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Selective Inhibition of NF-κB and Surfactant Therapy in Experimental Meconium-Induced Lung Injury

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Summary

Meconium aspiration syndrome (MAS) in newborns is characterized mainly by respiratory failure due to surfactant dysfunction and inflammation. Previous meta-analyses did not prove any effect of exogenous surfactant treatment nor glucocorticoid administration on final outcome of children with MAS despite oxygenation improvement. As we supposed there is the need to intervene in both these fields simultaneously, we evaluated therapeutic effect of combination of exogenous surfactant and selective inhibitor of NF-κB (IKK-NBD peptide). Young New Zealand rabbits were instilled by meconium suspension and treated by surfactant alone or surfactant in combination with IKK-NBD, and oxygen-ventilated for 5 h. PaO_2/FiO_2, oxygenation index, oxygen saturation and ventilation efficiency index were evaluated every hour; *post mortem*, total and differential leukocyte counts were investigated in bronchoalveolar lavage fluid (BALF) and inflammatory, oxidative and apoptotic markers were assessed in lung tissue homogenates. Exogenous surfactant combined with IKK-NBD improved oxygenation, reduced neutrophil count in BALF and levels of IL-1β, IL-6, p38 MAPK and caspase 3 in comparison with surfactant-only therapy. It seems that inhibition of inflammation may be strong supporting factor in surfactant treatment of MAS.

Key words

Meconium-induced lung injury • Surfactant treatment • NF-κB • Inflammation • IKK-NBD

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Introduction

Meconium-induced lung injury in newborns is widely associated with pulmonary inflammation. Meconium-stained amniotic fluid contains cytokines capable of triggering immune response (de Beaufort *et al.* 2003). Complement and toll-like receptors (TLR) 4 activation with subsequent cytokine production had been found *in vitro* in the presence of meconium (Salvesen *et al.* 2009, Salvesen *et al.* 2010). *In vivo*, polymorphonuclear activation, free radicals production, cytokine synthesis, phospholipase A_2-mediated tissue damage, cell apoptosis and edema formation accompanied main feature of this disease – surfactant dysfunction (Vydiasagar and Zagaryia 2008, Mikolka *et al.* 2013, Kopincová *et al.* 2014). What is clinically manifested as serious respiratory failure with the need of exogenous surfactant and ventilatory support, is on the molecular level largely result of many interrelated signaling pathways streaming through activation of nuclear factor κB (NF-κB; reviewed in Kopincova and Calkovska 2016). Since all these inflammatory processes are able to impair administered exogenous surfactant and mitigate the treatment efficacy, inhibition of inflammation is one of the targets of current therapeutic interventions.

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Selective NF-κB inhibition by IKKγ NEMO Binding Domain (IKK-NBD) inhibitory peptide as an experimental pharmacological tool has been the matter of the last decade. IKK-NBD is cell-permeable low molecular peptide which binds to the regulatory NEMO (NF-κB Essential Modulator) subunit of IKK (IκB kinase) complex, thus preventing IκBα (Inhibitor of κB α) protein phosphorylation and related NF-κB activation (von Bismarck *et al.* 2012). Recently, its *in vivo* administration has been examined in connection with various disorders as from rat cerebral ischemia-reperfusion injury model (Desai *et al.* 2010) through murine Duchenne muscular dystrophy (Reay *et al.* 2011) to trial in dogs with Diffuse large B-Cell lymphoma (Habineza Ndikuyeze *et al.* 2014). In respiratory tract, IKK-NBD had favorable effect in the treatment of LPS-induced pulmonary inflammation in mice (von Bismarck *et al.* 2012) and surfactant "fortifier" in lavage model of ARDS in newborn piglets (Ankermann *et al*. 2005, von Bismarck *et al.* 2007, von Bismarck *et al.* 2009). Considering that meconium aspiration syndrome (MAS) unites main features of both these conditions, surfactant dysfunction and TLR4-mediated inflammation, we were interested if IKK-NBD "upgrade" of surfactant therapy would have an additional benefit in the treatment of experimental MAS.

Methods

General design of experiments

Design of experiments was approved by the local Ethics Committee of Jessenius Faculty of Medicine, Comenius University, and National Veterinary Board. It had been published in details previously (Kopincová *et al.* 2014).

For this series of experiments, we used 21 young New Zealand white rabbits (Velaz s.r.o., Czech Republic) of both genders with body weight (b.w.) of 2.1±0.1 kg. After all previously described entry procedures (anesthesia and surgery), animals were stabilized for 15 min on artificial ventilation with frequency of 30/min, fraction of inspired oxygen (FiO_2) of 0.21, inspiration time Ti 50 %, peak inspiratory pressure (PIP) to keep a tidal volume (V_T) between 7-9 ml/kg b.w. and no positive end-expiratory pressure (PEEP). Then, arterial blood samples were obtained for basal values evaluation including blood gases (PaO_2, $PaCO_2$) and oxygen saturation ($SatO_2$) by RapidLab 348 (Siemens, Germany).

Meconium instillation

Rabbits were administered meconium suspension (25 mg/ml) in a dose of 4 ml/kg b.w. divided into two equal portions while the animals were positioned to the right and left. Following meconium administration, PEEP was adjusted to 0.25-0.3 kPa and FiO_2 to 1.0. Within 30 min respiratory failure occurred, manifested by decrease in dynamic lung-thorax compliance for more than 30 % and PaO_2<10 kPa. 30 min after meconium, all above-mentioned parameters were recorded.

Treatment protocol

Thirty minutes after meconium administration, the animals were randomly divided into three groups: 1) meconium without treatment (Mec group, n=7); 2) meconium with surfactant-only treatment (Surf group, n=7); 3) meconium with combined surfactant and IKKγ NEMO Binding Domain Inhibitory peptide (IKK-NBD) treatment (Surf+IKK-NBD group, n=7).

In surfactant-treated animals, modified porcine surfactant (Curosurf®, Chiesi Farmaceutici, Italy; 80 mg phospholipids (PL)/ml was administered in two-step procedure as described in details previously (Mikolka *et al.* 2013, Kopincová *et al.* 2014). In the first step, lung lavage with Curosurf diluted by saline (37 °C) at PL concentration of 5 mg/ml and volume of 10 ml/kg b.w. had been performed twice and was followed by undiluted Curosurf bolus at a dose of 100 mg PL/kg, 1.25 ml/kg b.w. using asymmetric high-frequency jet ventilation (f. 300/min, Ti 20 %, PIP/PEEP 1.5/0.3 kPa). In Surf+IKK-NBD group, 500 μg of IKK-NBD/kg b.w. (AnaSpec, Inc., CA, USA) was dissolved in 0.5 ml of 0.125 % dimethyl sulphoxide (DMSO) and added to surfactant bolus, according to von Bismarck *et al.* (2009).

Animals were oxygen-ventilated for following 5 h. At 30 min, 1, 2, 3, 4, and 5 h after the treatment, blood gases and respiratory parameters were recorded. At the end of experiments, animals were sacrificed by an overdose of anesthetics.

Measurement of lung functions parameters

Tracheal airflow was measured by a heated Fleisch head connected to a pneumotachograph. Airway pressure was registered *via* a pneumatic catheter placed in the tracheal tube and connected to electromanometer. Mean airway pressure (MAP) was calculated as:

MAP=(PIP+PEEP)/2.

Oxygenation index (OI) was calculated as:

OI = (MAPxFiO_2)/PaO_2);

Ventilation efficiency index (VEI) as:

VEI = 3800/[(PIP-PEEP] x frequency x PaCO_2.

Bronchoalveolar lavage fluid cells evaluation

Immediately after overdose of anesthetics, lungs and trachea were excised. Left lungs were lavaged by saline (0.9 % NaCl, 37 °C) 3x10 ml/kg and bronchoalveolar lavage fluid (BALF) was centrifuged at 1500 rpm for 10 min. Total number and differential count of leukocytes in BALF was determined microscopically.

Biochemical analyses in lung tissue homogenates

For biochemical evaluations, strips of the right lung tissue were homogenized in ice-cold PBS (0.02 mol/l, pH 7.2) for final concentration 10 % (weight/volume). Homogenates were subjected to two freeze-thaw cycles and centrifuged for 15 min at 1500 g. Then, supernatants were removed and analyzed.

For cytokine evaluation, ELISA kits for Rabbit IL-1β and IL-6 (USCN Life Science, Wuhan, China) were used according to the manufacturer's instructions. Data were expressed as pg/ml of homogenate.

Apoptotic markers were evaluated by p38 MAPK alpha ELISA Kit (Abcam, Cambridge, UK) and Rabbit Caspase 3 ELISA Kit (Cusabio, Wuhan, China) according to the manufacturer's instructions. Data were expressed as ng/ml of homogenate.

For evaluation of oxidative markers, OxiSelect TBARS Assay Kit (Cell Biolabs Inc., San Diego, USA) was used for malondyaldehyde/thiobarbituric reactive substances (TBARS; product of lipid peroxidation). Data were expressed as μmol/ml of homogenate. Total antioxidant capacity (TAC) was assessed using TAC assay (Abcam, Cambridge, UK) and data were expressed as Trolox equivalent per ml of homogenate.

Statistical analyses of results

Statistical analyses were performed by STATISTICA (StatSoft, Inc.; Czech Republic). Two-way analysis of variance (ANOVA) with Duncan *post hoc* test was used for PaO_2/FiO_2, OI, VEI (grouping factors "group" and "time"). Non-parametric analysis (Kruskal-Wallis ANOVA test) was used for comparison of total and differential leukocyte count in BALF, cytokine levels, p38 mitogen-activated protein kinase (MAPK), caspase 3, TBARS and TAC in homogenates of lung tissue. A value of $p<0.05$ was considered to be statistically significant. Numeric values are expressed as mean ± standard error of mean (SEM).

Results

The entry parameters (body weight, gender) as well as respiratory parameters did not differ before meconium instillation between groups.

Recovery of the therapeutic bronchoalveolar lavage (BAL) fluid

Therapeutic bronchoalveolar lavage in both surfactant-treated groups (Surf and Surf+IKK-NBD) was performed by 10 ml/kg of diluted exogenous surfactant. The recovery of therapeutic BAL fluid was similar in both groups (70.3±2.1 % for Surf group and 76.0±3.5 % for Surf+IKK-NBD group, $p=0.092$, Mann-Whitney U test).

Effect of therapy on the lung functions

PaO_2/FiO_2, VEI, SatO_2 were decreased and OI was increased in 30 min after meconium administration similarly in all experimental groups ($p<0.001$ vs. basal values; between-group differences were non-significant).

The administration of surfactant led to transient improvement in PaO_2/FiO_2 in the second hour of therapy ($p<0.05$ vs. Mec; Fig. 1A); however, when mean airway pressure was taken into account (parameter OI), the effectivity of surfactant monotherapy was significant from the first 30 min after administration and this improvement lasted until the end of experiment ($p<0.001$ vs. Mec; Fig. 1B). Oxygen saturation was improved from the first hour of the treatment to the end ($p<0.01$-0.001 vs. Mec; Fig. 1D). On the other hand, improvement in VEI became evident only in the fifth hour of experiment ($p<0.01$ vs. Mec; Fig. 1C).

In comparison with monotherapy, the combination of surfactant treatment and IKK-NBD brought benefit in all measured respiratory parameters. Foremost, the increase in PaO_2/FiO_2 and VEI occurred already in the first 30 min after administration and were significant not only in comparison with untreated group but also with surfactant-treated group ($p<0.01$ to 0.001 vs. Mec, $p<0.05$ vs. Surf; Fig. 1A, C). This treatment effect persisted for the whole experiment (Fig. 1A, B, C, D) and at the end exceeded the effect of surfactant monotherapy ($p<0.05$ vs. Surf; Fig. 1A, B).

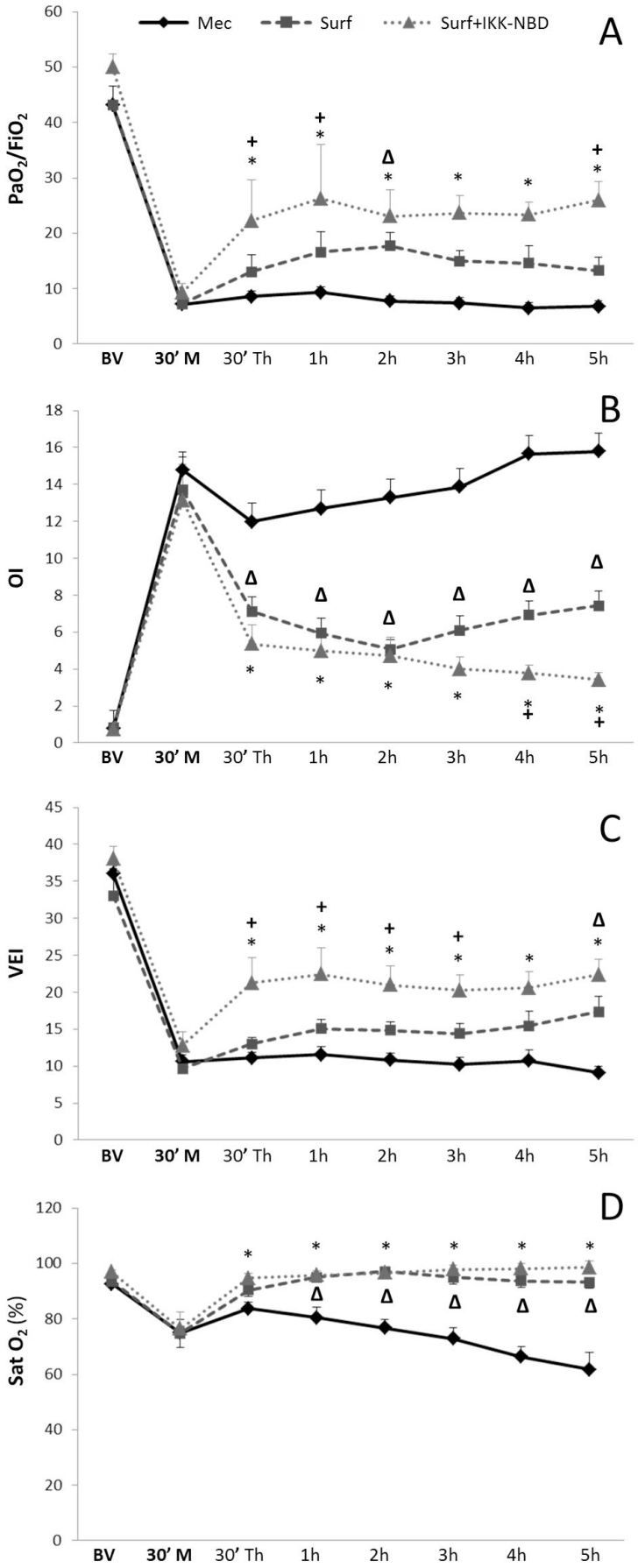

Fig. 1. PaO_2/FiO_2 (**A**), oxygenation index (**B**), ventilation efficiency index (**C**) and oxygen saturation (**D**) before meconium administration (BV), 30 min (30') after meconium (M) and therapy (Th) administration during 5 h of experiment. Mec – untreated group; Surf – surfactant-only treated group; Surf+IKK-NBD – surfactant and IKK-NBD-treated group; $^{\Delta}$ $p<0.05$ to 0.001 for Surf vs. Mec; * $p<0.05$ to 0.001 for Surf+IKK-NBD vs. Mec; $^{+}$ $p<0.05$ to 0.01 for Surf+IKK-NBD vs. Surf.

Effect of therapy on leukocyte sequestration into lungs

Post mortem, lavage of left lung was performed, using 3x10 ml/kg of saline. Recovery of BALF did not differ between groups (73.2±5.5 % for Mec; 76.6±3.2 % for Surf; 78.2±5.8 % for Surf+IKK-NBD, all p>0.05).

Meconium instillation led to massive influx of leukocytes into the lungs with total count up to 250±72 x 10^7 in ml of BALF. Both treatments decreased the number of leukocytes significantly (p<0.01 for Surf and Surf+IKK-NBD vs. Mec); however, differential leukocyte count was significantly shifted only when combined therapy was used (Table 1). Addition of IKK-NBD to surfactant prevented neutrophil sequestration into the lungs, thus the highest portion of white blood cells stands for monocytes/macrophages (p<0.01 vs. Mec). Relative counts of leukocytes and eosinophils were also decreased in Surf+IKK-NBD-treated group when compared with untreated group (p<0.01 for leukocytes and p<0.05 for eosinophils vs. Mec; Table 1).

Tab. 1. Total and differential leukocyte count in bronchoalveolar lavage fluid (basophil count not shown due to very rare occurrence).

	Total count (x10^7/ml)	**Neutrophils** (%)	**Monocytes/ Macrophages** (%)	**Lymphocytes** (%)	**Eosinophils** (%)
Mec	250.3±72.0	51.1±5.2	43.6±5.4	1.7±0.3	3.5±0.7
Surf	49.4±6.7[a]	47.8±7.5	49.2±7.5	1.2±0.2	1.8±0.4
Surf+IKK-NBD	51.3±8.3[a]	22.8±7.2[a]	75.4±7.4[a]	0.4±0.1[ac]	1.4±0.3[b]

Mec – untreated group; Surf – Surfactant-only treated group; Surf+IKK-NBD – Surfactant and IKK-NBD treated group; [a] p<0.01 vs. Mec; [b] p<0.05 vs. Mec; [c] p<0.05 vs. Surf.

Effect of therapy on lung tissue inflammation

Cytokine levels

Surfactant administration only led to non-significant (IL-1β) or completely no change (IL-6) in cytokine levels (Table 2). Combination of surfactant and IKK-NBD prevented meconium-induced interleukin (IL)-1β and IL-6 formation in lung tissue (p<0.01 for IL-1β and p<0.05 for IL-6 vs. Mec). Moreover, the decrease in IL-6 was pronounced in comparison with surfactant-only treated animals (p<0.05; Table 2).

Tab. 2. Levels of interleukin (IL)-1β, IL-6, p38 Mitogen-activated protein kinase (p38 MAPK), Caspase 3, Thiobarbituric acid reactive substances (TBARS) and Total antioxidant capacity (TAC) in lung tissue homogenates.

	IL-1β (pg/ml)	**IL-6** (pg/ml)	**p38 MAPK** (ng/ml)	**Caspase 3** (ng/ml)	**TBARS** (μmol/ml)	**TAC** (TE/ml)
Mec	332.2±19.7	4.7±0.5	78.1±6.6	5.2±0.6	30.3±3.4	14.0±0.7
Surf	272.8±32.5	4.5±0.8	81.6±3.9	5.7±0.4	21.9±1.5	16.7±0.7
Surf+IKK-NBD	166.8±8.7[a]	2.2±0.4[bd]	57.1±3.0[ac]	3.5±0.4[bd]	23.4±8.5	17.1±0.9[x]

Mec – untreated group; Surf – Surfactant-only treated group; Surf+IKK-NBD – Surfactant and IKK-NBD treated group; TE – Trolox equivalent; [a] p<0.01 vs. Mec; [b] p<0.05 vs. Mec; [c] p<0.01 vs. Surf; [d] p<0.05 vs. Surf; [x] p=0.07 vs. Mec.

Cytokine levels

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Apoptotic markers

Apoptotic signalization was diminished after IKK-NBD administration. Levels of both evaluated markers p38 MAPK and caspase 3 were decreased in group with combined therapy when compared to untreated group or surfactant-treated group (p<0.01 for

p38 MAPK and $p<0.05$ for caspase 3 vs. both Mec and Surf; Table 2).

Oxidative markers

Markers of oxidative stress in lung tissue presented by TBARS and TAC evaluation were not significantly affected by any of the treatment. Decrease in TBARS formation after therapeutic intervention was not significant, while in TAC there was just tendency to amelioration after combined therapy ($p=0.07$ vs. Mec; Table 2).

Discussion

Meconium present in newborn's lungs causes a number of alterations often resulting in severe respiratory failure with need of ventilatory support and exogenous surfactant treatment, as endogenous surfactant is degraded and dysfunctional (Mokra *et al.* 2013).

In our experiments, meconium led to marked deterioration in all respiratory parameters in very short time after instillation. Ventilation/perfusion mismatch in Mec group was reflected by reduced oxygen saturation up to 61 % at OI>15. Upon removal, lungs of untreated animals were atelectatic at sight and BALF was rich in cells with high portion of neutrophils.

Meconium-induced surfactant dysfunction is multilevel process, starting with direct effect of meconium on surfactant components and ending with harmful inflammatory and oxidative cascades (Kopincova and Calkovska 2016). Despite confirmed deleterious effect on endogenous surfactant, effectivity of exogenous surfactant given as in newborns with MAS is still discussed. Systematic reviews and meta-analyses did not prove its benefit on newborns' final outcome, regardless of improvement in pulmonary oxygenation (Lee and Kim 2013, El Shahed *et al.* 2014). On the other side, there is growing evidence of surfactant lavage benefit in both experimental and clinical MAS (Lista *et al.* 2006, Dargaville 2012a, Lin *et al.* 2014) with some authors finding the combination of surfactant lavage and bolus the most effective treatment (Henn *et al.* 2015). This resulted in the fact, that some current national guidelines recommend therapeutic use of exogenous surfactant in severe MAS (Castillo Salinas *et al.* 2015, Chettri *et al.* 2016).

In our study, combination of lavage with diluted surfactant followed by surfactant bolus turned out to be effective on some ventilatory parameters. Improvement in OI and oxygen saturation was seen in the first hour after the treatment; improvement in VEI was restricted to the last hour of the experiment probably due to persisting airway obstruction.

Lavage with surfactant is useful tool for washing out meconium from obstructed airways (Ohama and Ogava 1999). Together with meconium, cytokines, chemokines and phospholipases are removed, resulting in mitigation of chemotactic stimuli and inflammation. In accordance with this, we found marked decrease in leukocyte sequestration into lungs after surfactant treatment, though the portion of neutrophils was still higher compared to Surf+IKK-NBD group. However, cytokines were not reduced in Surf group what is in accordance with our other study (Mikolka *et al.* 2016c). Recently we have shown that surfactant treatment in experimental MAS may decrease cytokine expression on mRNA levels, nevertheless, it was not capable of preventing also translational process (discussed in Mikolka *et al.* 2016a). There was also only non-significant decrease in lipid peroxidation after surfactant; non-significance in this group might be, however, accounted to wide variance of data. When we compared only Mec and Surf group using non-parametric Mann-Whitney U test, significance was marked, in accordance with our previous results, where surfactant treatment prevented oxidative damage in lung tissue and systemic circulation (Kopincová *et al.* 2014, Mikolka *et al.* 2016b). And finally, no change in apoptotic markers suggests that small amount of residual meconium is able to initiate inflammatory cascades which in turn activate each other (Kopincova and Calkovska 2016).

Considering inferior effect of anti-inflammatory monotherapy (without surfactant) in MAS (Mikolka *et al.* 2013) and after comparison with other studies using IKK-NBD combined with surfactant (Ankermann *et al.* 2005, von Bismarck *et al.* 2009) we did not include IKK-NBD-treated animals without surfactant. Surfactant administration is now considered to be an integral component of the management of MAS, especially for those newborns who are intubated (Canadian Paediatric Society 2005, Stenson and Smith 2012) while corticoid monotherapy should not be considered standard practice in the meantime (Dargaville 2012b, Stenson and Smith 2012). Thus, solely IKK-NBD therapy, presumably, would not be in clinical use.

In MAS, surfactant dysfunction and lung inflammation are two main conditions to fight with, when obstructive effect of meconium is diminished by

suctioning. Surfactant replacement improves oxygenation, but may be additionally impaired by running inflammation. Damping inflammation without surfactant substitution seems to be weak tool to completely overcome deleterious effects of meconium on surface tension in airways. These might be the reasons, why the final outcome of newborns in systematic reviews did not change nor after corticosteroids neither after surfactant despite early improvement in oxygenation (Ward and Sinn 2003, El Shahed *et al.* 2014, Garg *et al.* 2016).

To the contrary, "upgrade" of surfactant therapy with anti-inflammatory agent may bring an additional benefit (Mikolka *et al.* 2013, Kopincová *et al.* 2014, Lin *et al.* 2016). IKK-NBD, selective inhibitor of NF-κB, combined with surfactant seems to be promising in the treatment of respiratory distress syndrome (Ankermann *et al.* 2005, von Bismarck 2007, von Bismarck 2009). In this study, quick onset and persisting effect of the therapy was seen in all measured respiratory parameters. Lower leukocyte migration to lungs, reduced portion of sequestrated neutrophils, decrease in inflammatory cytokine levels and apoptotic pathway markers were seen after successful NF-κB inhibition.

Combined therapy led to rapid improvement in oxygenation in the first 30 min after administration and reached values (especially in VEI and PaO_2/FiO_2) which were higher than in surfactant-only treated animals, suggesting that this effect cannot be ascribed just to meconium removal by lavage. To explain this observation is challenging issue. Such quick onset of therapeutic effect in MAS has been previously noticed by our group after treatment with surfactant + budesonide (Mikolka *et al.* 2013) and surfactant + N-acetylcysteine (Kopincová *et al.* 2014). In the case of glucocorticoid administration, prompt reply occurs probably due to non-genomic effects of corticoids which include interactions of glucocorticoids with cellular membranes, specific interaction with membrane-bound glucocorticoid receptors, and non-genomic effects mediated through binding to the cytosolic glucocorticoid receptors (Alangari *et al.* 2010) – none of these can be reached using IKK-NBD. In the latter case, changes in redox status might affect redox-sensitive p38 MAPK pathway leading to cyclooxygenase-2 (COX-2) induction and subsequent thromboxane production (Zafarullah *et al.* 2003, Bernatova 2014). Inhibition of COX-2 (and thromboxane) might prevent bronchoconstriction, lower airway resistance and improve lung function parameters (Uhlig *et al.* 1996, Kopincová *et al.* 2014).

Recently, it has been shown that COX-2 induction in airway smooth muscle cells and COX-2-derived lung inflammation may be triggered also *via* PI3K/Akt/NF-κB pathway after activation of EGFR/PDGFR (Yang *et al.* 2009). These receptors can be rapidly activated by adenosine released from injured epithelial cells, leading to immediate COX-2-mediated airway constriction (Zhou *et al.* 2013). Taken together, in meconium-polluted airways where multiple agents including phospholipase A2 can directly damage epithelial cells, inhibition of NF-κB-dependent COX-2 induction may prevent prostanoid bronchoconstriction and improve oxygenation. But of course, many other mechanisms can stand behind this fast effect of combined therapy and more studies are needed to clarify this observation.

Inhibition of inflammatory signalization led to reduction in cytokine production. Meconium is known to activate TLR4/MD-2/CD14 complex – that means, the starting point of LPS-induced inflammation and the first signal point for both IL-1β and IL-6 transcription (Lu *et al.* 2008). Downregulation of these pathways damped not only cytokine levels but also chemokine production, manifested by reduction in the number of migrating cells and the portion of neutrophils in the lungs. Hand in hand, decrease in p38 MAPK and caspase 3 levels were found in lung tissue homogenates after combined therapy. p38 MAPK expression is not NF-κB-dependent, but is increased after cytokine stimulation of the cell (Pelaia *et al.* 2005). Its induction results in apoptosis mediated by caspase 3 (Damarla *et al.* 2014). Reduction in these substances suggests less apoptotic signalization in injured lungs.

Finally, there is some unclarity about low effect of combined therapy on oxidative parameters in the lung tissue. No change in TBARS formation and only tendency to improvement in antioxidant capacity in the tissue were seen after Surf+IKK-NBD administration, though it is known that inductive nitric oxide synthase (iNOS) induction is NF-κB-dependent (Kopincová *et al.* 2011) and expression of some subunits of nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase requires NF-κB activity (Anrather *et al.* 2006, Manea *et al.* 2007). Oxidative damage had not been prevented even after restriction of neutrophil activity in the lungs, so perhaps some other activated cells, e.g. epithelial or endothelial, were engaged in this process. As free radicals have strong potential not only to harm the tissue, but are

also essential in inflammatory signaling, down-regulation of their production in MAS is desirable.

Taken together, combination of exogenous surfactant and IKK-NBD was superior to surfactant monotherapy as regards lung function and some inflammatory markers. Anti-inflammatory "upgrade" of surfactant therapy may thus be taken into account in the management of MAS in the future.

Conflict of Interest

There is no conflict of interest.

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