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Total Body Response to Mechanical Ventilation of Healthy Lungs: an Experimental Study in Piglets

J. KOBR [1], J. FREMUTH [1], K. PIZINGEROVÁ [1], S. FIKRLOVÁ [1], P. JEHLIČKA [1], P. HONOMICHL [1], L. ŠAŠEK [1], J. RACEK [2], O. TOPOLČAN [3]

[1]Department of Pediatrics, Pediatric Intensive Care Unit, Charles University in Prague, Faculty of Medicine in Pilsen, Czech Republic, [2]Department of Clinical Biochemistry and Hematology, Charles University in Prague, Faculty of Medicine in Pilsen, Czech Republic, [3]Laboratories of Immunoanalysis, Charles University in Prague, Faculty of Medicine in Pilsen, Czech Republic

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Summary

The objective of our study was to assess the influence of mechanical ventilation on healthy body organs. Fifteen piglets (aged 6 weeks, 19-27 kg) were anesthetized, instrumented, and divided into three groups: Group A – spontaneously breathing, group B – mechanically ventilated with tidal volume 6 ml/kg, and group C – ventilated with tidal volume 10 ml/kg for 12 hours. The parameters of lung, heart, liver and kidney functions neurohumoral regulation and systemic inflammatory reaction were recorded initially (time-1) and after 12 hours (time-12) of mechanical ventilation. At the onset of experiment (time-1) the levels of soluble adhesive molecules were higher (CAM; $P<0.01$), glomerular filtration index and free water clearance were lower ($P<0.05$) in both ventilated groups than in group A. Right ventricle myocardial performance index was higher (RIMP; $P<0.05$) in group C when compared with group A. Levels of CAM ($P<0.05$) and creatinine clearance ($P<0.01$) were higher, free water clearance was lower ($P<0.05$) in group C when compared to group B. At time-12 the RIMP ($P<0.05$) and levels of CAM were increased ($P<0.01$), creatinine clearance was decreased ($P<0.05$) in both ventilated groups compared to the same parameter at time-1. Ventilation index was higher ($P<0.05$), and hypoxemic index was lower ($P<0.01$) in group C when compared to group B. In conclusion, this study showed that mechanical ventilation induced changes compatible with early inflammatory response in healthy animals. Higher tidal volumes had detrimental effect on ventilatory parameters, reduced myocardial performance and potentiated adverse reaction of other organs.

Key words

Mechanical ventilation • Inflammatory reaction • Neurohumoral regulation • Organ function

Corresponding author

J. Kobr, Faculty Hospital in Pilsen, Alej Svobody 80, 304 60 Pilsen, Czech Republic. E-mail: kobr@fnplzen.cz

Introduction

In our clinical practice, we observe extravasal fluid retention in critically ill newborns and infants with pulmonary disease. The question is whether this fluid retention is mediated by the response of extrapulmonary organs associated with pulmonary pathology or whether it is a consequence of the ventilatory treatment.

The expression of soluble adhesive molecules and the release of cytokines from alveolar macrophages, endothelium of pulmonary vascular bed and lung tissue follow intrathoracic positive pressure changes during mechanical ventilation. Circulating inflammatory mediators induce molecular interactions, vascular permeability as well as organ microcirculation changes (Thomas 1997, Flori *et al.* 2003, Hiro *et al.* 2007). Intrathoracic volume and pressure changes limit diastolic cardiac ventricular filling and activate neurohumoral regulation to stabilize the blood circulation (Zellers *et al.* 1997). Aldosterone decreases urinary excretion of sodium, whereas brain natriuretic peptide increases

urinary sodium excretion. Activated autonomous nervous system receptors, angiotensin and catecholamines influence renal perfusion, whereas connexins influence microcirculation in the renal medulla. Renal microcirculation, membrane receptors, transport channels, and cell organels functions are regulated by signal molecules and influenced by organ, tissue, cell, and molecular interactions (Haeflinger *et al.* 2004, Wölfle *et al.* 2007, Schmit *et al.* 2008). The resulting renal functions are influenced mostly at the regional interactions (Hoste *et al.* 2006, Morgan 2007).

Our healthy animal model allowed for regular examination of global cardiac ventricular performance, function of lung, liver, and kidneys as well as neurohumoral regulation and inflammatory response. To select representative parameters for such assessment we followed our own previous experimental experience (Kobr *et al.* 2008).

Material and Method

This experimental, comparative study was approved by the multidisciplinary ethical committee of Faculty of Medicine in Pilsen, Czech Republic according to the valid regulations of the Czech Republic and European Union (Helsinki Declaration 2004), and conducted at an EU accredited experimental laboratory of the Faculty of Medicine in Pilsen.

The study included 15 white piglets, aged 6 weeks, average weight of 23 kg (range 18.8-27 kg), and sex ratio 6:9 in favor of females.

Animal model

All animals were premedicated with atropine 0.07 mg/kg (Atropin; Hoechst-Biotika, Slovakia) and azaperon 5.0 mg/kg (Stresnil; Janssen Pharmaceutica N.V., Belgium) intramuscularly. Thiopental 10.0 mg/kg (Thiopental, VUAB Pharma, Czech Republic) was used intravenously for the induction of anesthesia and tracheal intubation (endotracheal tube with internal diameter 5.5 mm, Kendall; GPS Prague, Ltd.) (Jacson and Cockcroft 2007). Thiopental at 2.0 mg/kg/h by continuous intravenous administration and bolus doses of fentanyl (5.0 μg/kg Fentanyl; Hexal AG, Germany) were used for continuous analgesia and sedation. Central venous catheter was inserted into the internal jugular vein (Certofix Paed 5F; B.Braun, Germany), arterial cannula (Arrow 22G; International CR, Germany) was surgically inserted into femoral artery. Urinary drainage was achieved by surgical cystostomy using permanent urinary catheter (Arrow Cystofix 10F; International CR, Germany). All animals were given intravenous infusion of Ringer`s solution at 2.0 ml/kg/h (Ringer, Infusia, Czech Republic) for the duration of the study.

Animals were divided into three groups based on different strategies of ventilation. Group A used as the control group included tracheally intubated and spontaneously breathing piglets, connected to the ventilator for one hour to assess lung mechanics. Animals in Group B and C were tracheally intubated and mechanically ventilated in supine position for 12 h using different tidal volumes, in Group B 6 ml/kg and in Group C 10 ml/kg. Animals were mechanically ventilated using pressure controlled setting (Elema 900C, Siemens, Germany) with constant respiratory rate 26 breaths/min, positive end-expiratory pressure 6 cm H_2O, and fraction of inspired oxygen 0.21.

Measurements and calculations

The following parameters were continuously monitored in all animals (Life Scope 9, Nihon Kohden, Japan): ECG, heart rate (HR), pulse oxymetry (SpO_2), end-tidal carbon dioxide ($etCO_2$; kPa), central venous pressure (CVP; mm Hg), systemic systolic arterial blood pressure (SBP; mm Hg), mean systemic arterial blood pressure (MABP; mm Hg) and diastolic systemic arterial blood pressure (DBP; mm Hg). Urine output (UO; ml/kg/h) and core temperature (°C) were recorded using permanent urinary catheter. The values of peak inspiratory pressure (PIP; cm H_2O), mean airway pressure (Paw; cm H_2O), respiratory rate (RR), end-expiratory pressure (PEEP; cm H_2O), tidal volume (V_T; ml/kg), minute ventilation (V_E; l/min), and fraction of inspired oxygen (FiO_2) were recorded. Calculation of the following indices was performed: alveolar-arterial oxygen tension difference ($AaDO_2$; kPa), arterio-alveolar oxygen tension difference (a/ADO_2; kPa), oxygenation index (OI), hypoxemic index ($PaO2/FiO_2$), and dead space to tidal volume ratio (V_D/V_T;%), ventilation index (VI), dynamic compliance of lung (C_{dyn}; ml/cm H_2O/kg), airway resistance (R_{aw}; cmH_2O/l/s). Echocardiographic examination was performed repeatedly (probe 3.5-5.0 Hz, Sono Line; Siemens, Germany) and the following indices were recorded: left ventricular shortening fraction (SF), and Tei-index of myocardial performance of the right (RIMP) and left (LIMP) ventricles, [IMP = (isovolumic relaxation time + isovolumic contraction time)/ejection time]. Tei-index evaluates global systolic and diastolic

function of each ventricle. Increase of the index value represents myocardial functional impairment (Tei *et al.* 1995).

The following parameters were measured in urine and serum samples: urea (mmol/l), creatinine (μmol/l), sodium (mmol/l), potassium (mmol/l), chloride (mmol/l), osmolality (mmol/kg). Alanine-aminotransferase (ALT; μkat/l), aspartate-aminotransferase (AST; μkat/l), total bilirubin (μkat/l), glucose (mmol/l), and fibrinogen (g/l) were measured in serum and plasma samples. Blood gas and acid-base analysis were obtained from systemic arterial blood.

Creatinine clearance [CrCl = (u-creatinine x urine output)/s-creatinine; ml/min], fractional excretion of sodium [FeNa = (u-Na/s-Na)/(u-creatinine/s-creatinine)], glomerular filtration index [GFI = u-Na/(u-creatinine/s-creatinine)] and free water clearance [Cfw = urine output – (urine output x u-osmolality)/s-osmolality] were calculated (Quigley and Alexander 1997).

The following immunoanalysis was performed in serum and plasma samples: interleukin 6 (IL-6; pg/ml; RD-ELISA), tumor necrotizing factor alpha (TNFα; pg/ml; RD-ELISA), intercellular adhesion molecule-1 (ICAM; ng/ml; Bender-ELISA), vascular cell adhesion molecule-1 (VCAM; ng/ml; Bender-ELISA) and brain natriuretic peptide (BNP; ng/ml; Bachem-EIA).

Study protocol

The recovery interval after endotracheal intubation and insertion of intravascular and urinary catheters took 60 min.

Clinical assessment, respiratory and circulatory parameters were recorded, and blood samples were obtained in all animal groups after recovery interval (time-1) (A, B_1, C_1); and after 12 hours (time-12) in mechanically ventilated groups (B_{12}, C_{12}). The blood samples were collected from the arterial line, urine from the urinary catheter at the same time points.

All the animals were killed at the end of the study by intravenous administration of a bolus dose of cardioplegic solution at 15 ml/kg (Infuse Thomas cum procain; Ardapharma, Czech Republic).

Statistical analysis

Parametric data were expressed as mean, 95 % CI, SEM, standard deviation. Non-parametric data were expressed as median, 95 % CI, range, interquartile range. For qualitative analysis of accuracy of the variables reference interval dispersion (Wilcoxon-Shapiro), the linearity (linear fit), average (Anderson-Darling), reproducibility (Bland-Altman) agreement were used.

The data of groups B and C were compared to a control group A; within each group before and after the 12-h interval and between groups B and C. $P<0.05$ values were considered statistically significant. All the data were analyzed using statistical software (Analyze-it 211 Software Ltd.).

Results

All measured values, laboratory analysis results, and calculated parameters from spontaneously breathing animals in group A were used as control data.

Time-1

In the parameters of ventilation the arterio-alveolar oxygen tension difference (a/ADO_2) was lower in Group C_1 when compared to Group A (0.55±0.22 vs. 0.89±0.19; P=0.019), and airway resistance (R_{aw}) was higher in Group C_1 when compared to Group B_1 (1.21±0.28 vs. 1.01±0.36; P=0.049).

The observed extrapulmonary parameters and their differences between mechanically ventilated groups (B, C) and spontaneously breathing group (A) at time-1 are summarized in Table 1. When the data in groups B and C were compared, the following differences were detected: higher ICAM levels in group C_1 compared to group B_1 (41.86±1.34 vs. 25.2±0.33; P=0.032), higher creatinine clearance in group C_1 compared to group B_1 (0.609±0.04 vs. 0.234±0.02; P=0.0002), and lower free water clearance (Cfw) in group C_1 compared to group B_1 (–0.12±0.76 vs. –0.78±0.31; P=0.044).

Data variations of other monitored parameters did not reach the level of statistical significance.

Time-12

Arterio-alveolar oxygen tension difference (a/ADO_2) was lower in group C_{12} compared to group B_{12} (0.67±0.39 vs. 0.87±0.24; P=0.021), hypoxemic index (PaO_2/FiO_2) was lower in group C_{12} compared to group B_{12} (322.97±173.50 vs. 414.12±107.62; *P*=0.040), and higher oxygenation index (OI) in group C_{12} compared to group C_1 (4.33±2.73 vs. 2.10±1.38; *P*=0.041).

The observed extra-pulmonary parameters and their differences between groups B and C at time-12 are summarized in Table 2.

Table 1. Summarized extrapulmonary parameters and their differences compared with group A in the time-1 (n=15).

Variables	A	B_1	P values	C_1	P values
	Mean ± S.D. (95 % CI)	Mean ± S.D. (95 % CI)		Mean ± S.D. (95 % CI)	
TNFα (pg/ml)	72.96 ±7.08 (6.207)	68.65 ± 13.72 (12.026)	NS	96.94 ± 51.60 (45.200)	0.050
IL-6 (pg/ml)	32.82 ± 7.75 (6.795)	32.79 ± 6.75 (5.916)	NS	29.50 ± 1.10 (1.000)	NS
BNP (ng/ml)	1.04 ± 0.56 (0.492)	1.64 ± 0.22 (0.189)	NS	1.78 ± 0.42 (0.365)	<0.01
VCAM (ng/ml)	25.88 ± 1.09 (0.951)	37.70 ± 1.02 (0.896)	<0.05	38.86 ± 0.92 (0.808)	<0.01
ICAM (ng/ml)	6.9 ± 2.27 (1.993)	25.2 ± 0.33 (0.288)	<0.01	41.86 ± 1.34 (1.176)	<0.01
ALT (μkat/l)	0.63 ± 0.13 (0.115)	0.82 ± 0.21 (0.182)	NS	0.76 ± 0.23 (0.202)	NS
AST (μkat/l)	0.62 ± 0.30 (0.264)	1.16 ± 1.35 (1.183)	NS	0.50 ± 0.12 (0.103)	NS
Bilirubin (μmol/l)	4.00 ± 1.55 (1.358)	3.40 ± 0.49 (0.429)	NS	3.60 ± 0.80 (0.701)	NS
Fibrinogen (g/l)	1.17 ± 0.22 (0.194)	1.33 ± 0.49 (0.429)	NS	1.51 ± 0.23 (0.206)	NS
Cfw	-0.68 ± 0.51 (0.448)	-0.78 ± 0.31 (0.274)	NS	-0.12 ± 0.76 (0.664)	<0.05
GFI	1101 ± 1181.60 (1035.703)	3063 ± 4537.90 (3977.543)	<0.05	2885 ± 3057 (3572.70)	<0.05
FeNa	7.56 ± 7.78 (6.822)	22.67 ± 33.89 (29.707)	<0.05	25.36 ± 36.78 (32.240)	<0.05
UO (ml/kg/h)	2.12 ± 0.53 (0.465)	1.92 ± 1.01 (0.886)	NS	1.70 ± 0.60 (0.526)	NS
CrCl (ml/min)	0.183 ± 0.03 (0.195)	0.234 ± 0.02 (0.226)	NS	0.609 ± 0.04 (0.624)	<0.01
MABP (mm Hg)	74 ± 10.46 (9.167)	77 ± 15.12 (13.251)	NS	73 ± 5.12 (4.490)	NS
SF	0.43 ± 0.12 (0.092)	0.64 ± 0.07 (0.063)	<0.05	0.57 ± 0.09 (0.082)	<0.05
RIMP	0.22 ± 0.02 (0.015)	0.24 ± 0.01 (0.012)	NS	0.25 ± 0.02 (0.020)	<0.05
LIMP	0.30 ± 0.03 (0.024)	0.30 ± 0.04 (0.031)	NS	0.30 ± 0.04 .034)	NS

A – Spontaneous breathing, B_1 – Mechanical ventilation V_T 6 ml/kg in the time-1, C_1 – Mechanical ventilation V_T 10 ml/kg in the time-1, TNFα – tumor necrotizing factor alpha, IL-6 – interleukin 6, BNP – brain natriuretic peptide, VCAM – vascular cell adhesion molecule, ICAM – intercellular adhesion molecule, ALT – alanin-aminotransferase, AST – aspartate-aminotransferase, Bilirubin – total bilirubin value, Fibrinogen – fribrinogen value, Cfw – free water clearance, GFI – glomerular filtration index, FeNa – fractional excretion of sodium, UO – one-hour diuresis, CrCl – creatinine clearance, MABP – mean arterial blood pressure, SF – shortening fraction of left ventricle, RIMP – myocardial performance index of right ventricle, LIMP – myocardial performance index of left ventricle. Values are given as mean ± S.D., 95 % confidence interval (95 % CI). NS - Not significant.

Significant differences in renal indices were observed only in group C with free water clearance (Cfw) lower in group C_{12} compared to group C_1 (P=0.040), one-hour diuresis (UO) lower in group C_{12} compared to group C_1 (P=0.008). Significant changes in myocardial function were observed in the left ventricular shortening fraction being lower in group B_{12} compared to group B_1 (P=0.037) and group C_{12} compared to group C_1 (P=0.041). RIMP was higher in group B_{12} compared to group B_1 (P=0.0085), and in group C_{12} compared to group C_1 (P=0.019). LIMP was higher in group C_{12} compared to group C_1 (P=0.032).

The summarized data (n=25) were analyzed and levels of relevance, casual relation, odds and the reliability were determined. Interesting correlation, regression, level of reliability and confidence interval of targeted dependent and independent variables are summarized in Table 3. The multivariate analysis of renal parameters as target of dependent variables demonstrated that free water clearance did not correlate with FeNa, GFI, CrCl or UO; glomerular filtration index did not correlate with LIMP, UO or MABP, and one-hour diuresis did not correlate with GFI, CrCl, LIMP or SF.

Regression analysis clarified following findings. Glomerular filtration was correlated with creatinine clearance (r=0.723; p<0.01) but not with one-hour diuresis (r=0.029; p=0.842), mean arterial pressure (r=0.321; p=0.588) or left ventricle performance (r=0.097; p=0.923). One-hour diuresis ware was influenced by mean of arterial pressure (r=0.487; p<0.05) but not by glomerular filtration (r=0.029; p=0.772), shortening fraction or the left ventricle performance. An excretion of sodium correlated with glomerular filtration (r=0.994; p<0.01). The influence of the aldosterone on decrease of sodium and increase of potassium urinary excretion was present after 12 h demonstrated by their urine losses (ratio of values sodium/potassium in urine permanently below 1.0).

Discussion

Respiratory failure is the most common indication of mechanical ventilation in the pediatric intensive care units. In contrast, indications for mechanical ventilation of otherwise healthy children are rare. Scientific literature does not provide enough information about the direct impact of mechanical ventilation on changes in the functions of extrapulmonary organs or systems.

Table 2. Extrapulmonary parameters and their differences in the time-12 (n=10).

Variables	B_{12} Mean ± S.D. (95 % CI)	C_{12} Mean ± S.D. (95 % CI)	P values
TNFα (pg/ml)	49.42 ± 24.24 (21.244)	36.58 ± 14.93 (13.083)	NS
IL-6 (pg/ml)	92.18 ± 15.14 (13.269)	37.50 ± 15.37 (13.468)	NS
BNP (ng/ml)	1.38 ± 0.39 (0.344)	1.46 ± 0.26 (0.226)	NS
VCAM (ng/ml)	37.52 ± 0.77 (0.673)	40.04 ± 0.66 (0.575)	<0.05
ICAM (ng/ml)	30.22 ± 1.77 (1.556)	66.66 ± 1.59 (1.393)	<0.01
ALT (μkat/l)	0.74 ± 0.26 (0.224)	0.72 ± 0.22 (0.190)	NS
AST (μkat/l)	1.18 ± 1.32 (1.156)	0.99 ± 0.14 (0.125)	NS
Bilirubin (μmol/l)	11.60 ± 13.88 (12.165)	4.60 ± 2.24 (1.968)	NS
Fibrinogen (g/l)	1.22 ± 0.39 (0.343)	1.39 ± 0.38 (0.330)	NS
Cfw	0.14 ± 1.53 (1.345)	-1.04 ± 0.23 (0.203)	NS
GFI	390.18 ± 588.33 (515.680)	251.10 ± 128.84 (112.930)	NS
FeNa	2.54 ± 4.35 (3.810)	1.89 ± 1.01 (0.890)	NS
UO (ml/kg/h)	1.30 ± 0.48 (0.419)	0.96 ± 0.70 (0.614)	NS
CrCl (ml/min)	0.123 ± 0.02 (0.661)	0.254 ± 0.05 (0.821)	<0.05
MABP (mm Hg)	89 ± 6.97 (6.108)	81 ± 7.20 (6.311)	NS
SF	0.52 ± 0.05 (0.047)	0.43 ± 0.13 (0.118)	NS
RIMP	0.31 ± 0.03 (0.029)	0.30 ± 0.03 (0.028)	NS
LIMP	0.35 ± 0.05 (0.043)	0.38 ± 0.05 (0.047)	NS

B_{12} – mechanical ventilation V_T 6 ml/kg in the time-12, C_{12} – mechanical ventilation V_T 10 ml/kg in the time-12, TNFα – tumor necrotizing factor alpha, IL-6 – interleukin 6, VCAM – vascular cell adhesion molecule, ICAM – intercellular adhesion molecule, ALT – alanine-aminotransferase, AST – aspartate-amino-transferase, Bilirubin – total bilirubin values, Fibrinogen – fribrinogen values, BNP – brain natriuretic peptide, Cfw – free water clearance, GFI – glomerular filtration index, FeNa – fractional excretion of sodium, UO – one-hour diuresis, CrCl – creatinine clearance, MABP – mean arterial blood pressure, SF – shortening fraction of left ventricle, RIMP – myocardial performance index of right ventricle, LIMP – myocardial performance index of left ventricle. Values are stated as mean ± S.D., 95 % confidence interval (95 % CI). NS - Not significant.

Table 3. Correlation, regression and data reliability (n=25).

Dependent variables	Independent variables	R^2	p	t	95 % CI intervals	F	P values
Cfw	*FeNa*	0.085	NS	0.007	-13.79 to 9.25	0.166	NS
	GFI	0.061	NS	0.004	-1412 to 1214	0.085	NS
	UO	0.115	NS	0.013	-0.27 to 0.47	0.307	NS
FeNa	*GFI*	0.994	<0.01	0.998	115.93 to 127.51	19.04	<0.05
	TNFα	0.667	<0.01	0.444	0.48 to 1.36	18.39	<0.01
	$AaDO_2$	0.163	NS	0.027	-0.19 to 0.43	0.630	<0.05
GFI	*UO*	0.029	NS	0.001	-0.0001 to 0.0001	0.019	NS
	CrCl	0.723	<0.01	0.637	0.288 to 0.455	3.019	<0.05
	LIMP	0.097	NS	0.009	-0.001 to 0.002	0.219	NS
	MABP	0.327	NS	0.107	-0.003 to 0.0003	2.747	NS
UO	*ICAM*	0.426	<0.01	0.181	-0.03 to -0.001	5.099	<0.05
	MABP	0.487	<0.05	0.238	-0.01 to -0.06	7.165	<0.05
	CrCl	0.505	<0.05	0.331	0.388 to 0.405	4.028	<0.05
	GFI	0.029	NS	0.001	-1514 to 1732	0.019	NS
	LIMP	0.288	NS	0.083	-0.046 to 0.057	2.080	NS
	SF	0.065	NS	0.004	-0.077 to 0.288	0.099	NS

Cfw – free water clearance, FeNa – fractional excretion of sodium, GFI – glomerular filtration index, UO – one-hour diuresis, CrCl – creatinine clearance, TNFα – tumor necrotizing factor alpha, $AaDO_2$ – alveolar-arterial oxygen tension difference, LIMP – myocardial performance index of left ventricle, MABP – mean arterial blood pressure, ICAM v intercellular adhesion molecule, SF – shortening fraction of left ventricle. NS – Not significant.

The aim of our study was to assess the influence of mechanical ventilation of healthy lungs on the function of extrapulmonary organs. We used healthy experimental animals to exclude the influence of other primary organ pathology and to eliminate the confounding influence of hypoxia and/or hypercapnia.

Several clinical and experimental studies focusing on renopulmonary interaction during mechanical ventilation of diseased lungs were published in the last few years. Clinical study demonstrated a protective influence of the airway pressure release ventilation on renal function in patients with acute lung injury (Hering *et al.* 2002), renal failure as a result of cardio-renal syndrome in acute or chronic heart failure (Liang *et al.* 2007), and the need for optimal interventional treatment strategy including mechanical ventilation in patients with renal failure (Kuiper *et al.* 2005). Experimental studies with lung impairment in murine model referred to an effect of a particular strategy of mechanical ventilation on development of systemic organ inflammation (Gurkan *et al.* 2003) and mechanical ventilation induced inflammatory reaction leading to pulmonary, hepatic and renal dysfunctions in experimental pneumonia (Dhanireddy *et al.* 2006). There is also experimental evidence for renal functional impairment in dogs with acute lung injury after aspiration of gastric content (Hoag *et al.* 2008) or non-protective strategy of mechanical ventilation causing acute lung injury and exclusively renal functional changes without morphological changes (Wolthuis *et al.* 2009). All the so far published data from clinical and experimental studies could not fully exclude the effect of underlying lung pathology and/or abnormal cardiopulmonary interactions on the function of extrapulmonary organ systems.

The results of our study which support the so far published data, bring some new findings. Remarkable changes were found after only one hour of mechanical ventilation leading to the development of systemic inflammatory response, reduction of the right ventricular myocardial performance, and subsequent activation of neurohumoral reaction. Early development of systemic inflammatory response was confirmed by higher serum levels of both soluble adhesive molecules compared with the group of spontaneously breathing piglets (P<0.01). Deterioration of the right ventricular myocardial performance was detected by higher values of Tei-index in comparison with spontaneously breathing piglets (P<0.05). Interestingly, this right ventricular functional

myocardial deterioration during the first hour of mechanical ventilation did not correlate with brain natriuretic peptide levels. This could be explained by subtle changes of right ventricular myocardial performance and/or activation of other neurohumoral autoregulations preventing cardiac atrial dilatation.

After 12 h of mechanical ventilation this situation changed completely. Over time, mechanical ventilation resulted in a further reduction of biventricular myocardial performance ($P<0.01$), development of systemic and pulmonary venous congestion, and expected elevation of serum levels of brain natriuretic peptide. Tei index of myocardial performance assesses both systolic and diastolic function of cardiac ventricles and appears to be sensitive to changes of preload and afterload in the acute clinical and experimental settings (Tei *et al.* 1995, Cheung *et al.* 2004). One can also speculate about mechanical ventilation affecting systolic function or ventricular afterload to a larger extent than preload and diastolic function, therefore not leading readily to atrial distension and increased brain natriuretic peptide levels. This explanation is supported by the fact that no correlation was found between the values of Tei index and levels of brain natriuretic peptide in our study.

Activation and time-related changes of the neurohumoral regulation can explain higher left ventricular contractility ($P<0.05$) and higher creatinine clearance; urinary sodium excretion as well as glomerular filtration ($P<0.05$) in the first hour compared with a group of spontaneously breathing animals.

Mechanical ventilation using higher tidal volumes led to progressive changes in inflammatory response in our study. Increased tidal volumes during artificial ventilation cause alveolar distension. This mechanical stimulus induces expression of soluble adhesive molecules and cytokine activation (Barton and Mahony 1997, Thomas 1997). The effect of mechanical ventilation on oxygenation, systemic blood pressure, one-hour diuresis, and on liver function was minimal. As expected, one-hour diuresis was dependent on systemic mean arterial pressure.

We have to admit that our selected set of biochemical parameters provides only indirect information about the activation of hormonal renin-angiotensin-aldosterone system. The changes of sodium excretion and presence of neurohumoral regulation should be supported by a direct measurement of peptides and hormones. Indices like free water clearance and fractional excretion of sodium are remarkable indicators of aldosterone effect.

We are aware that the results of our study could been partially limited by a dispersion of the renal data (Anderson-Darling; $p\geq0.05$) caused by either post-analytical mathematical calculations of the laboratory values, or pre-analytical stress in individual animals influenced by invasive interventions. Indexed data were sufficiently accurate (Bland-Altman; $p<0.01$) as the concept was not based on absolute values assessment but their differences.

Results of our study show that mechanical ventilation of healthy lungs activates early inflammatory response and triggers neurohumoral regulation. After 12 h of mechanical ventilation, effect of aldosterone persisted with reduction of free water clearance. Detrimental mechanical ventilatory strategy with higher tidal volume contributed to reduction of both ventricular myocardial performance, to reduction of endogenous creatinine clearance and one-hour diuresis. Based on our data, the use of protective strategy in mechanical ventilation and an early extubation have priority in daily pediatric clinical practice.

Conflict of Interest

There is no conflict of interest.

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