

EXPERIMENTAL STUDY

Dexamethasone treatment improved lung functions in meconium-instilled rabbits, but influenced cardiovascular variables

Mokra D, Calkovska A, Tonhajzerova I, Petraskova M, Bulikova J, Redfors B

Department of Physiology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia.

mokra@jfmed.uniba.sk

Abstract: *Background:* In this study, the effects of single and repetitive dexamethasone administration on lung functions in meconium-instilled rabbits were compared. In addition, short-term adverse effects of dexamethasone on blood pressure, heart rate, and heart rate variability were evaluated.

Methods: Artificially ventilated adult rabbits intratracheally received 4 ml/kg of meconium suspension (25 mg/ml). When respiratory failure developed, animals received single-dose of dexamethasone (0.5 mg/kg i.v.) 0.5 h after meconium instillation (Dex1), two doses of dexamethasone (each 0.5 mg/kg i.v.) 0.5 and 2.5 h after meconium instillation (Dex2) or were left without treatment (Mec). Animals were oxygen-ventilated for 5 h after the first dose of treatment.

Results: Dexamethasone treatment significantly improved gas exchange and reduced right-to-left pulmonary shunts, central venous pressure, and lung edema, whereas trend to more pronounced improvement in some parameters was observed after two doses of dexamethasone. There were no significant between-group differences in blood pressure, however, decreased heart rate and increased heart rate variability were observed particularly after repetitive dexamethasone administration.

Conclusion: Intravenous dexamethasone, especially when given in two doses, improved lung functions, but influenced cardiovascular variables in meconium-instilled rabbits (Tab. 2, Fig. 3, Ref. 34). Full Text (Free, PDF) www.bmj.sk.

Key words: meconium aspiration, inflammation, glucocorticoids, dexamethasone.

Meconium aspiration syndrome (MAS) is a serious respiratory disorder in the term and post-term neonates. Acute lung injury due to meconium aspiration is initially characterized by mechanical obstruction of the airways, ventilation/perfusion mismatch, hypoxemia, and acidosis. Subsequently, progressive inflammation, alveolar exudation, surfactant dysfunction, and pulmonary hypertension complicate the clinical course and treatment of MAS (1, 2). About 30 % of infants with MAS require mechanical ventilation, whereas severe cases need invasive therapy such as extracorporeal membrane oxygenation. Due to rather frequent failure of conventional therapy, new approaches including lung lavage with diluted exogenous surfactant (3–5) and anti-inflammatory treatment, such as glucocorticoids (GCs) (6–9) have been tested.

Department of Physiology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia

Address for correspondence: D. Mokra, MD, PhD, Dept of Physiology, Jessenius Faculty of Medicine, Comenius University, Mala Hora 4, SK-037 54 Martin, Slovakia.
Phone/Fax: +421.43.4131426

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Potent anti-inflammatory properties of GCs result from diminishing the migration and activation of neutrophils, eosinophils, mononuclears, and other cells as well as from modulation of action of the mediators released from activated cells. Free glucocorticoid (GC) molecules cross cell membrane into the cytoplasm, where they bind to a specific glucocorticoid receptor (GR). After migration into the cell nucleus, the GR complex interacts with nuclear factor (NF)- κ B and protein activator (AP)-1, and thereby inhibits expression of cytokines, pro-inflammatory enzymes, and other biologically active substances (10), important in the pathogenesis of MAS (11–14). Influencing the vasoactive substances, such as arachidonic acid metabolites, endothelin-1, and platelet-activating factor, GCs modulate also the pulmonary vascular tone. Nevertheless, reduction in alveolar-arterial oxygen gradient and in venous admixture after administration of GCs may in part result from steroid-induced redistribution of the pulmonary blood flow to better ventilated areas. By stabilizing membranes GCs diminish the endothelial injury and microvascular permeability (10). In addition, by reducing polymorphonuclear inflammation dexamethasone effectively decreases meconium-induced increase in airway responsiveness to histamine (15).

Taken together, neonates with severe MAS could benefit from systemic GCs, however, timing of GC administration is critical for ideal pulmonary response (16). Although pretreatment with

methylprednisolone (6) and dexamethasone (8) before meconium instillation reduced pulmonary hypertension, venous admixture, edema formation and improved oxygenation in piglets with MAS, early treatment with dexamethasone after meconium instillation just slightly improved oxygenation and lung edema (8). On the other side, double dose dexamethasone treatment in piglets enhanced gas exchange and reduced oxygen requirements (7). Similarly in newborns with MAS, dexamethasone given for several days in a reduced schedule improved lung functions and facilitated weaning from ventilator (9). Nevertheless, high doses of systemic GCs or their repetitive administration may adversely influence cardiovascular functions. Although changes in blood pressure, heart rate, and cardiac rhythm may be critical for neonates with meconium-induced respiratory failure, side effects of repetitive systemic high-dose GCs administration in MAS have not been studied yet. Therefore, the aim of these experiments was to evaluate effects of one and two doses of dexamethasone on pulmonary functions as well as on blood pressure, heart rate, and heart rate variability within 5 hours of the treatment.

Methods

Ethical approval: The study was approved by local Ethics Committee of Jessenius Faculty of Medicine, Comenius University and the experiments followed the instructions for animal care and use.

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

Design of the study: Chinchilla rabbits of mean body weight (b.w.) 2.4 ± 0.4 kg of both genders were anesthetized with intramuscular ketamine (20 mg/kg b.w.; Narkamon, Spofa, Czech Republic) and xylazine (5 mg/kg b.w.; Rometar, Spofa, Czech Republic) followed by continuous intravenous (i.v.) infusion of ketamine (20 mg/kg b.w./h). Tracheotomy was performed and catheters into the femoral artery and right atrium for sampling blood, and into the femoral vein to administer anesthetics were inserted. Animals were then paralyzed with pipecuronium bromide (0.3 mg/kg b.w./30 min; Arduan, Gedeon Richter, Hungary) and subjected to pressure-controlled ventilator (Beat-2, Chirana, Slovakia). At the beginning of experiments, all animals were ventilated with a frequency of 30/min, fraction of inspired oxygen (FiO_2) was 0.21, peak inspiratory pressure (PIP) to keep a tidal volume (VT) between 7–9 ml/kg b.w. and no positive end-expiratory pressure (PEEP) in this stage of experiments. After stabilization, cardiopulmonary parameters were recorded and samples of arterial and mixed venous blood were taken for blood gas analysis and estimation of hemoglobin (Rapidlab™348, Bayer Diagnostics, Germany). Suspension of meconium (25 mg/ml, 4 ml/kg b.w.) was instilled into the tracheal tube during positioning of the animal. From this moment on, animals were ventilated with 100 % oxygen (FiO_2 1.0) and PEEP 0.3 kPa. Sodium bicarbonate (4.2 % inf. sol., 2 ml/kg b.w., Braun, Germany) was administered to keep blood pH and base excess in the physiological range 2 min after instillation of meconium as well as dur-

ing further course of the experiment, if needed. Within 30 min respiratory failure developed, defined as >30 % decrease in dynamic lung-thorax compliance (C_{dyn}) and arterial partial pressure of oxygen (PaO_2) <10 kPa at FiO_2 1.0. Blood samples were taken and parameters recorded again. Animals received a single-dose of dexamethasone i.v. (0.5 mg/kg b.w.; Dexamed, Medochemie, Cyprus) 0.5 h after meconium instillation (Dex1 group, $n=7$), or two doses of dexamethasone i.v. (each 0.5 mg/kg b.w.) 0.5 and 2.5 h after meconium instillation (Dex2 group, $n=7$), or were left without treatment (Mec group, $n=7$). Dexamethasone was diluted with saline in a ratio 1:2 and administered slowly within 5 min. All animals were oxygen-ventilated for additional 5 hours with recording of cardiopulmonary parameters at 0.5, 1, 2, 3, 4, and 5 h after administration of the first dose of treatment.

Measurements: Tracheal airflow and VT were measured by a Fleisch head connected to pneumotachograph. Airway pressure was registered via a pneumatic catheter placed 0.5 cm below the distal tip of tracheal tube and connected to electromanometer. C_{dyn} was calculated as a ratio between VT adjusted per kg b.w. and airway pressure gradient (PIP-PEEP). Mean airway pressure (MAP) was calculated as $\text{MAP}=(\text{PIP}+\text{PEEP})/2$, ventilation efficiency index (VEI) as $\text{VEI}=(\text{PIP}-\text{PEEP}) \times \text{ventilatory rate} \times \text{PaCO}_2$, and oxygenation index (OI) as $\text{OI}=(\text{MAP} \times \text{FiO}_2)/\text{PaO}_2$. Right-to-left pulmonary shunts were calculated by computer program using the Fick equation (4). Systolic (SBP) and diastolic (DBP) blood pressures were measured via catheter in the femoral artery connected to electromanometer, and mean arterial blood pressure (MABP) was calculated as $\text{MABP}=\text{DBP} + 1/3 (\text{SBP} - \text{DBP})$. Central venous pressure (CVP) was registered through catheter inserted in the right atrium connected to electromanometer. Heart rate (HR) was calculated from ECG recorded using subcutaneous electrodes. Heart rate variability (HRV) was evaluated by computer system (VariaPulse TF3, Sima Media, Czech Republic). Variables of frequency domain HRV, i.e. spectral powers in low frequency (LF: 0.05–0.15 Hz) and high frequency (HF: 0.15–2.0 Hz) bands as well as total power (TP) were evaluated. At the end of experiments, animals were killed by an overdose of anesthetics. Lungs were excised and dried at 60°C for 24 h to determine lung edema by estimating the lung wet/dry weight ratio.

Statistical analysis: All data were tested for normality of distribution by Kolmogorov-Smirnov test. Since the distribution of variables of frequency domain of HRV was extremely skewed, logarithmic transformation of these data was used to improve normality before the statistical analysis was performed. Then, between-group differences were analysed by ANOVA with post-hoc LSD test. Within-group differences were evaluated by Wilcoxon test. A $p < 0.05$ was considered statistically significant. Data were expressed as means \pm SEM.

Results

Lung function parameters

Meconium instillation worsened gas exchange, right-to-left pulmonary shunts, and ventilatory pressures compared to values

Tab. 1. Parameters of gas exchange, pH and ventilatory parameters before and after instillation of meconium (M) and at 0.5, 1, 2, 3, 4, and 5 hours of the treatment in non-treated (Mec) group and groups treated by one dose (Dex1) and two doses (Dex2) of dexamethasone.

	Before M	After M	0.5 h	1 h	2 h	3 h	4 h	5 h
PaO₂/FiO₂ (kPa):Mec	42.2±1.6	6.8±0.3	7.1±0.3	6.8±0.2	7.0±0.5	6.9±0.4	6.5±0.3	6.2±0.4
PaO₂/FiO₂ (kPa):Dex1	42.6±2.4	7.0±0.4	7.8±0.5	9.1±1.0*	8.3±0.4*	7.9±0.3*	7.8±0.5*	8.0±0.6*
PaO₂/FiO₂ (kPa):Dex2	42.6±1.7	7.0±0.4	8.8±0.2 [†]	8.8±0.2*	8.9±0.3 [†]	8.6±0.2 [‡]	8.5±0.3 [‡]	8.8±0.4 [‡]
PaCO₂ (kPa):Mec	4.4±0.4	6.6±0.5	7.0±0.4	8.3±0.3	7.2±0.4	7.5±0.5	7.4±0.4	7.8±0.5
PaCO₂ (kPa):Dex1	3.9±0.4	5.8±0.4	5.9±0.5	6.5±0.6 [†]	6.5±0.5	6.5±0.4	6.9±0.7	6.9±0.7
PaCO₂ (kPa):Dex2	4.0±0.2	6.1±0.5	6.0±0.4	6.1±0.4 [‡]	6.0±0.3*	6.3±0.3*	5.2±0.2 ^{‡a}	6.7±0.3
pHa:Mec	7.42±0.02	7.21±0.03	7.16±0.03	7.20±0.03	7.18±0.04	7.19±0.04	7.21±0.04	7.20±0.04
pHa:Dex1	7.44±0.02	7.23±0.03	7.27±0.02 [†]	7.26±0.04	7.28±0.03*	7.26±0.03	7.26±0.04	7.29±0.04
pHa:Dex2	7.45±0.02	7.22±0.02	7.27±0.01 [†]	7.28±0.02*	7.28±0.01*	7.29±0.02*	7.30±0.02	7.30±0.02*
PIP (kPa):Mec	0.58±0.03	1.37±0.06	1.54±0.06	1.57±0.05	1.56±0.07	1.59±0.07	1.60±0.06	1.65±0.07
PIP (kPa):Dex1	0.57±0.04	1.34±0.04	1.39±0.03*	1.42±0.03*	1.40±0.05*	1.36±0.06*	1.44±0.03*	1.48±0.03*
PIP (kPa):Dex2	0.56±0.03	1.28±0.05	1.35±0.03 [†]	1.35±0.03 [‡]	1.39±0.04*	1.44±0.04	1.43±0.04*	1.44±0.04 [†]
PEEP (kPa):Mec	0	0.26±0.02	0.31±0.01	0.33±0.01	0.32±0.01	0.34±0.01	0.31±0.01	0.33±0.02
PEEP (kPa):Dex1	0	0.27±0.02	0.27±0.02	0.30±0.02	0.30±0.01	0.31±0.01	0.30±0.03	0.33±0.01
PEEP (kPa):Dex2	0	0.26±0.01	0.30±0.01	0.28±0.01*	0.28±0.01	0.31±0.02	0.29±0.01	0.29±0.01 ^a
MAP (kPa):Mec	0.29±0.02	0.82±0.03	0.93±0.03	0.96±0.02	0.94±0.03	0.97±0.04	0.96±0.03	0.99±0.03
MAP (kPa):Dex1	0.28±0.02	0.83±0.03	0.85±0.02*	0.86±0.02 [†]	0.85±0.06*	0.84±0.02 [†]	0.87±0.03*	0.91±0.02*
MAP (kPa):Dex2	0.28±0.01	0.80±0.03	0.83±0.02 [†]	0.83±0.02 [†]	0.84±0.03*	0.88±0.02*	0.86±0.02*	0.87±0.02 [†]

Abbreviations: PaO₂: arterial partial pressure of oxygen, FiO₂: fraction of inspired oxygen, PaCO₂: arterial partial pressure of carbon dioxide, pHa: arterial pH, PIP: peak inspiratory pressure, PEEP: positive end-expiratory pressure, MAP: mean airway pressure. For differences between Mec vs. Dex1 and Mec vs. Dex2 groups: *P<0.05, †P<0.01, ‡P<0.001; for Dex1 vs. Dex2 groups aP<0.05. Data are expressed as means±SEM.

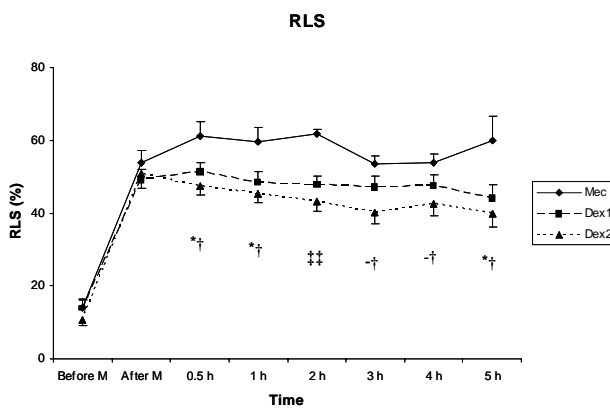


Fig. 1. Right-to-left pulmonary shunts (RLS) before and after meconium instillation (M) and at 0.5, 1, 2, 3, 4, and 5 hours of the treatment in non-treated (Mec) group and treated groups by one dose (Dex1) and two doses (Dex2) of dexamethasone. Significant between-group differences: left mark for Dex1 vs Mec, right mark for Dex2 vs Mec, *p<0.05, †p<0.01, ‡p<0.001, -p>0.05.

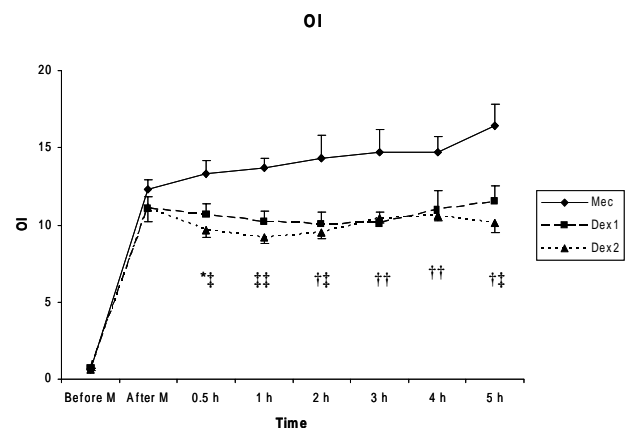


Fig. 2. Oxygenation index (OI) before and after meconium instillation (M) and at 0.5, 1, 2, 3, 4, and 5 hours of the treatment in non-treated (Mec) group and treated groups by one dose (Dex1) and two doses (Dex2) of dexamethasone. Significant between-group differences: left mark for Dex1 vs Mec, right mark for Dex2 vs Mec, *p<0.05, †p<0.01, ‡p<0.001.

before meconium instillation (all p<0.05) (Tab. 1). Dexamethasone administration significantly reduced shunting (Fig. 1), enhanced oxygenation expressed as PaO₂, PaO₂/FiO₂ (Tab. 1), and oxygenation index (Fig. 2) and improved VEI (Fig. 3) and PaCO₂ (Tab. 1). In some evaluated parameters, trend to more pronounced

improvement was observed in Dex2 group than in Dex1 group (Tab. 1). Higher arterial pH was found in both treated groups compared to Mec group (Tab. 1), despite lower volume of bicarbonate administered in Dex1 (19.3±2.3 ml, p>0.05) and Dex2 (13.3±1.7 ml, p<0.05) groups in comparison with Mec group

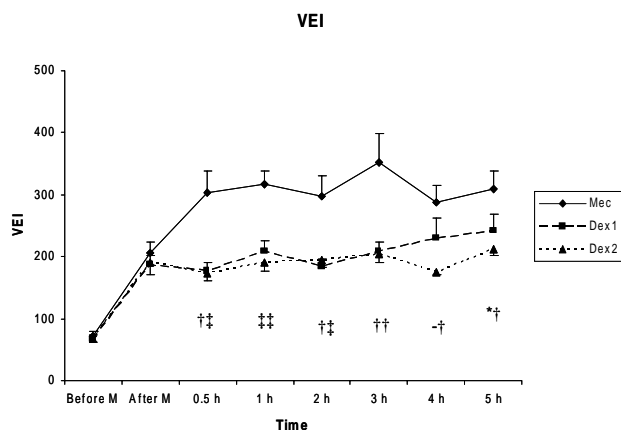


Fig. 3. Ventilation efficiency index (VEI) before and after meconium instillation (M) and at 0.5, 1, 2, 3, 4, and 5 hours of the treatment in non-treated (Mec) group and treated groups by one dose (Dex1) and two doses (Dex2) of dexamethasone. Significant between-group differences: left mark for Dex1 vs Mec, right mark for Dex2 vs Mec, * $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$, - $p > 0.05$.

(21.3 ± 2.6 ml). In treated groups, improvement in gas exchange allowed to reduce the ventilatory pressures compared to non-treated group (Tab. 1). Single-dose dexamethasone treatment diminished lung edema formation compared to Mec group (7.06 ± 0.20 in Mec group vs. 6.21 ± 0.20 in Dex1 group, $p < 0.01$), while repeated administration of dexamethasone amplified reduction of lung edema compared to Mec group (6.03 ± 0.20 in Dex2 group, $p < 0.001$).

Cardiovascular parameters

Central venous pressure decreased after one-dose dexamethasone treatment in comparison to Mec group, whereas more obvious decrease was found in Dex2 group (Tab. 2). Administration of dexamethasone slightly increased arterial blood pressure, but the changes were not significant (Tab. 2). Lower heart rate and higher spectral power in LF band were observed at 5 h of the treatment in Dex2 group compared to Mec group ($p < 0.05$) (Tab. 2). Spectral power in HF band and TP increased after dexamethasone administration in both treated groups compared to Mec ($p < 0.001$) (Tab. 2), but to higher extent in Dex2 group.

Discussion

Lung function parameters

Progressive hypoxia, hypercapnia and acidosis in severe MAS often requires ventilatory support or mechanical ventilation with higher oxygen concentrations. These may further contribute to inflammatory and oxidative damage of the lung tissue. Nevertheless, demonstration of inflammation as a key element in meconium-induced acute lung injury has revealed new perspectives for use of anti-inflammatory drugs, e.g. glucocorticoids, in the treatment of MAS.

In these experiments, already a single-dose dexamethasone given 0.5 h after meconium instillation significantly decreased right-to-left pulmonary shunts and central venous pressure, improved gas exchange, and reduced lung edema and ventilatory pressures compared to non-treated group. In the study by Holopainen et al., the same dose of dexamethasone given 1 h after meconium instillation in piglets showed just non-significant improvement in oxygenation from 30 min of the treatment and no between-group differences in PaCO_2 , pH, and lung edema (8). Discrepancy between our results and findings by Holopainen et al indicate that changes following meconium aspiration become severe very early and there is only limited chance to overcome the progressing inflammation. More effective gas exchange after dexamethasone treatment was probably related to better matching of ventilation and perfusion, as a result of reduced lung edema and less bronchoconstriction.

Our finding of improved lung functions already within 0.5 h after dexamethasone administration is consistent with results by Zhou et al, who demonstrated inhibition of allergic reaction in guinea pigs within 10 min after GCs inhalation (17). Because such rapid effects of GCs cannot be explained by the above mentioned genomic mechanisms, which require at least 4 h till some changes may be observed, we suppose the participation of nongenomically mediated mechanisms: specific interaction with the cytosolic GC receptor (cGCR), non-specific interactions with cellular membranes, and/or specific interactions with membrane-bound glucocorticoid receptors (mGCR). GCs may exert rapid effects on various tissues and cells modulating hormone secretion, neuronal excitability, carbohydrate metabolism, cell morphology, cell behaviour, and other processes within seconds or minutes (18, 19). The GC action may be related to affecting ion cycling (20), cellular energy metabolism (21), or to direct influence on neuronal activity (22), as well as to rapid effects on second messenger systems (21). Nongenomically-mediated effects of GCs have been recently proven also in respiratory system, e.g. rapid inhibition of histamine-induced contractions of airway smooth muscle (23) or stimulation of Na^+/H^+ exchange activity in bronchial epithelial cells (20). It is supposed that nongenomic mechanisms may be responsible for GCs action until the effects mediated by genomic mechanisms occur (19).

Since in our pilot experiments rapid improvement in lung functions was followed by worsening in gas exchange and ventilatory parameters from 2 hours after dexamethasone administration (24), in this study we administered the second dose of dexamethasone already 2 hours after the first dose to partially eliminate the impact of on-going lung injury. Consequently, more pronounced tendency to enhance some pulmonary functions was observed after two doses of dexamethasone compared to single-dose treatment. Similarly to our results, two-dose administration of dexamethasone (each of 0.5 mg/kg b.w.) 2 and 8 h after meconium instillation reduced oxygen requirements, OI and PaCO_2 , and improved VEI and lung compliance in piglets (25). In newborns with MAS, dexamethasone given for nine days in a reducing schedule (0.5 mg/kg b.w./d in two divided doses for 3 days, 0.25 mg/kg/d for 3 days, and 0.125 mg/kg/d for 3 days) signifi-

Tab. 2. Cardiovascular parameters before and after instillation of meconium (M) and at 0.5, 1, 2, 3, 4, and 5 hours of the treatment in non-treated (Mec) group and groups treated by one dose (Dex1) and two doses (Dex2) of dexamethasone.

	Before M	After M	0.5 h	1 h	2 h	3 h	4 h	5 h
CVP (kPa):Mec	0.35±0.03	0.64±0.05	0.73±0.05	0.75±0.06	0.74±0.04	0.72±0.04	0.81±0.05	0.86±0.06
CVP (kPa):Dex1	0.39±0.03	0.61±0.04	0.61±0.06	0.59±0.05*	0.64±0.04	0.70±0.04	0.63±0.05 [†]	0.64±0.05 [‡]
CVP (kPa):Dex2	0.41±0.03	0.59±0.04	0.60±0.03*	0.58±0.04*	0.59±0.04*	0.61±0.03*	0.62±0.03 [†]	0.59±0.02 [‡]
MABP (kPa):Mec	8.8±0.2	9.3±0.7	9.4±0.6	9.6±0.6	9.6±0.4	9.0±0.5	9.3±0.4	9.2±0.9
MABP (kPa):Dex1	8.5±0.6	9.0±0.6	9.5±0.8	8.8±0.9	9.0±0.6	8.9±0.8	9.9±1.4	9.4±1.1
MABP (kPa):Dex2	8.9±0.4	9.3±0.8	10.0±0.7	10.3±0.7	9.5±0.6	9.9±0.7	10.0±0.8	9.7±0.6
HR (bpm):Mec	199±7	202±8	219±7	208±7	219±7	233±9	235±10	250±11
HR (bpm):Dex1	206±12	214±6	212±6	210±5	221±6	235±8	241±11	243±10
HR (bpm):Dex2	191±8	203±10	210±9	219±9	232±14	226±10	226±7	218±6*
logLF:Mec	-2.22±0.43	-1.16±0.66	-0.93±0.50	-1.04±0.64	-0.28±0.37	-0.29±0.38	-0.67±0.50	-0.62±0.25
logLF:Dex1	-2.79±0.54	-0.87±0.78	-0.32±0.76	-0.14±0.43	-0.01±0.41	0.10±0.41	-0.39±0.47	0.02±0.35
logLF:Dex2	-2.27±0.43	-1.10±0.63	-0.02±0.48	-0.19±0.44	-0.46±0.42	-0.16±0.35	-0.13±0.49	0.25±0.30*
logHF:Mec	0.11±0.32	-0.08±0.32	-0.03±0.25	-0.07±0.36	0.07±0.31	-0.29±0.34	-0.20±0.30	-0.22±0.41
logHF:Dex1	0.05±0.33	-0.19±0.46	1.31±0.17 [‡]	2.25±0.24 [‡]	2.34±0.24 [‡]	1.72±0.28 [‡]	1.57±0.32 [‡]	1.42±0.20 [‡]
logHF:Dex2	0.12±0.29	0.44±0.18	1.38±0.14 [‡]	2.07±0.31 [‡]	2.30±0.25 [‡]	2.56±0.25 [‡]	2.26±0.16 [‡]	1.93±0.20 [‡]
logTP:Mec	0.30±0.28	0.46±0.41	0.33±0.26	0.37±0.42	0.67±0.32	0.48±0.33	0.34±0.35	0.31±0.29
logTP:Dex1	0.19±0.31	0.61±0.38	1.71±0.20 [‡]	2.43±0.18 [‡]	2.48±0.23 [‡]	2.02±0.22 [‡]	1.80±0.28 [‡]	1.70±0.17 [‡]
logTP:Dex2	0.30±0.27	0.85±0.24	1.69±0.19 [‡]	2.20±0.31 [‡]	2.47±0.21 [‡]	2.70±0.22 [‡]	2.42±0.14 [‡]	2.16±0.18 [‡]

Abbreviations: CVP: central venous pressure, MABP: mean arterial blood pressure, HR: heart rate, logHF, LF, TP: logarithmic values of spectral powers in HF band, LF band, and total power. For differences between Mec vs. Dex1 and Mec vs. Dex2 groups: *P<0.05, [†]P<0.01, [‡]P<0.001. Data are expressed as means±SEM.

cantly improved the lung functions and facilitated weaning from ventilator (9). These results suggest that GCs may be effective also in well-established MAS, but repetitive or higher doses are needed.

Cardiovascular parameters

Administration of GCs may be associated with adverse effects on cardiovascular variables depending on the timing, dose, way of administration, as well as on the individual properties of GC. In this study, slight increase in blood pressure was observed after dexamethasone administration in evaluated time intervals, however, differences to non-treated group were not significant. Similarly, in piglet models of MAS no significant changes in MABP were observed after GCs compared to controls (6, 7, 25). GCs may influence the arterial blood pressure regulation through multiple peripheral mechanisms, including upregulation of both sympathetic nervous system and renin-angiotensin-aldosterone system (26). Bound to mineralocorticoid receptors, GCs may cause Na⁺ and water retention and subsequently induce an increase in blood pressure due to genomic renal action of aldosterone. In addition, aldosterone may exert also direct vascular effects mediated by nongenomic mechanisms, leading to increase in peripheral vascular resistance and blood pressure within several minutes (27). Nevertheless, non-significant changes in blood pressure seen after dexamethasone may be explained by its negligible mineralocorticoid activity compared to other GCs, as well

as by suppression of endogenous cortisol with substantial mineralocorticoid effects (28).

In addition, we have observed decreased heart rate after two doses of dexamethasone, with significant differences to non-treated group at 5 hours of the treatment. Similarly, Soukka et al found lower heart rate in methylprednisolone-pretreated group compared to non-treated group, with significant differences at 2 and 6 h after meconium instillation (6). Brotman et al demonstrated increase in systolic blood pressure and decrease in heart rate after dexamethasone administration in healthy volunteers (28). Decrease in heart rate by an average of 20–35 beats/min was observed also in one third of patients with pemphigus treated by pulse GCs treatment (29). Similarly to the effects on blood pressure, the mechanisms of GC influence on heart rate are also poorly understood. Decrease in heart rate can be mediated by arterial baroreflex mechanism as a response to increased blood pressure.

Nevertheless, the most pronounced cardiovascular changes related to dexamethasone administration in our study were observed in variables of frequency domain of heart rate variability. Significantly higher spectral power in HF band and total power from administration of the first dose of dexamethasone till the end of the experiment in both treated groups and higher spectral power in LF band in two-dose dexamethasone-treated group compared to non-treated group were found. Analysis of the variation

of heart rate around the mean value (heart rate variability) is used as a supplement to common investigation of the cardiovascular system to reveal already slight cardiovascular autonomic dysregulation (30–32). In humans, high-frequency fluctuations of heart rate are related to vagal activity reflecting respiratory sinus arrhythmia, while low frequency variability of heart rate is determined by sympathetic and parasympathetic activity and is related to baroreflex (30). Similarly in rabbits, Moguilevski et al using analysis of power spectrum distinction with autonomic cardiac blockade showed that parasympathetic-related component of HRV is reflected by the right side and sympathetic influence by the left side of the spectrogram (33). Nevertheless, only rare information is available about effects of GCs on the cardiovascular control mechanisms in the term newborns. The changes found in our study may indicate that dexamethasone increases predominantly the activity of parasympathetic-mediated control of the heart, but due to interspecies differences between humans and rabbits (34) we cannot exclude the participation of sympathetic influence also in HF band. The finding that similarly to rapid changes in pulmonary functions also the cardiovascular variables have changed within 30 min after dexamethasone administration further supports the participation of nongenomically mediated mechanisms in the action of dexamethasone.

Nevertheless, since cardiovascular dysregulation in MAS and effects of dexamethasone on the control mechanisms are firstly corroborated in this study, interpretation of the results is questionable. In addition, value of our results may be influenced by interspecies differences between humans and rabbits as well as by big inter-individual differences, often seen in evaluation of heart rate variability. Moreover, clinical applicability of the information obtained in meconium-instilled rabbits may be limited in newborns with MAS. Unfortunately, due to technical problems, neonatal animals are rarely used as models of MAS. Nevertheless, with respect to comparable changes observed after meconium instillation in animals and after meconium aspiration in newborns, several-week-old rabbits (3, 4, 15) and piglets (6, 8) were proven to be suitable animal models of MAS.

In conclusion, intravenous administration of dexamethasone effectively improved the lung functions in meconium-instilled rabbits, with slight trend to more pronounced improvement after two doses of dexamethasone. However, adverse effects of systemically administered GCs on cardiovascular variables should be considered, especially when GCs are used repetitively.

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