EXPERIMENTAL STUDY

Surfactant lung lavage using asymmetric high-frequency jet ventilation followed by conventional ventilation in rabbits with meconium aspiration

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Abstract

Background: **Severe impairment of lung functions in meconium aspiration syndrome (MAS) often needs the application of combined therapeutic approach. In our recent study, surfactant lung lavage during asymmetric high-frequency jet ventilation (HFJV) removed more meconium than surfactant lavage during conventional ventilation, however, after the lavage excessive CO_2 elimination was observed during HFJV.**
Objectives: **We hypothesized that the combination of asymmetric HFJV during surfactant lung lavage and conventional ventilation in the post-lavage period may be of benefit in a rabbit model of MAS.**
Methods: **Suspension of human meconium in saline (25 mg/ml, 4 ml/kg) was instilled into the tracheal tube of conventionally ventilated (frequency, f, 30/min, inspiration time, Ti, 50 %) anesthetized rabbits to cause a respiratory failure. Animals were then lavaged (10 ml/kg in 3 portions) with diluted surfactant (Curosurf, 100 mg of phospholipids/ml) or saline during asymmetric HFJV (f, 300/min, Ti, 70 %). After the lavage, animals were ventilated conventionally (f, 30/min, Ti, 50 %) for next 1 hour.**
Results: **Surfactant lung lavage during asymmetric HFJV removed more meconium pigments and solids than saline with HFJV ($p<0.05$ or $p<0.01$, respectively). Moreover, application of asymmetric HFJV facilitated the lavage fluid removal in both groups. In the post-lavage period, improved oxygenation, lung compliance, right-to-left pulmonary shunts, and reduced ventilatory requirements were found in the surfactant group ($p<0.05$), while pCO_2 was kept in the normal range.**
Conclusions: **Surfactant lung lavage by asymmetric HFJV followed by conventional ventilation is advantageous combination in rabbits with MAS and may be tested in neonatal MAS *(Tab. 2, Fig. 2, Ref. 12).***
Key words: high-frequency jet ventilation, lung lavage, mechanical ventilation, meconium aspiration, surfactant.

Respiratory failure resulting from meconium aspiration often requires the use of combined therapeutic approach. However, while surfactant lung lavage was recently shown to be beneficial in many experimental (1–4) and clinical studies (5–7), other methods potentially improving the lung functions and meconium removal are rarely being published. In our recent study, asymmetric high-frequency jet ventilation (HFJV) had an additive effect on meconium removal by means of surfactant lung lavage (8). Nevertheless, in HFJV-ventilated group lower oxygenation and higher elimination of CO_2 was found in the post-lavage period than in conventionally ventilated group. The present experiments combined the advantages of two different types of ventilation together with surfactant lung lavage, i.e. surfactant lung lavage performed during asymmetric HFJV and conventional ventilation in the post-lavage treatment period in rabbits with MAS.

Materials and methods

Materials: Meconium was collected from 20 healthy term neonates, lyophilized and stored at -20 °C. Before use, meconium was suspended in 0.9 % NaCl at a concentration of 25 mg/

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ml. Modified porcine surfactant (Curosurf, Chiesi Pharmaceuticals, Italy) was diluted in saline at a concentration of 10 mg phospholipids/ml.

Methods: The study design was approved by the Local Ethics Committee. Adult rabbits (chinchilla) with body weight (b.w.) of 1.8±0.1 kg were anesthetized with intramuscular ketamine (Narkamon, Spofa, Czech Republic) at a dose of 20 mg/kg b.w., and xylazine (Rometar, Spofa, Czech Republic) at a dose of 5 mg/kg b.w. followed by intravenous (i.v.) infusion of ketamine at a dose of 20 mg/kg b.w./h. Tracheotomy was performed and tracheal tube was inserted. Femoral artery was cannulated for arterial blood sampling, femoral vein for administration of drugs and anesthetics and jugular vein for the sampling of mixed venous blood. Animals were paralyzed with pipecuronium bromide (Arduan, Gedeon Richter A.G., Hungary) at a dose of 0.3 mg/kg b.w./30 min i.v. to avoid spontaneous breathing and ventilated with pressure-controlled ventilator Beat-2 (Chirana, Slovakia) with frequency (f) of 30/min, fraction of inspired oxygen (FiO_2) of 0.21, positive end-expiratory pressure (PEEP) 0 kPa, 50 % inspiration time (Ti), and peak inspiratory pressure (PIP) adjusted to keep the tidal volume (VT) of 8–10 ml/kg b.w. Baseline values of VT, PIP and PEEP were recorded, and partial pressures of O_2, CO_2 and pH were measured by blood gas analyzer (Radiometer, Denmark). A suspension of meconium at a dose of 4 ml/kg b.w. was instilled proportionally into the left and right lungs during lateral positioning of the animal. FiO_2 was then increased to 1.0 and PEEP to 0.3 kPa, and PIP was adjusted to keep VT of 8–10 ml/kg b.w. After this, 5 ml of 4.2 % sodium bicarbonate (Braun, Germany) to keep blood pH in the normal range and furosemide (Furosemid, Hoechst-Biotika, Slovakia) at a dose of 5 mg/kg b.w. to promote diuresis were administered i.v. Additional dose of meconium (1 ml/kg) was given to animals not showing evidence of respiratory failure 15 min after the first dose. Within 30 min after the meconium instillation, respiratory failure developed, defined as >30 % decrease in dynamic lung-thorax compliance (Cdyn) and PaO_2 <10 kPa at FiO_2 of 1.0. Blood samples were analysed and lung function parameters measured at this time point. Then, the lung lavage with saline (Sal group) or diluted Curosurf (Surf group) at a dose of 10 ml/kg b.w. in 3 portions was performed, so that within 30 seconds one third of the lavage fluid was instilled by syringe into the tracheal tube of animals situated in the right and left lateral positions. Subsequently, asymmetric HFJV (f 300/min, 70 % Ti) was applied for 5 min, combined with suction (Suction professional, Elettromedicali, Italy) with negative pressure of 60

Tab. 1. Removal of the lavage fluid and meconium in saline (Sal) and surfactant (Surf) groups.

	Sal group	Surf group
Removed lavage fluid (ml/kg b.w.)	7.5±0.3	7.6±0.3
Removed lavage fluid (%)	74.7±3.4	75.7±2.8
Removed meconium pigments (mg/kg b.w.)	14.4±1.0	18.7±0.8 *
Removed meconium pigments (%)	13.9±1.1	18.0±0.6 *
Removed meconium solids (mg/kg b.w.)	6.3±0.4	30.8±3.1 **
Removed meconium solids (%)	3.4±0.3	16.1±1.2 **

For between-group comparisons: * $p<0.05$, ** $p<0.01$

Tab. 2. Lung function parameters in saline (Sal) and surfactant (Surf) groups during experiments.

	Before M	After M	10 min	30 min	60 min
PaO_2/FiO_2-Sal (kPa)	51.47 ± 2.44	7.89 ± 0.54	9.51 ± 0.68 *	10.69 ± 1.22	9.26 ± 1.04 **
PaO_2/FiO_2-Surf (kPa)	47.75 ± 3.91	7.39 ± 0.30	15.34 ± 2.40	17.97 ± 2.39	17.90 ± 1.92
$PaCO_2$-Sal (kPa)	3.41 ± 0.35	5.63 ± 0.82	6.05 ± 0.61	5.30 ± 0.33	5.72 ± 0.60
$PaCO_2$-Surf (kPa)	3.56 ± 0.33	5.55 ± 0.45	4.87 ± 0.40	4.54 ± 0.23	4.46 ± 0.24
pH-Sal	7.52 ± 0.02	7.35 ± 0.03	7.26 ± 0.04	7.31 ± 0.03	7.29 ± 0.03
pH-Surf	7.50 ± 0.02	7.37 ± 0.02	7.34 ± 0.03	7.36 ± 0.02	7.37 ± 0.03
PIP-Sal (kPa)	0.53 ± 0.02	1.22 ± 0.03	1.32 ± 0.02 **	1.32 ± 0.02 *	1.32 ± 0.04
PIP-Surf (kPa)	0.53 ± 0.02	1.22 ± 0.02	1.13 ± 0.03	1.17 ± 0.06	1.15 ± 0.08
PEEP-Sal (kPa)	0.0	0.30 ± 0.0	0.47 ± 0.02 *	0.43 ± 0.02	0.43 ± 0.02
PEEP-Surf (kPa)	0.0	0.3 ± 0.0	0.35 ± 0.02	0.38 ± 0.04	0.37 ± 0.05
MAP-Sal (kPa)	0.30 ± 0.0	0.78 ± 0.02	0.90 ± 0.0 **	0.90 ± 0.0 *	0.90 ± 0.03
MAP-Surf (kPa)	0.30 ± 0.0	0.80 ± 0.0	0.75 ± 0.02	0.78 ± 0.05	0.77 ± 0.06
C_{dyn}-Sal (ml/kPa/kg)	16.3 ± 0.8	9.1 ± 0.3	10.1 ± 0.6	10.0 ± 0.6	9.6 ± 0.6 *
C_{dyn}-Surf (ml/kPa/kg)	15.8 ± 0.7	9.0 ± 0.2	10.9 ± 0.3	11.5 ± 0.5	12.0 ± 0.7
RLS-Sal (%)	7.7 ± 1.1	44.9 ± 3.7	47.9 ± 4.4	47.5 ± 3.1	47.3 ± 3.2 *
RLS-Surf (%)	11.7 ± 2.1	49.7 ± 2.9	40.3 ± 2.4	39.5 ± 4.0	35.1 ± 3.5

Abbreviations: Before M, After M – before and after meconium administration, 10, 30, 60 min – minutes of ventilation after the lavage.
Between-group comparisons: * $p<0.05$, ** $p<0.01$.

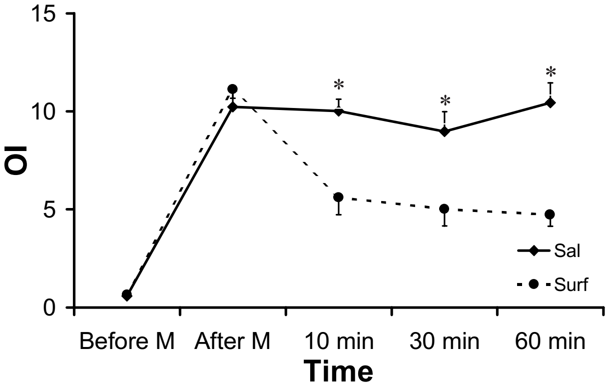

Fig. 1. Oxygenation index (OI) in saline (Sal) and surfactant (Surf) groups before and after meconium administration (M) and 10, 30 and 60 minutes after the lavage. Between-group comparisons: *p<0.05, **p<0.01.

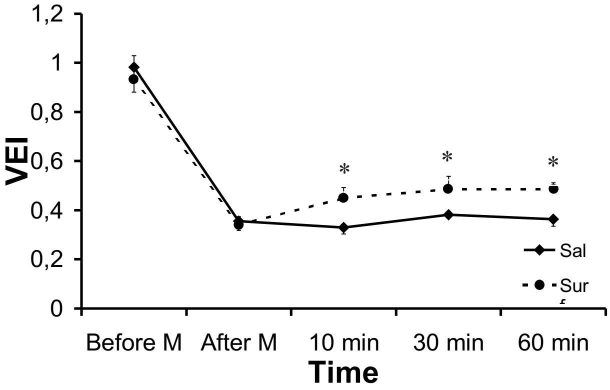

Fig. 2. Ventilation efficiency index (VEI) in saline (Sal) and surfactant (Surf) groups before and after meconium administration (M) and 10, 30 and 60 minutes after the lavage. Between-group comparisons: *p<0.05, **p<0.01.

kPa 1 and 5 minutes after the fluid administration. In both groups, the procedure was repeated twice under the same conditions and the volume of recovered liquid was determined. All animals were then ventilated conventionally (f 30/min, 50 % Ti) for one additional hour, and blood gases and lung function parameters were measured 10, 30 and 60 min after the lavage. At the end of experiment, animals were killed by an overdose of anesthetics.

Lung function measurements

Tracheal airflow and VT were measured by heated Fleisch head connected to the pneumotachograph (UMMT SAV, Slovakia), placed temporarily between the tracheal tube and the outlet of ventilator. Airway pressure was registered via a pneumatic catheter placed 0.5 cm below the distal tip of the tracheal tube and connected to the electromanometer (Tesla, Czech Republic), and transfered to multi-channel recorder (RFT, Germany). Mean airway pressure (MAP) was calculated as MAP= (PIP+PEEP)/2, ventilation efficiency index (VEI) as VEI=3800/[(PIP-PEEP) (cmH_2O) x ventilatory frequency x $paCO_2$ (mmHg)], and oxygenation index (OI) as OI=MAP x FiO_2/PaO_2. Dynamic lung-thorax compliance (Cdyn) was calculated as a ratio of the tidal volume adjusted per kg b.w. and airway pressure gradient (PIP-PEEP).

Calculation of right-to-left pulmonary shunts

Shunts were calculated using Fick equation: (CcO_2 - CaO_2/ CcO_2 - CvO_2) x 100, where CcO_2, CaO_2 and CvO_2 are oxygen concentrations in pulmonary capillaries, arterial and mixed venous blood. CcO_2 was calculated using PaO_2 (alveolar partial pressure of oxygen) from the equation: PaO_2 = (PB - PH_2O) x FiO_2 - $PaCO_2$ x [FiO_2 + (1-FiO_2)/R], where PB is barometric pressure and PH_2O represents vapor pressure of water. Respiratory exchange ratio (R) was assumed to be 0.8, and hemoglobin was measured spectrophotometrically by Specol 11 (Carl Zeiss, Germany).

Evaluation of removed meconium

The amount of removed meconium pigments was estimated spectrophotometrically (1). The aspirate was centrifuged at 40,000 g for 1 hour. The optical density (OD) values of supernatant read at 260 and 300 nm were added to the formula: OD300-(0.13 x OD260). According to the linear relationship between the optical density and amount of meconium pigments in the sample, the content of pigments in the lavage fluid was calculated. The amount of removed meconium solids was estimated by meconium-crit method (2). Three samples of lavage fluid were taken into the microhematocrit glass tubes and centrifuged at 10,000 rpm for 5 min. The percentage of solid content was read and the average value of solids calculated.

Data analysis

Mann-Whitney's and Wilcoxon's tests were applied for between-group and within-group comparisons, respectively. Values are shown as means±SEM and p<0.05 was considered statistically significant.

Results

Twelve adult rabbits entered the study and were used for final data analysis, six in each group. There were no significant differences in the body weight, gender rate, baseline blood gases or lung function parameters.

All animals received 100 mg/kg b.w. (25 mg/ml, 4 ml/kg) of meconium suspended in saline. One animal in each group showing insufficient response to meconium received an additional dose of meconium (25 mg/ml, 1 ml/kg). Totally, 104.2±4.2 mg/kg b.w. of meconium was administered in both groups. All animals were lavaged with 10 ml/kg b.w. of saline or diluted surfactant. The recovery of lavage fluid was comparable between groups (p>0.05). Surfactant lung lavage removed more meconium pigments and solids than saline lavage (p<0.05 or p<0.01, respectively) (Tab. 1).

The instillation of meconium significantly decreased the dynamic lung-thorax compliance, worsened the gas exchange as well as increased the right-to-left pulmonary shunts and ventilatory pressures, comparably in both groups (Sal group vs Surf group, p>0.05). After the surfactant lavage, the lung compliance gradually increased and the shunting decreased with significant differences when compared to the Sal group at 60 min

(Tab. 2). Subsequent impacts in form of improved oxygenation ($p<0.05$) (Fig. 1), slightly decreased $PaCO_2$ and elevated pH in comparison with the saline-lavaged group were found (Tab. 2). Furthermore, the surfactant lavage was observed to reduce the ventilatory pressures and VEI, when compared to the Sal group ($p<0.05$ or $p<0.01$, respectively) (Tab. 2, Fig. 2).

Discussion

Since meconium is responsible for the inactivation of pulmonary surfactant (9), vasoconstriction and inflammatory lung injury (10–11), its removal from the lungs became the fundamental task in the management of MAS. However, due to its high tenacity, meconium is hardly accessible by conventional suction even in short time after the aspiration, and alternative approaches improving its removal are often needed. Recently, the lung lavage with diluted exogenous surfactant significantly improved lung functions and oxygenation (1, 5–7). Moreover, the surfactant lavage increased the removal of meconium from the lungs (2–4). Our recent experiments showed that surfactant lung lavage improved the meconium removal during both conventional ventilation (CV) and asymmetric HFJV when compared to the saline lavage (8). In addition to the latter, the surfactant lavage combined with asymmetric HFJV removed more meconium than surfactant lavage during CV, however, in the post-lavage period, HFJV was found to be less beneficial in gas exchange than CV (8). Therefore, the present study was performed to combine favourable properties of the two mentioned modes of ventilation – asymmetric HFJV by improving the meconium removal and conventional ventilation by keeping the oxygenation and elimination of CO_2 in the normal range.

In this study, the combination of surfactant lung lavage with asymmetric HFJV removed significantly higher amounts of meconium pigments and solids than the saline lavage. The rather high removal of meconium pigments, but low removal of solids by saline lavage indicate that saline removes particularly the pigmented parts of meconium soluble in water, such as gastrointestinal enzymes, bile acids, and bilirubin. On the other hand, exogenous surfactant lowers the surface tension of meconium and particularly removes the parts of meconium, which are hardly cleared by saline – mucopolysaccharides, free fatty acids, triglycerides, cholesterol etc. Similarly, Dargaville et al found higher removal of pigments than solids by both saline and surfactant lavages in piglets although these authors used different methods for meconium evaluation, type and concentration of surfactant, and larger volume of the lavage fluid (4).

In addition, in our experiments, asymmetric HFJV combined with suction removed a significant portion of administered fluid in both surfactant and saline-lavaged animals, as was proved also by our recent study (8). These findings confirm the original hypothesis of Brychta et al that due to inspiration time being longer than 60 % of the respiratory cycle, HFJV asymmetry in airflow forces the movement of materials outside the airways (12). Furthermore, high minute ventilation during HFJV decreased the $PaCO_2$ and stabilized the pH and PaO_2 in several minutes. Under the clinical conditions, slight hypercarbia to hypocarbia is prefered to prevent the changes in cerebral blood flow. However, rapid elimination of CO_2 and stabilization of PaO_2 and pH by HFJV may be of benefit during the lung lavage procedure, when the ventilation is interrupted during the fluid administration and suction, and neonates may suffer from hypoxia and hypercarbia. In the post-lavage period, conventional ventilation with moderate tidal volumes and ventilatory pressures was well tolerated in all animals. Moreover, the distal spreading of surfactant and the opening of collapsed alveoli increased the lung compliance. The improvement in oxygenation led to the diminishing of right-to-left pulmonary shunts and to further improvement in oxygenation. In surfactant-lavaged group, the gas exchange was stabilized and values of blood gases kept in an acceptable range despite the fact that reduced ventilatory pressures were applied.

Surfactant lung lavage using asymmetric HFJV improved the meconium removal, and conventional ventilation applied after the lavage contributed to further improvement in lung functions. We conclude that the combination of asymmetric HFJV during the lung lavage and conventional ventilation in the post-lavage period was of benefit in a rabbit model of MAS and in future may be tested also in neonatal MAS.

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