of Pasturella-free New Zealand white rabbits (2.0 ± 0.2 kg) at 5.4 g PFC/kg. Controls received 6.0 mL/kg i.v. saline. After 3 days, lungs were excised and IPRV was measured (total lung volume by fluid displacement). IPRV occurred in all PFC rabbits while controls showed no effects. Low VP (0.1-8.3 torr) PFCs produced the least IPRV (15-37% increase) while higher VP (9.8-10.5 torr) PFCs caused significantly greater IPRV (134-188% increase) compared to control. Emulsion particle size also appeared to directly influence degree of IPRV. Since low VP (≤3 torr) PFCs have extended tissue retention while high VP (>9 torr) PFCs cause significant IPRV, neither are suitable as "blood substitutes". Perflubron-based emulsions with VPs ≈ 8 torr and mean particle size <0.13 μm, however, are ideally suited since they cause little IPRV in rabbits and have improved biological properties including prolonged blood half-life and acceptable tissue residence times.

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Morphometric and Cytochemical Analysis of Neutrophil and its Granules in Broncho-Pulmonary Lavages of ARDS Patients
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Adult Respiratory Distress Syndrome (ARDS) has a high mortality rate despite significant advances in support care. Infectious complications in the lung indicated that the function of neutrophils (PMN) obtained from patients with ARDS is significantly impaired. We hypothesize that PMN from the blood stream which migrate into the lung airspace lose their function ability. We compared the PMN granule populations in circulation with BAL fluid. BAL fluids of 36 patients with early ARDS and 16 with late ARDS were collected. Cells (2-6x 10^6) from each patient are used for EM and morphometric studies. All of the ARDS lavage fluids have high protein concentrations and predominantly contain PMNs (81.7 ± 8.1%) whose viability uniformly exceed 90% by trypan blue exclusion. We examined enzyme markers of primary and secondary granules in PMNs using cytochemistry and immunocytochemistry. Specific granule degranulation (≤ 80%) occurred in PMNs of ARDS patients. In 16 cases of paired studies, the PMNs in second lavages showed only a mild loss of specific granules but a significant loss of azurophilic granules. Normal PMNs lose only a few specific granules as they migrate into the lung. In conclusion, the rapid degranulation of PMNs in BAL of ARDS is expressed by the loss of its functional activity. The extent of degranulation of BAL vs blood PMNs indicated the loss of primary granules as the PMN changes their function during migration. Supported by NIH HL30542.

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SILICA EXPOSURE INCREASES NITRIC OXIDE (NO) PRODUCTION IN RAT ALVEOLAR TYPE II CELL FRACTIONS.
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Alveolar type II epithelial (type II) cells play an important role in the pulmonary response to irritant challenges. Recent evidence suggests that these cells produce NO which may, in turn, participate in defense reactions in response to foreign substances. In the present study, we assessed whether the intratracheal (IT) instillation of silica might be associated with an increase in NO production by rat type II cells. Male rats received IT instillations of endotoxin-free saline or silica (10 mg/100 gBW). After 24 hrs, type II cell-enriched fractions were isolated from perfused/lavaged lungs by elastase/collagenase digestion and purified by centrifugal elutriation. Inducible NO synthase (iNOS) mRNA levels in freshly isolated type II cell fractions were evaluated using Northern blot analysis. Media nitrate and nitrite levels were indexed after a 20 hr incubation period of type II cells in culture. iNOS mRNA levels were elevated in the type II cell fractions isolated from silica-treated rats compared to saline-controls. After culture, total media nitrate and nitrite levels were three-fold higher in silica vs saline-exposed type II cell fractions. These results suggest that NO synthase expression is induced in rat alveolar type II epithelial cells in response to an in vivo silica challenge.

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PHOSPHOLIPID-BINDING PROTEINS AND PHOSPHOLIPASE A_2 IN RADIATION- INDUCED INCREASE IN PROSTAGLANDIN SYNTHESIS.
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Annexin I, a member of a family of calcium-dependent phospholipid-binding proteins (PLBP), has been suggested as a regulator of prostaglandin metabolism by its inhibitory effect on phospholipase A_2 (PLA_2). Prostaglandin synthesis is increased in irradiated tissues, but the mechanism underlying the increase has not been delineated. We studied the possible involvement of a lung 36 KD PLBP, which possesses characteristics of annexin I, and PLA_2 in the increased thromboxane synthesis in the irradiated rat lung. The right lung of rats was irradiated with 0, 15 and 30 Gy of x-rays and the animals were sacrificed after 3 months. PLBP was determined by its ability to transfer unilamellar liposomes to multilamellar liposomes and by immunoblotting against the anti-36 KD PLBP antisera. Thromboxane production by minced lung tissue was determined by RIA of TXB_2. PLA_2 activity was assayed by the hydrolysis of ^{14}C-dioleoylphosphatidylcholine. Our results showed that the PLBP activity of the 30 Gy lungs was lower than that of the 0 and 15 Gy lungs (8.82±0.47 compared to 9.73±0.49 and 9.95±0.78 nmol phospholipid transferred/mg protein, respectively). However, these differences are not statistically significant. Western blotting also demonstrated a reduction of PLBP in the 30 Gy irradiated lungs. PLA_2 activity was lower in the 30 Gy lung as compared to that in the 0 Gy lung (0.23±0.01 vs 0.32±0.01 nmol fatty acid liberated/mg protein/min, $p < 0.001$), in spite of a 2.8-fold increase in thromboxane synthesis (36.7±6.5 vs 107.6±14.3 pg TXB_2/mg tissue/min for 0 and 30 Gy lungs). These results suggest that PLBP and PLA_2 are not likely involved in the radiation-induced increase in prostaglandin metabolism.

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ABSTRACTS

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