Selective NF-κB inhibition, but not dexamethasone, decreases acute lung injury in a newborn piglet airway inflammation model

Philipp von Bismarck a, Karsten Klemm a,1, Carlos-Francisco García Wistädt a,1, Supandi Winoto-Morbach b, Stefan Schütze b, Martin F. Krause a,*

a Department of Paediatrics, Universitätsklinikum Schleswig-Holstein, Campus Kiel, Schwanenweg 20, 24105 Kiel, Germany
b Institute of Immunology, Universitätsklinikum Schleswig-Holstein, Campus Kiel, Schwanenweg 20, 24105 Kiel, Germany

A R T I C L E   I N F O
Article history:
Received 22 July 2008
Received in revised form 20 January 2009
Accepted 19 February 2009

Keywords:
Lung inflammation
Nuclear factor-κB
Polymorpho-nuclear leukocytes
Chemokines
Dexamethasone
IKK-NBD peptide
Acute lung injury
Respiratory failure
Newborn piglets

A B S T R A C T
Acute respiratory failure in neonates (e.g. ARDS, meconium aspiration pneumonitis, pneumonia) is characterized by an excessive inflammatory response, governing the migration of polymorpho-nuclear leukocytes (PMNLs) into lung tissue and causing consecutive impairment of gas exchange and lung function. Critical to this inflammatory response is the activation of nuclear factor-κB (NF-κB) that is required for transcription of the genes for many pro-inflammatory mediators. We asked whether the inhibition of NF-κB activity using either a selective inhibitor (IKK-NBD peptide) or dexamethasone would be more effective in decreasing NF-κB activity and chemokine expression in pulmonary cells. Changes in lung function were repeatedly assessed for 24 h following induction of acute respiratory failure and therapeutic intervention.

We conducted a randomized, controlled, prospective animal study with mechanically ventilated newborn piglets which underwent repeated airway lavage (20 mL/kg of balanced saline) to remove surfactant and to induce lung inflammation. Admixed to 100 mg kg−1 surfactant, piglets then received either IKK-NBD peptide (5–IKK), a selective inhibitor of NF-κB activation, its control peptide without intrinsic activity, dexamethasone (5–Dexa), its solvent aqua, or an air bolus only (all groups n = 8). After 24 h of mechanical ventilation, the following differences were measured: PaO2/FiO2 (S-Dexa vs. 0.14 ± 0.01 [SEM]) to remove surfactant and to induce lung inflammation. Admixed to 100 mg kg−1 surfactant, piglets then received either IKK-NBD peptide (5–IKK), a selective inhibitor of NF-κB activation, its control peptide without intrinsic activity, dexamethasone (5–Dexa), its solvent aqua, or an air bolus only (all groups n = 8). After 24 h of mechanical ventilation, the following differences were measured: PaO2/FiO2 (S-Dexa vs. 0.14 ± 0.01 [SEM]) to remove surfactant and to induce lung inflammation. Admixed to 100 mg kg−1 surfactant, piglets then received either IKK-NBD peptide (5–IKK), a selective inhibitor of NF-κB activation, its control peptide without intrinsic activity, dexamethasone (5–Dexa), its solvent aqua, or an air bolus only (all groups n = 8)

1 Resolution of Pulmonary Interstitial Edema in Neonatal Respiratory Distress Syndrome: The Role of Glucocorticoids and NF-κB, Pulmonary Pharmacology & Therapeutics 22 (2009) 297–304

1. Introduction
In neonatal inflammatory lung diseases with respiratory failure such as acute respiratory distress syndrome (ARDS), an increased expression of cytokines, selectins, integrins, and chemokines can be found to cause the invasion of inflammatory cells into lung tissue, which contribute to the degradation of surfactant films to hyaline membranes within the alveoli, and to the formation of a proteinaceous edema within the pulmonary interstitial space [1,2]. Therefore, glucocorticoids have been used to alleviate respiratory failure due to the damage caused by polymorpho-nuclear leukocytes (PMNLs) and alveolar macrophages in their metabolically activated states. They promote surfactant synthesis in type II pneumocytes [3], and inhibit the production of pro-inflammatory mediators through gene expression by activated glucocorticoid receptor binding to negative glucocorticoid response elements in the 5′-flanking region of pro-inflammatory cytokine genes [4], thereby...
depressing the activity of the nuclear factor kappa B (NF-κB), a transcription factor that is required for transcription of the genes for many pro-inflammatory mediators, including adhesion molecules, cytokines, and chemokines [5,6]. However, a recent large clinical trial on adult ARDS patients could not demonstrate a decreased mortality with the use of systemic methylprednisolone in the hyperinflammatory phase [7].

In contrast, animal trials of different types of acute inflammatory lung injury using surfactant [8] or liposomes [9,10] as carrier substances for dexamethasone showed positive results, as the overall glucocorticoid dose related to body weight was reduced and homogenous distribution of the drug within the lung was achieved. The use of intratracheal dexamethasone without a lipophil vehicle homogenous distribution of the drug within the lung was achieved. Overall glucocorticoid dose related to body weight was reduced and

The use of intratracheal dexamethasone without a lipophil vehicle homogenous distribution of the drug within the lung was achieved. Overall glucocorticoid dose related to body weight was reduced and

The aim of this study was to compare the short-term effects of intratracheally administered IKK-NBD peptide (a selective cell-permeable oligopeptide that selectively inhibits the NF-κB activation; see chapter 2.3) with dexamethasone on lung function in a newborn inflammatory model of acute lung injury induced by repeated airway lavage. The drugs were admixed to surfactant that was used as a carrier substance. The rational for this comparison was the assumption that selective NF-κB inhibition might act faster than dexamethasone and would exert its effects within 24 h of mechanical ventilation, then being a reasonable choice of treatment in a setting of acute respiratory failure. The piglets were ventilated for 24 h while gas exchange, lung mechanics, lung volumes, and extravascular lung water (EVLW) were repeatedly determined, followed by ex vivo analyses of BALF for PMNLS and mononuclear cells, chemokine concentrations, and NF-κB activity in pulmonary cells.

2. Materials and methods

2.1. Animal preparation and clinical care

The experimental protocol was approved by the local review board for the care of animal subjects in accordance with the German law on animal protection (BGBI I, page 1319). 40 male piglets (mixed cures) weighing 2.2 ± 0.1 [SEM] kg were studied in pairs of two between days 2 and 5 of age. Initially 0.025 mg kg⁻¹ atropine, 10 mg kg⁻¹ ketamine and 1 mg kg⁻¹ midazolam hydrochloride were administered to allow oral intubation with an uncuffed 3.5 mm endotracheal tube (ETT). Anesthesia and muscle paralysis were maintained by continuous infusion of 16 mg kg⁻¹ propofol and 0.17 mg kg⁻¹ pancuronium bromide per hour. To prevent leakage the tube was tightly secured in place by a peritracheal ligature. A thermodilution catheter was inserted into the left femoral artery for monitoring of hemodynamic parameters and arterial blood gases. The right internal jugular vein was catheterized for infusion therapy and for cold bolus injection for performing thermodilution measurements.

2.2. Mechanical ventilation and airway lavage

Mechanical ventilation was provided by two time-cycled pressure-limited infant ventilators (Babylog 1, Dräger, Germany) at FiO₂ = 0.5, positive end-expiratory pressure (PEEP) = 0.6 kPa, flow = 8.1 min⁻¹, inspiratory time = 0.4 s, ventilator rate = 25 min⁻¹, before lavage and 35 min⁻¹ during the sequence of repeated airway lavage until intervention. Peak inspiratory pressure (PIP) was adjusted every hour to keep tidal volume (V₅₇) at 7 ml kg⁻¹.

Repeated airway lavage was carried out by the instillation and removal of 30 ml kg⁻¹ of warmed normal saline via the ETU over a 30 s period. Airway lavage was then repeated every 3–5 min until both the PaO₂/FiO₂ decreased to ~85 mmHg, and a PIP ≥ 2.0 kPa was required to maintain V₅₇ at 7 ml kg⁻¹. To maintain PaCO₂ within a range of 35–50 mmHg, the ventilator rate was changed according to the following protocol: reduced by ~5 min⁻¹ if PaCO₂ < 35 mmHg; reduced by ~10 min⁻¹ if PaCO₂ < 30 mmHg; increased by ~5 min⁻¹ if PaCO₂ > 50 mmHg; increased by ~10 min⁻¹ if PaCO₂ > 60 mmHg. FiO₂ was adjusted every hour to stay within the limits for PaO₂ of >40–<150 mmHg using the following protocol: FiO₂ increased by 0.1, if PaO₂ < 40 mmHg; FiO₂ reduced by 0.05, if PaO₂ > 150 mmHg. Besides surfactant depletion and iatrogenic pulmonary edema, repeated airway lavage acts as a potent pro-inflammatory stimulus causing the expression of chemokines and the migration of PMNL and mononuclear cells in their metabolically activated state into lung parenchyma, even to an extent that peripheral neutropenia is reached [14].

2.3. Experimental protocol, randomization, surfactant and drugs

Gas exchange parameters (PaO₂ and PaCO₂) were measured every 1–2 h; lung function parameters (functional residual capacity (FRC), dynamic compliance of the respiratory system (Cₕ), alveolar portion of the tidal volume (V₅₇), and tidal volume (V₅₇)) and hemodynamic parameters (notably extravascular lung water (EVLW)) were measured before airway lavage, after airway lavage at baseline and at 1, 6, 12, 18 and 24 h after intervention.

At baseline the animals were randomized in pairs of two (n = 8 per group) to minimize differences in physiology caused by age, weight and litter. Prepared combinations for randomization were, as defined below: either piglet 1 S–IKK/piglet 2 S–CP, or piglet 1 S–CP/piglet 2 S–IKK, or piglet 1 S–Dexa/piglet 2 S–Aqu, or piglet 1 S–Aqu/piglet 2 S–Dexa, or piglet 1 C/piglet 2 C.

The surfactant preparation HL-10 (Halas Pharma BV, Rotterdam, The Netherlands) was administered at a dose of 100 mg kg⁻¹ at 2.5 ml kg⁻¹. It is a freeze-dried surfactant isolated from minced porcine lungs and consists of approximately 90% phospholipids, 0.6% surfactant protein B, and 0.4% surfactant protein C.

In the S–IKK group (group 1), 1.25 mg per piglet (i.e. 568 µg kg⁻¹ as an average) of a selective NF-κB inhibitor (IKK-NBD peptide, Biomol, Hamburg, Germany) was dissolved in 50 µl DMSO 0.125% and was added to the surfactant preparation by gentle stirring. IKK-NBD peptide is a selective cell-permeable inhibitor of NF-κB activation. It blocks the interaction of NEMO (NF-κB Essential Modulator, an IkB complex regulatory protein) with the IkB kinase complex. The synthesis of the binding molecule IkBα (IκB) is the key event in stopping NF-κB activity, however, the rate-limiting step in activation of NF-κB appears to lie in the activity of IKK [15]. IKK-NBD peptide (NBD stands for NEMO Binding Domain) is an inhibitor of NF-κB activation which selectively blocks the interaction of NEMO with the IKK complex [16]. The peptide consists of 28 amino acids and forms two domains, an antenapedia homeodomain, which confers cell permeability, and the T735 to E745 region of IKKβ, which is the NEMO binding domain (NBD) [17].

The S–CP group (group 2) piglets were treated with surfactant and an IKK-NBD control peptide (Biomol) which is structurally similar but rendered biologically inactive by replacing tryptophan with alanine in positions 22 and 24.

Dexamethasone was purchased from Sigma–Aldrich (München, Germany). 0.25 mg per piglet (i.e. 108 µg kg⁻¹ as an average) was dissolved in 1 ml aqua and mixed with the surfactant preparation (S–Dexa, group 3). Dexamethasone inhibits NF-κB translocation, but also plays an integral role in stimulating surfactant synthesis.
through activation of the rate-regulatory enzyme for phosphatidylycholine synthesis: the cholinephosphate cytidylyltransferase [18,3]. S-Aqua group (group 4) piglets received 1 ml aqua mixed with surfactant, as a control. Another control group (C, group 5) received 2.5 ml kg⁻¹ air into a second lumen of the ETT.

Surface tension-increasing properties of IKK-NBD peptide and dexamethasone as well as their solvents DMSO and aqua at their defined doses and dissolved in surfactant could be excluded by minimum and maximum surface tension measurements of 100 μl of the surfactant preparations on the saline filled trough of a modified Wilhelmy balance (E. Biegler, Mauerbach, Austria), as previously described in Ref. [19] (data not given).

### 2.4. Measurement of gas exchange, haemodynamics and lung function

\[ \text{PaO}_2 \] and \[ \text{PaCO}_2 \] were measured in blood samples taken from the femoral arterial catheter. \[ \text{PaCO}_2 \] was used for calculating a ventilation efficiency index \( (\text{VEI} = \frac{3800}{(\text{PIP} - \text{PEEP}) \cdot \text{f} \cdot \text{PaCO}_2}) \) according to Notter et al. [20].

Cardiac output monitoring (PC 8000 PiCCO monitor, Pulsion, München, Germany) was performed using the transpulmonary indicator dilution technique (cold bolus of 2 ml normal saline \(-8^\circ \text{C}\), as previously described in Ref. [21].

To calculate FRC, \( C_t, V_a \), and \( V_t \), we used the nitrogen washout technique for lung volumes and the single breath least squares method for lung mechanics, as previously described in Ref. [22].

### 2.5. Broncho-alveolar lavage fluid (BALF) and blood cell counts

Two lavages were performed for diagnostic purposes: the initial lavage in the sequence of lavages to establish lung injury, and a lavage after 24 h of mechanical ventilation immediately before killing the piglets. BALF was filtered to remove gross particles and secretions from the airways, and was centrifuged for 4 min at 4°C and 2500 rev min⁻¹ to separate cells and cellular debris from the surfactant and lavage fluid.

### 2.6. Clinical care

A heating pad was used to maintain a constant core temperature in the piglets of 38–39°C. Each piglet received an infusion of D5W in 1/2 normal saline at a fluid intake of 80 ml kg⁻¹ d⁻¹. Two hours after intervention, 50 mg kg⁻¹ of Ampicillin/Sulbactam was given intravenously to prevent bacterial infections.

### 2.7. Interleukin-8 (IL-8) and leukotriene B₄ (LTB₄) assays

IL-8 concentrations in BALF were determined using the QuantiTrol® porcine IL-8/CXCL8 immunoassay (R&D Systems, Wiesbaden, Germany). LTB₄ concentrations were also measured by immunoassay (R&D Systems) which is based on the competition between LTB₄ and the LTB₄-alkaline phosphate conjugate for a limited amount of LTB₄ antisemur.

### 2.8. Ex vivo lung processing

After killing the piglets with an overdose of 1-m KCl in deep sedation, the lungs were removed from the thorax, and all the blood was cleansed from the pulmonary circulation by infusing normal saline into the main pulmonary artery. Small specimens of tissue from a ventral (non-dependent) position of the left lower lobe were taken to determine wet–dry-ratios by drying overnight at 60°C to remove any fluid.

### 2.9. NF-κB activity in pulmonary cells

#### 2.9.1. Preparation of cytosolic and nuclear extracts

Frozen lung tissue samples were pulverized and melted. The pulverized material was incubated in 10 mM TRIS, 5 mM MgCl₂, 10 mM KCl, 1 mM EGTA, and 10% Sucrose for 30 min at pH 7.4 and 4°C. Then, 10% NP-40 was added (Vol. 10/1). Samples were centrifuged for 1 min at 4°C and 16.000 × g, and 20 μg of supernatant protein was subjected to Western blot analysis of cytosolic proteins. To obtain nuclear extracts, the pellets were resuspended in 20 mM TRIS, 5 mM MgCl₂, 320 mM KCl, 0.2 mM EGTA, and 25% Glycerol and incubated at pH 7.4 for 15 min at 4°C for nuclear lysis. Samples were centrifuged for 5 min at 4°C and 16.000 × g, and 20 μg supernatant protein was analyzed by Western blot for nuclear proteins.

#### 2.9.2. Western blot analysis

Equal amounts of protein were separated on 12.5% SDS-PAGE and electroblotted onto nitrocellulose membranes for 1 h at 100 V and 4°C. Blots were blocked in 5% nonfat dry milk in TBS containing 0.1% Tween 20 (TBST) for 1 h at room temperature and probed with anti NF-κB p65 p105/p50 (AB # 3035, Cell Signaling Technology, Boston, USA), in the same buffer. After washing three times with TBST, blots were incubated with anti-rabbit IgG, HRP-linked as secondary antibodies (AB #7074, Cell Signaling Technology) for 1 h at room temperature. Protein bands were visualized by enhanced chemiluminescence using Super Signal (GE Healthcare, München, Germany).

### 2.10. Statistical methods

We used a one-way analysis of variance to assess differences between the five groups for parameters measured at a single time point. Repeated measures two-way analysis of variance (ANOVA) was used to assess differences between groups in changes over time, thereby separating the first (1–12 h) and the second (12–24 h) half of the experimental period. An unpaired \( t \)-test was used for direct comparisons of S-IKK vs. S-Dexa.

Mean values are expressed ±SEM. Differences in values of \( p < 0.05 \) were considered to be significant.

### 3. Results

#### 3.1. Comparability of study groups

A total of 40 piglets weighing 2.2 ± 0.1 kg was studied in pairs of two according to a randomization protocol after airway lavage. One piglet was excluded from the C group because of insufficient surfactant removal from the airways due to a left bronchial dynamic stenosis. There were no significant differences between the five groups with regard to body weight, the number of lavages performed, loss of lavage fluid in the airways, final \( \text{PaO}_2/\text{FiO}_2 \) levels (Fig. 1) were superior in the S-IKK group (when compared to S-Dexa) only in the second half of the observational period (group difference \( p < 0.05 \), time factor \( p = \text{ns} \)); at 24 h the following results were obtained: S-IKK 230 ± 9 mmHg vs. S-Dexa 188 ± 14, \( p < 0.05 \) (unpaired \( t \)-test). VEI (Fig. 2) differed between the two intervention groups within both time periods (1–12 h: group difference \( p < 0.01 \), time factor \( p < 0.001 \); 12–24 h: group difference \( p < 0.001 \), time factor \( p = \text{ns} \)). At 24 h the following
results were obtained: S+IKK 0.18 ± 0.01 [3800/(PIP-PEEP) ÷ f × PaCO2] vs. S+Dexa 0.14 ± 0.01, p < 0.05.

Table 2 shows baseline, 1, 12, and 24 h data for lung function parameters (FRC, CP, and V̇A) and EVLW. While there were no statistical differences between the five groups at baseline (one-way ANOVA), at 24 h the following differences were observed: FRC between the five groups at baseline (one-way ANOVA); direct comparison of the two intervention groups, however, showed the following differences: S+IKK 23 ± 7 pg ml⁻¹ vs. S+Dexa 71 ± 11, p < 0.01.

3.4. Chemokines in BALF

IL-8 concentrations (Fig. 4) at 24 h differed significantly between the five groups (p < 0.01, one-way ANOVA); but comparison of the two intervention groups was not significant: S+IKK 351 ± 117 pg ml⁻¹ vs. S+Dexa 491 ± 144 (unpaired t-test). In contrast, as treatment differences of LTB4 concentrations were not significant (p = ns), comparison of the two intervention groups, however, showed the following differences: S+IKK 23 ± 7 pg ml⁻¹ vs. S+Dexa 14 ± 2, S+Aqua 15 ± 2, C 10 ± 1.

3.5. Wet lung weight/dry lung weight ratio

Wet-dry-ratios from small lung specimens were: S+IKK 7.6 ± 0.2, S+CP 8.4 ± 0.2, S+Dexa 8.7 ± 0.2, S+Aqua 8.7 ± 0.4, C 9.2 ± 0.2 (p < 0.05, one-way ANOVA; S+IKK vs. S+Dexa p < 0.01, unpaired t-test).

3.6. NF-κB activity in pulmonary cells

NF-κB activity in the nucleus and cytosol of pulmonary cells differed significantly (p < 0.001; one-way ANOVA; Fig. 5); direct

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical parameters at baseline.</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
<tr>
<td>Lavages (n)</td>
</tr>
<tr>
<td>Saline lost in Airways (%)</td>
</tr>
<tr>
<td>PaO₂/FiO₂ (mmHg)</td>
</tr>
<tr>
<td>PIP (kPa)</td>
</tr>
</tbody>
</table>

Treatment groups: surfactant + IKK-NBD peptide (S+IKK); surfactant + IKK-NBD control peptide (S+CP); surfactant + dexamethasone (S+Dexa); surfactant + aqua (S+Aqua); control (C). Mean ± SEM.

Saline lost in Airways denotes the percentage of lavage fluid that was not recovered from the airways.
after 24h

C

rs (ml kg

C6

Mean

C6

SEM. Comparisons of all 5 groups (treat, overall) and of S+IKK vs. S+Dexa by repeated measurement analysis, describing differences between hours 1–2 and 12–24 (treatment effect/time influence).

Unpaired t-test at 24 h comparing S+IKK vs. S+Dexa: p < 0.05.

| Treatment groups: surfactant + IKK-NBD peptide (S+IKK); surfactant + IKK-NBD control peptide (S+CP); surfactant + dexamethasone (S+Dexa); surfactant + aqua (S+Aqua); control (C). FRC = functional residual capacity; Crs = dynamic compliance of the respiratory system; V̅A = alveolar portion of tidal volume; EVLW = extra-vascular lung water. Mean ± SEM. Comparisons of the 5 groups at baseline: not significant (ns). Comparisons of all 5 groups (treat, overall) and of S+IKK vs. S+Dexa by repeated measurement analysis, describing differences between hours 1–2 and 12–24 (treatment effect/time influence). |

<table>
<thead>
<tr>
<th>Table 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood leukocytes after 24 h.</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
| Total cell count (cells μl

1

) | 7163 ± 766 | 8086 ± 676 | 7488 ± 702 | 8443 ± 1796 | 11,207 ± 1655 | ns | ns |
| Bands (%) | 3.5 ± 0.9 | 7.4 ± 2.6 | 6.6 ± 0.8 | 8.0 ± 2.2 | 8.4 ± 1.7 | ns | 0.03 |
| PMNL (%) | 55 ± 4 | 54 ± 4 | 59 ± 4 | 56 ± 3 | 53 ± 5 | ns | ns |

Treatment groups: surfactant + IKK-NBD peptide (S+IKK); surfactant + IKK-NBD control peptide (S+CP); surfactant + dexamethasone (S+Dexa); surfactant + aqua (S+Aqua); control (C). Mean ± SEM. Comparisons of all 5 groups (treat, overall) by one-way ANOVA, and of S+IKK vs. S+Dexa by unpaired t-test.

comparison of the two intervention groups yielded the following results: nucleus S+IKK 32 ± 5%, S+Dexa 55 ± 3% (p < 0.01, unpaired t-test); cytosol S+IKK 205 ± 2%, S+Dexa 170 ± 2, p = ns.

Fig. 3. Total cell count in broncho-alveolar lavage fluid (BALF) of the intact lung and after 24 h of mechanical ventilation. Data are mean ± SEM. One-way ANOVA for comparison of all 5 groups, unpaired t-test for comparing S+IKK vs. S+Dexa.

Fig. 4. Interleukin-8 (IL-8) and leukotriene B4 (LTB4) in broncho-alveolar lavage fluid (BALF) after 24 h of mechanical ventilation. Data are mean ± SEM. Overall comparisons of all 5 groups by one-way ANOVA, comparison of S+IKK vs. S+Dexa by unpaired t-test.
4. Discussion

The encouraging results of a previous study with IKK-NBD peptide [23] – notably reductions in EVLW and in PMNL concentration in BALF –, prompted us to compare the immunosuppressive short-term effects of IKK-NBD peptide with those of dexamethasone in a similar model, but with an even higher degree of lung edema (average EVLW for all piglets at baseline: 51±4 ml kg⁻¹ vs. 42±5 in the previous study) and for a much longer observation period (24 h vs. 6 h). IKK-NBD peptide admixed to a porcine surfactant preparation and compared to the effects of dexamethasone in surfactant resulted in significantly improved gas exchange and lung function, probably due to lower chemokine liberation and a decreased PMNL migration into the lung. Taking into account the time slope of our study, clinical improvements using IKK-NBD treatment did not occur before 12 h (exceptions: VEI, C₃a, Vₐ̄) after its administration, while most of the dexamethasone effects was equal to the effects of surfactant treatment alone. Most importantly, reduction in NF-κB activity in the nucleus of pulmonary cells was convincingly low in the S+IKK group and differed significantly from the values obtained in S+Dexa group pulmonary cells.

Two editorials [24,25] have proposed the suppression of inflammatory activity during the hyperinflammatory phase of acute lung injury by targeting NF-κB. NF-κB seems to be a logical choice for investigation, as it is at the center of the signal transduction network and governs the expression of pro-inflammatory cytokines and chemokines and of pro-apoptotic factors. This is even more the case in neonatal pulmonary diseases, because studies with newborn PMNL vs. adult PMNL showed that, e.g. TNF-α induced NF-κB activation was even more pronounced in newborn PMNL [25], which underlines the significance of this pro-inflammatory pathway in newborn organisms.

The most obvious explanation for improvements in lung function in our present study is the suppression of the chemokines IL-8 and LTB₄ (Fig. 4) followed by decreased cell migration into the lung (Fig. 3). Other authors found that after LPS stimulus, IKK-NBD peptide counteracts reduced expression of G-protein-coupled receptor kinase by chemokine receptor desensitization and lowers PMNL migratory response [26]. It also prevents the increased toll-like receptor2 mRNA expression after LPS challenge [27]. In a mouse model, cyclooxygenase-2 levels and expression of TNF-α were reduced at a dose of 500 μg per mouse (i.e. ~20 mg kg⁻¹) together with a reduction in carrageenan induced paw edema [28]. IKK-NBD peptiides from other manufacturers were shown to prevent LPS-induced CD11b protein expression and PMNL adhesion [29]. These experiments differ from ours, as IKK-NBD peptide administrations were carried out in adult animals and via the systemic route. The attenuation of PMNL migration into lung tissue is probably more effective using the systemic approach; however, systemic side effects might also occur more often in the delicate neonatal organism.

Dexamethasone, however, did not exert any improvements in PaO₂/FiO₂, VEI or lung inflammation, when compared to the administration of Surfactant alone. This is explained by the fact, that nuclear NF-κB activity was not suppressed after 24 h. As there are no clinical trials on treatment of newborn or pediatric ARDS with corticosteroids, adult animal models with intratracheal administration must be taken as surrogates: different authors have described attenuation of PMNL concentrations in BALF in an oleic-acid rat model [13,12]; mild improvements in gas exchange and plasma malonyl dialdehyde in a hydrochloric acid rabbit model [11]; and improvements in lung compliance in an oleic-acid rat model [12].

Taken together, lung function was better preserved by IKK-NBD peptide than by dexamethasone as expressed by significant improvements in PaO₂/FiO₂ and VEI. A requisite for this “surfactant fortifying” effect is decreased PMNL migration into lung parenchyma [30] and attenuation of alveolar macrophages [31] (not tested in this study), and capillary leakage and transmission of large molecule peptides into lung tissue [32]. The approach of making exogenous surfactant less vulnerable to inflammatory attack within the airways was successfully tried with substances that share the property of binding water, such as, e.g. hyaluronan in a rat model of meconium injury [33], or in vitro by plamalogens, an alk-1-etyl-acyl subclass of phosphatidylethanolamine and phosphatidylcholine [34].

4.1. Limitations

4.1.1. Dosage

The IKK-NBD peptide dose of 568 μg kg⁻¹ dissolved in surfactant was derived from our previous experience in a similar model [23] when an average dose of 666 μg kg⁻¹ was chosen. May et al. [35] used a much larger dose of 200 μg per mouse (average body weight 25 g, i.e. approximately 8000 μg kg⁻¹) intraperitoneally to treat mechanical ear edema and zymosan-induced peritonitis. Lower doses may be equally effective, but a dose-response relationship has not yet been established. The average dexamethasone dose of 113 μg kg⁻¹ was adapted from clinical practice in the 80th and 90th with preterm infants developing BPD [36,37] and single studies with term infants receiving dexamethasone for inflammatory lung...
1.4.2. Kinetics

There is little knowledge about how long the anti-inflammatory effect of IKK-NBD peptide lasts when given in a single dose. In contrast, in an adult rat model, free intratracheal dexamethasone peaks at 1 h after its administration within the lung parenchyma while this peak is delayed to 4 h by using liposomes as a vehicle [10]. 24 h recovery of the drug is close to 0% of the initial dose given. This finding explains the observation of improved lung function in chronic lung disease of prematurity not before several doses of dexamethasone over a 72 h interval [40].

4.3. Drug interactions

Intermittent or permanent binding of some IKK-NBD peptide or its control peptide (both small molecules composed of 28 amino acids, molecular weight 3694 and 3464, respectively) or of dexamethasone (molecular weight 516) with surfactant components cannot be excluded. However, Nimmo et al. [8] proved unchanged surface tensions in films with dexamethasone admixed to the artificial surfactant Survanta®.

4.4. Solvents

As the IKK-NBD peptide/control peptide solvent DMSO (average dose 0.04 mg kg$^{-1}$) might induce NF-$\kappa$B activation [41] in pulmonary cells, we thought it important to describe a second control group with aqua only (the solvent of dexamethasone) to prove absent intrinsic effects of DMSO in our model (S-CP group).

5. Conclusion

Admixture of IKK-NBD peptide, but not dexamethasone, to surfactant improved gas exchange, lung function, and pulmonary edema by reducing chemokine generation and PMNL migration into the lung. Given as a single intratracheal dose, the effects of selective NF-$\kappa$B inhibition on surfactant “fortification” were obvious after approximately 12 h and lasted for the whole observational period of 24 h of mechanical ventilation. Contrary to the effect on smooth muscle relaxation by topical administration within several hours [42], dexamethasone failed to exert any significant clinical improvements in this neonatal animal model of respiratory failure. Our findings suggest that “fortification” of exogenous surfactant by selectively inhibiting NF-$\kappa$B might be a valuable therapeutic alternative to glucocorticoid treatment in the treatment of severe neonatal inflammatory lung disease.

Acknowledgements

Supported by a grant from Fritz-Bender-Stiftung, München, Germany, and by intramural funding (Dr. Krause). The surfactant preparation HL-10 was generously provided by Halas Pharma BV, Rotterdam, The Netherlands.

References


