Pulmonary surfactant is a complex of highly active phospholipids and proteins that cover the alveolar epithelial surface of the lungs [King and Clements, 1972b] and acts to promote lung stability by reducing surface tension within the lung, while also protecting against inhaled pathogens [Goerke, 1998]. Surfactant is synthesized in the alveolar type-II cells, stored in the lamellar bodies, and secreted to the alveolar space where it undergoes complex physiological changes [Gross et al, 1988]. The composition of lung surfactant in humans is very constant, although it may change in the diseased states [Hallman et al, 1982]. Phospholipids account for 85% of its composition and the main component is dipalmitoylphosphatidylcholine (DPPC). In addition, surfactant contains different apoproteins (SP-A, SP-B, SP-C and SP-D), neutral lipids, and carbohydrates [Jobe and Ikegami, 1987]. The reduction of surface tension within the lung is a result of the interaction between surfactant phospholipids and the two hydrophobic surfactant proteins, SP-B and SP-C [Goerke, 1998], while the two hydrophilic proteins, SP-A and SP-D, are members of a family of innate immune molecules called collectins. Collectins opsonize bacteria and viruses and enhance their phagocytosis by macrophages and neutrophils [Crouch and Wright, 2001].

Pulmonary-surfactant dysfunction can lead to acute lung injury and is characterized by alveolar instability, floating and collapse. Multiple studies of patients with a variety of lung diseases have shown that surfactant levels are decreased in the inflamed, injured or infected lung [Pison et al, 1990; Gregory et al, 1991; Gunther et al, 1996]. In agreement, decreases in alveolar surfactant lipid pools have also been observed in several animal models of lung inflammation induced by both direct insults to the lung such as bacterial infection [Vanderzwan et al, 1998], oxygen toxicity [Bailey et al, 2002], endotoxin administration [McIntosh et al, 1996; Sugahara et al, 1996] and by indirect insults [Lewis et al, 1990], and cecal ligation and perforation [Malloy et al, 1997]. These abnormalities have been shown to occur in acute/adult respiratory distress syndrome (ARDS) [Ashbaugh et al, 1967; Lewis and Jobe, 1993] and infant/neonatal respiratory distress syndrome (IRDS/nRDS) [Avery and Mead, 1959]. Petty and co-workers [1977; 1979] later reported both qualitative and quantitative abnormalities in the surfactant content of ARDS patients. It is now known that surfactant dysfunction plays a major role in the pathophysiology of ARDS [Gregory et al, 1991] and functional changes have been described not only in patients with established ARDS but also in
patients at risk [Gunther et al, 1994].

The main biochemical abnormalities include an 80% fall in the total phospholipid content, decline in the fractional content of DPPC and phosphatidylglycerol and other fractions, and loss of apoproteins (90% of surfactant protein SP-A and SP-B) [Avery and Mead, 1959; Lewis and Jobe, 1993]. This loss of alveolar surfactant is due to several factors including the presence of plasma proteins [Holm et al, 1998], cleavage of phospholipids by serum phospholipases [Holm et al, 1991], formation of free radical species (including nitrates, lipid peroxidation etc.) [Gilliard et al, 1994; Cifuentes et al, 1995] and conversion to nonfunctional surfactant with more phospholipid aggregates [Holm et al, 1991]. Alveolar metabolism of surfactant is a complex process, primarily involving type II epithelial cells that synthesize, secrete and clear surfactant from the airspaces [Wright and Dobbs, 1991], along with phagocytic cells such as macrophages and neutrophils that participate in surfactant clearance [Wright, 1990]. In a situation of pulmonary inflammation, altered type II cell metabolism has been thought to play a role in the alterations of surfactant lipid levels. Viviano et al, [1995] observed a decrease in alveolar surfactant levels and a corresponding increase in intracellular surfactant after lipopolysaccharide (LPS) administration. The airway compartment with alveolar macrophages (AMs) and epithelial cells as the predominant cell types is a physiological barrier to a variety of environmental agents including gases, particulates and microbes. AMs are located at the air-tissue interface in the lung and are therefore the first cells which interact with inhaled organisms and antigens [Sibille and Reynolds, 1990]. During the development of an acute inflammatory lung injury, AMs play a very important role upon their activation. It has been shown in several lung injury models that activated pulmonary macrophages release the cytokines, tumor necrosis factor-a (TNF-a) and interleukin-1β (IL-1β) as well as the chemokines, monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1β (MIP-1β) [Monton and Torres, 1998]. All these inflammatory mediators together play a crucial role in the orchestration of an inflammatory response, particularly in neutrophil recruitment. Additionally, exposure of rat lungs ventilated ex vivo to LPS resulted in the presence of giant lamellar bodies within the type II cells [Uhlig et al, 1995; Fehrenbach et al, 1998]. Collectively these studies suggest that type II cell metabolism is altered after LPS administration and that a possible explanation for decreased surfactant pool size may be an increased clearance of surfactant lipids by the type II cells. Additional experimental evidence has also implicated recruited inflammatory cells as having an impact on surfactant pool sizes. Both neutrophils and
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Macrophages recovered from LPS exposed lungs had a greater capacity to internalize surfactant-like lipids compared to control cells in vitro [Quintero and Wright, 2002]. Therefore these recruited inflammatory cells may also have a significant impact on surfactant pool size by increasing the overall surfactant lipid clearance.

Exogenous-surfactant replacement has been successfully achieved in IRDS [Avery and Mead, 1959], but clinical trials in ARDS have had mixed results. The surfactant preparations normally used are natural surfactants of porcine (Curosurf) or bovine (Alveolfact, Survanta) origin, or synthetic protein free preparations. Surfactant preparations derived from natural sources contain the hydrophobic peptides SP-B and SP-C but none—or very low levels—of the much larger hydrophilic surfactant proteins SP-A and SP-D which are lost during the extraction procedure [Clark and Reid, 2003]. Earlier reports have shown that exogenous artificial surfactant (Exosurf) [Wiedemann et al, 1992] or bovine surfactant (Survanta) [Gregory et al, 1997] can improve oxygenation and lung mechanics in ARDS. Many investigations have also provided several important contributions to the literature regarding surfactant replacement therapy. First, the optimal surfactant preparation is very important and should contain apoproteins [Gilliard et al, 1994]. Second, in order to achieve a physiological response, surfactant must be administered in large volumes into the adult lungs [Gunther et al, 2002]. Direct bronchoscopic instillation may be one way to achieve these effects [Anzueto, 2002]. Surfactant administration by such means resulted in a marked increase in the phospholipid pool. The surface tension lowering properties were markedly improved, but not fully recovered. The bronchoscopic administration of surfactant resulted in an improvement of some of the biochemical and biophysical characteristics of surfactant in patients with ARDS [Wiswell et al, 1999]. Although surfactant preparations are not approved by the US Federal Drug Administration for use in patients with acute respiratory distress syndrome, these preparations show promises that it may be an important adjunctive therapy in patients with acute respiratory distress syndrome and refractory hypoxaemia.

Lacunae in the study

In view of the above facts there is a gap in the present knowledge such as the LPS induced lung injury and surfactant therapy. Also, the information regarding the cellular & molecular immunopathologic characteristics in response to endotoxin challenged rat ARDS and the amelioration of such effect by intratracheal instillation of artificial and natural heterologous...
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surfactant are not available. In particular, the apoptosis in the lung derived cells and the signal transduction pathways in the different study group needs to be evaluated.

Objectives

To study the inflammatory and immune response of the lung derived cells, in experimental lung surfactant therapy in relationship with endotoxin mediated lung injury in adult rats, following objectives have been pursued.

- Isolation of lung surfactant from heterologous source such as the porcine lung lavage and preparation of synthetic (designer) surfactants of defined phospholipid compositions.
- Characterization of isolated porcine lung surfactant by various biochemical methods including enzyme assays, chemical composition, isolation and analysis of lipid profile along with isolation of surfactant associated proteins.
- To develop the animal model of lung injury by surgical intervention of endotoxin (LPS) in adult rat and the reduction in inflammatory response induced by LPS through surfactant instillation.
- To assess the degree of lung injury by various parameters including wet/dry (W/D) weight ratio, myeloperoxidase (MPO) activity, histological characterization in lung tissue, generation of reactive oxygen/nitrogen species like nitric oxide (NO), along with citrulline levels.
- To isolate the bronchoalveolar lavage (BAL) fluid and, isolation and characterization of the inflammatory cell population in the BAL fluid, such as neutrophil and macrophage by Giemsa staining and also type-II epithelial cells isolation.
- To examine the changes in the expression of surfactant proteins (SP-A, SP-B) during LPS induced ARDS and subsequently with surfactant treatments.
- To study the cytokines/chemokines release such as the interleukins (IL-1β, IL-2, IL-4, TNF-α, IFN-γ) and the macrophage chemotactic factors (monocyte chemoattractant protein, MCP-1 and macrophage inflammatory protein, MIP) in the BAL fluid by quantitative ELISA, protein expression in lung tissue by Western blot and
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immunofluorescence as well as gene expression in lung tissue by reverse transcriptase-polymerase chain reaction (RT-PCR).

➢ To study the inflammatory pathway of COX and iNOS activation along with NF-κB transcription by measuring PGE₂ generation, COX-1 and COX-2 activity, NF-κB binding activity, Western blot and immunohistolocalization of COX-1, COX-2, iNOS, NF-κB and its downstream targets, IkBα and IKKα proteins.

➢ To study the cascade of signaling events, including activation of phosphatidylinositol 3-kinases and its downstream targets Akt phosphorylation and GSK-3β regulation by Western immunoblot and immunocytochemistry.

➢ To study the role of the intracellular Ca²⁺-homeostasis and intracellular alkalosis (pHi) by using fluorescent probes in the different study group.

➢ To study the mitochondrial dysfunction through mitochondrial permeability transition by evaluating changes in cytoplasmic proteins (Bcl-2, Bax) as well as mitochondrial proteins (cytochrome-c and Apaf-1) expression by Western immunoblot along with immunocytochemistry and through disruption of mitochondrial transmembrane potential (ΔΨm) by fluorescent microscopy along with spectrofluorimetric analysis.

➢ To measure the activity of caspase 1 and caspase 3 by proteolytic cleavage of the fluorogenic substrates (YVAD-AMC and DEVD-AMC) and expression of caspase-9 and caspase-3 by Western blot along with immunofluorescence as an index of apoptosis.

➢ To study the DNA damage in the lung tissue in response to caspase-3 activation by PARP cleavage, DNA fragmentation analysis, terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay, morphological examination of type-II pneumocytes and BALF cells by fluorescent dyes and externalization of phosphatidylserine.

➢ To explore the reparative and proliferation mechanism by studying the expression of VEGF, PPAR-γ, and PCNA by Western immunoblot and by immunohistochemistry.