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18:1/18:1-Dioleoyl-phosphatidylglycerol prevents alveolar epithelial apoptosis and profibrotic stimulus in a neonatal piglet model of acute respiratory distress syndrome

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ABSTRACT

Background: 18:1/18:1-Dioleoyl-phosphatidylgycerol (DOPG) is a surfactant phospholipid that is nearly non-detectable in neonatal surfactant films. When alveolar macrophages are exposed to DOPG *in vitro*, secretory phospholipase A2 (sPLA2) production is blocked, resulting in suppressed macrophage activity and improved surfactant function. We investigated whether the addition of DOPG to a commercially available surfactant preparation would improve lung function in a neonatal piglet model of acute respiratory distress syndrome.
Materials and methods: Respiratory failure was achieved by triple-hit lung injury (repeated broncho-alveolar lavage, injurious ventilation, tracheal lipopolysaccharide instillation, each intervention 24 h apart) in twenty-four domestic piglets aged 2–6 days and subject to mechanical ventilation. Following each lung injury protocol the piglets were treated with surfactant alone or with surfactant + DOPG.
Results: Within 72 h of mechanical ventilation, we observed significantly improved gas exchange (oxygenation and ventilation), lung mechanics (compliance and resistance of the respiratory system), and pulmonary oedema (extra-vascular lung water index) in the surfactant + DOPG group. This favourable clinical effect could be attributed to improved surfactant function, reduced sPLA2 secretion, inhibition of macrophage migration, reduced alveolar epithelial apoptosis, and suppression of amphiregulin and TGF-β1 expression in pulmonary tissues as a prerequisite for fibrous lung repair.
Conclusions: We conclude that surfactant fortified by DOPG preserves lung function, and prevents alveolar epithelial injury and fibrous stimulus by reduction of sPLA2 in a neonatal model of acute respiratory distress syndrome without any relevant discernable side effects. Hence, DOPG supplementation in a neonatal lung exerts important function protecting effects and seems to be justified in cases of overwhelming pulmonary inflammation.

Abbreviations: DOPG, 18:1/18:1-Dioleoyl-phosphatidylgycerol; sPLA2, secretory phospholipase A2; nARDS, neonatal acute respiratory distress syndrome; AECs, airway epithelial cells; EMT, epithelial-to-mesenchymal transition; PGs, phosphatidylglycerols; BALF, broncho-alveolar lavage fluid; MAP, mean airway pressure; PEEP, positive end-expiratory pressure; Peak, peak inspiratory pressure; OI, oxygenation index; VEI, ventilation efficiency index; V_T, tidal volume; sC_{rs}, specific (dynamic) compliance; R_{rs}, resistance of the respiratory system; SVRI, systemic vascular resistance index; EVLWI, extra-vascular lung water index; ITBVI, intrathoracic blood volume index; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labelling; rBAL, repeated bronchoalveolar lavage.

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1. Introduction

Surfactant replacement therapy in acute respiratory distress syndrome (ARDS) requiring mechanical ventilation is usually of limited effect because inflammation of the airway epithelium causes apoptosis, migration of blood-borne cells, and leakage of blood proteins into the airways; all of these components are capable of destroying the surfactant film [1–4]. Large clinical trials involving hundreds of adult [5] and paediatric patients [6] disappointingly could not detect significant treatment effects such as improvements in lung function, length of mechanical ventilation, or survival (young infants excepted). In a different clinical setting, *i.e.*, respiratory distress syndrome of prematurity, surfactant replacement therapy has been the treatment standard for almost three decades; however, as soon as inflammation of the airway epithelium is involved, the beneficial effects rapidly wear off due to accelerated surfactant metabolism [4,7,8]. Hence, only short-term improvements in oxygenation in the early phase of neonatal ARDS (nARDS) have been observed [9] in typical neonatal conditions, such as congenital pneumonia, sepsis, meconium aspiration syndrome, bile aspiration, and pulmonary haemorrhage.

Furthermore, surfactant therapy failure implies prolonged mechanical ventilation and a high likelihood of secondary damage to the neonatal lung by induction of fibrosis and the demise of alveolar-capillary units in the distal airspaces. As a consequence, nARDS mortality remains unacceptably high (~30% [10]).

nARDS may therefore be regarded as an overwhelming inflammation of airways, airway epithelial cells (AECs), and lung parenchyma that overrules the immune-modulating effects and the biophysical properties of endogenous/exogenous surfactant in neonates on mechanical ventilation. Usually, surfactant-induced host defence is attributed to the pulmonary collectins surfactant protein A and D [11]; however, almost all commercially available surfactant preparations do not contain these surfactant proteins. In contrast to the phosphatidylglycerols (PGs), the main surfactant phospholipid 16:0/16:0-Dipalmitoyl-phosphatidylcholine (DPPC) reaches extremely low surface tensions but absorbs only slowly to air/water interfaces at the mucosal level [12]. Whereas DPPC most likely does not exert any anti-inflammatory effects, 18:1/18:1-Dioleoyl-phosphatidylglycerol (DOPG) and some more saturated and unsaturated PGs (e.g., 12:0/12:0-DLPG, 14:0/14:0-DMPG, 16:0/18:1-POPG) have been shown to suppress secretory phospholipase A2 (sPLA2) activity and TNF-α production in alveolar macrophages in a dose-dependent manner [13–15]. DOPG is a naturally occurring PG in surfactant films of mammalian organisms and represents ~20% of total PGs in adult human surfactant films [16]. However, in newborn piglets on their first day of life, diunsaturated PGs represent only a minor fraction of total PG [17], a finding suggestive of gradual adaptation to extra-uterine life characterized by exposure to infectious agents.

Because sPLA2 is largely responsible for the inhibition of pulmonary surfactant function in ARDS [4,18] and causes migration and stimulation of macrophages while simultaneously being synthesized by macrophages, blocking this pathway with DOPG at the mucosal site might also prevent induction of pulmonary fibrosis at the lung parenchymal site (alveolar epithelial-to-mesenchymal transition, EMT [19,20]), as regularly observed in the subacute, proliferative stage of ARDS.

In a translational study mimicking the clinical condition of a newborn baby subject to mechanical ventilation, we used newborn piglets as a model of nARDS with respiratory failure because pigs, like humans, have a resident population of mature macrophages in the pulmonary capillary bed and are sensitive to lipopolysaccharides (LPS) [21]. Following exposure to triple-hit lung injury with severe pulmonary inflammation (repeated airway lavage, injurious ventilation, and tracheal LPS administration), the piglets being mechanically ventilated for 72 h received surfactant (poractant alfa) with or without additional DOPG every 24 h. This is the first preclinical study that evaluates the beneficial effects of topical DOPG administration on gas exchange and lung functions while focussing on surfactant surface tensions, cell type specificity in bronchoalveolar lavage fluid (BALF), AECs apoptosis, and pro-fibrotic growth factors in the tissues of excised lungs. We hypothesized that DOPG would reduce airway epithelial inflammation and alveolar EMT by reduced gene expression of pro-fibrotic growth factors.

2. Materials and methods

The review board for the care of animal subjects of the Schleswig-Holstein government approved the experimental protocol. All investigations were performed in accord with the German law for animal protection (BGBI 1, p. 1319) and the European Community guidelines (EU Directive 2010/63/EU). Twenty-four domestic piglets aged 2–6 days were included in the study. All piglets were older than 36 h at the study start to exclude open ductus of Botalli, which interferes with haemodynamic measurements due to shunting (see below).

Anaesthesia and analgesia were initially achieved by an intramuscular injection of 15 mg/kg ketamine, 1.5 mg/kg midazolam, and 0.025 mg/kg atropine. For continuous sedation, all piglets received an intravenous infusion of 5 mg/kg/h ketamine, 0.5 mg/kg/h midazolam, and 0.4 mg/kg/h vecuronium bromide throughout the whole study. After intubation with a 3.5 mm endotracheal tube, mechanical ventilation was started with the following ventilator parameters: FiO_2 = 0.5, PEEP = 6 mbar, rate = 25/min. Peak (inspiratory pressure) was adjusted every hour to keep tidal volume (V_T) at 7 mL/kg. $PaCO_2$ was kept within a range of 35–50 mmHg by variations in rate, and PaO_2 was maintained within 50–150 mmHg by variations in FiO_2 throughout the whole study.

Repeated airway lavage (rBAL, first step of the triple-hit lung injury protocol, Fig. 1) was performed with warmed normal saline until PaO_2/FiO_2 decreased to ~100 mmHg. 24 h later, injurious ventilation (second step) consisted of ventilation with zero-PEEP for 1 h, which was followed by 1 h of ventilation with a doubled V_T (i.e., 14 mL/kg). Another 24 h later (third step), all piglets received a tracheal instillation of 2.5 mg LPS (*Escherichia coli* serotype O127:B8; Sigma–Aldrich, München, Germany).

After baseline measurements the piglets were randomized to one of the following groups:

- control-group (C) received an air bolus;
- surfactant-group (S) received 50 mg/kg of surfactant (poractant alfa, Chiesi, Parma, Italy) at a concentration of 20 mg/mL after dilution with normal saline (i.e. 2.5 mL/kg);
- S + DOPG-group received 7.5 mg DOPG powder (1,2-Dioleoyl-*sn*-glycero-3phospho-*rac*-(1-glycerol) sodium salt, Sigma–Aldrich) solved in 1 mL normal saline and admixed to 50 mg/kg surfactant.

The DOPG dosage was derived from *in vitro* studies with alveolar macrophages using concentrations of 20 μg/mL in the culture dishes [13]. The theoretical DOPG concentration in the airways of the studied piglets after repeated airway lavage was estimated as follows: average weight (2.5 kg) × estimated FRC (~20 mL/kg) = 50 mL; dosage administered 7.5 mg; concentration = 150 μg/mL in the air/fluid phase of the airways. Although Berger et al. [13] could not demonstrate a linear sPLA2-reducing effect when increasing DOPG concentrations from 20 to 30 μg/mL, we thought it necessary to use a higher concentration to overcome potential inhibitors related to airway inflammation.