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Original Article

Lung-borne systemic inflammation in mechanically ventilated infant rats due to high PEEP, oxygen, and hypocapnia

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Abstract: Background: Intensive care practice calls for ventilator adjustments due to fast-changing clinical conditions in ventilated critically ill children. These adaptations include positive end-expiratory pressure (PEEP), fraction of inspired oxygen (FiO_2), and respiratory rate (RR). It is unclear which alterations in ventilator settings trigger a significant systemic inflammatory response. Methods: Fourteen-day old Wistar rat pups were randomized to the following groups: (a) "control" with tidal volume ~8 mL/kg, PEEP 5 cmH_2O, FiO_2 0.4, RR 90 min^{-1}, (b) "PEEP 1", (c) "PEEP 9" (d) "FiO_2 0.21", (e) "FiO_2 1.0", (f) "hypocapnia" with RR of 180 min^{-1}, and (g) "hypercapnia" with RR of 60 min^{-1}. Following 120 min of mechanical ventilation, plasma for inflammatory biomarker analyses was obtained by direct cardiac puncture at the end of the experiment. Results: Interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were driven by FiO_2 0.4 and 1.0 (P=0.02, P<0.01, respectively), tissue plasminogen activator inhibitor type-1 (tPAI-1) was increased by high PEEP (9 cmH_2O, P<0.05) and hypocapnia (P<0.05), and TNF-α was significantly lower in hypercapnia (P<0.01). Tissue inhibitor of metalloproteinase-1 (TIMP-1), cytokine-induced neutrophil chemoattractant 1 (CINC-1), connective tissue growth factor (CTGF), and monocyte chemoattractant protein-1 (MCP-1) remained unaffected. Conclusion: Alterations of PEEP, FiO_2, and respiratory frequency induced a significant systemic inflammatory response in plasma of infant rats. These findings underscore the importance of lung-protective ventilation strategies. However, future studies are needed to clarify whether ventilation induced systemic inflammation in animal models is pathophysiologically relevant to human infants.

Keywords: Interleukin-6, tumor necrosis factor-α, tissue plasminogen activator inhibitor type-1, hyperoxia, positive end-expiratory pressure, hypocapnia

Introduction

Mechanical ventilation (MV) is fundamental for treatment of critically ill adult and pediatric patients with respiratory failure [1, 2]. In fact, most admissions to the intensive care units during the current coronavirus disease 2019 (COVID-19) are associated with an exaggerated immune response leading to severe respiratory distress requiring MV [3]. Importantly, susceptibility to ventilator associated lung injury seems to differ in adults and children. Clinical studies in children did not confirm an association between higher V_T and mortality [4-7]. One study by Sousse et al., evaluating supraphysiologic V_T of 15 ml/kg in pediatric burn patients [8], showed a higher pneumothorax rate, but also fewer days on the ventilator, lower positive end-expiratory pressures (PEEP), and a lower incidence of atelectasis. These findings were replicated in translational two-hit infant rat *in-vivo* models comparing V_T 21 ml/kg and PEEP 1 cmH_2O to physiologic V_T of 7 ml/kg and PEEP 5 cmH_2O. After 4 h of ventilation, lung mechanics

improved the most in the high tidal group without histologic lung compromise [9, 10]. The published data suggest that age specific differences in susceptibility towards ventilator induced lung injury (VILI) exist likewise in humans and rats.

Lung injury has multiple facets and includes local and systemic biotrauma. The term biotrauma refers to pulmonary inflammatory responses to well-known physical stressors such as baro-, volu-, and atelectrauma [11]. Physiological V_T of 6-8 mL/kg and PEEP levels of 5-10 cmH_2O are generally believed to prevent or minimize biotrauma [12]. However, clinical practice with critically ill children demands multiple adjustments of respirator settings due to rapidly changing conditions such as severe hypoxemia, acidosis, air leaks, or cardiovascular deterioration. As a result, PEEP levels, fraction of inspired oxygen (FiO_2), and respiratory rates (RR) will frequently be modified. Although crucial for the outcome of critically ill children, it is not clear whether or how clinically necessary changes of ventilator settings trigger or promote systemic inflammatory cascades.

There is evidence that even short periods of ventilation (2 h) induce systemic inflammatory reactions in healthy adult rats [13] and that the magnitude of the inflammatory reaction in ventilated patients is age-dependent [7]. For ethical reasons, it is not possible to systematically test the impact of the above-mentioned ventilator settings on systemic inflammation in human infants. Therefore, highly standardized animal models hold important translational value.

As most animal studies in this research field were performed with adult animals, we aimed to evaluate plasma immune response to clinically relevant variations in PEEP, FiO_2, and RR in healthy infant rats. We hypothesized that these deviations from a recommended standard open-lung strategy would cause early and significant increases of lung-borne inflammatory biomarkers (interleukin-6 (IL-6); tumor necrosis factor-α (TNF-α); tissue plasminogen activator inhibitor type-1 (tPAI-1); tissue inhibitor of metalloproteinase-1 (TIMP-1); cytokine-induced neutrophil chemoattractant 1 (CINC-1); connective tissue growth factor (CTGF); and monocyte chemoattractant protein-1 (MCP-1)) in the systemic circulation of infant Wistar rats.

Methods

Ethical approval

All animal experiments mentioned in this study were approved by the cantonal authority of Zurich according to the Swiss Animal Welfare Legislation (reference number 50/2010).

Experimental design

Details about animal deliveries and housing, experimental design, animal preparation, and blood sample processing were published previously [14, 15]. In brief, 14 days old infant Wistar rats of either sex were anesthetized with inhalative isoflurane and a consecutive intraperitoneal (IP) injection of ketamine (75 µg/g body weight, BW) and xylazine (12 µg/g BW) solution. Tracheostomy was performed and animals were connected by tracheal tube to a computer-controlled ventilator (flexiVent®, Scireq, Montreal, Canada) using the following baseline settings: tidal volume (V_T) ~8.0 mL/kg, PEEP 5 cmH_2O, RR 90 min^{-1}, and FiO_2 0.4. Initially, lung volume history was standardized with two lung volume recruitment maneuvers (up to 40 cmH_2O with 9-s ramp and 3-s plateau) separated by a pressure-volume (PV) maneuver (from 0 cmH_2O to 25 cmH_2O, 7-s ramp and 7-s decrease) within 2 minutes. Heart rate (HR) and peripheral oxygen saturation (SpO_2) were continuously monitored, body temperature was checked and actively controlled. Animals were randomized to the following groups. "Control" (i.e. MV with V_T ~8 mL/kg, PEEP 5 cmH_2O, FiO_2 0.4, RR 90 min^{-1}), "PEEP 1" (PEEP 1 cmH_2O, rest of settings unchanged) and "PEEP 9" (PEEP 9 cmH_2O, RR 120 min^{-1}, rest of settings unchanged). RR in the "PEEP 9" group was set to 120 min^{-1} in order to achieve standard blood gas values. In "FiO_2 0.21" and "FiO_2 1.0" inspired fractions of 0.21 and 1.0 were applied with the rest of ventilator settings as set at baseline. Lastly, "hypocapnia" and "hypercapnia" were induced by RR of 180 min^{-1} and 60 min^{-1}, respectively, with the rest of ventilator settings unchanged. At 30 min after the start of the experiment, all animals received 0.5 mL 0.9% NaCl IP to avoid dehydration. The targeted base-exchange condition was verified by mixed blood gas analysis drawn from direct cardiac puncture after a partial laparotomy and sternotomy at the end of

Table 1. Biomarker concentrations in plasma of infant rats after two hours of mechanical ventilation

Biomarker / Group	IL-6 (ng/mL)	TNF-α (ng/mL)	t-PAI-1 (ng/mL)	TIMP-1 (ng/mL)	CINC-1 (ng/mL)	CTGF (ng/mL)	MCP-1 (ng/mL)
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Control (n=7)	2.0 (1.2-2.5)	0.11 (0.08-0.18)	6.5 (4.1-9.4)	84.2 (71.8-103.0)	12.0 (11.7-15.7)	9.4 (0.19-13.5)	3.6 (3.0-3.8)
PEEP1 (n=8)	1.7 (0.8-1.8)	0.03 (0.02-0.07)	4.1 (3.7-6.1)	86.5 (59.4-99.4)	15.0 (10.7-19.5)	4.3 (0.19-14.5)	3.4 (2.6-4.1)
PEEP9 (n=9)	1.6 (0.7-2.5)	0.02 (0.02-0.94)	11.5* (7.5-15.1)	103.1 (66.6-120.1)	12.0 (9.5-12.7)	0.2 (0.2-8.3)	3.8 (2.7-4.1)
FiO_2 0.21 (n=10)	1.3* (0.1-1.5)	0.03* (0.02-0.09)	10.1 (6.1-15.1)	88.0 (79.8-102.3)	10.4 (7.1-14.3)	2.8 (1.6-19.8)	3.4 (2.7-3.8)
FiO_2 1.0 (n=9)	1.8 (1.5-2.3)	0.08 (0.03-0.09)	6.4 (5.6-11.9)	99.5 (88.0-118.4)	14.2 (12.2-17.2)	4.9 (1.4-10.0)	3.9 (2.3-4.4)
Hypocapnia (n=10)	1.8 (0.3-2.2)	0.05 (0.02-0.15)	15.8* (9.8-26.8)	87.2 (50.1-105.5)	9.7 (8.8-12.3)	1.4 (0.2-10.3)	4.0 (3.1-4.1)
Hypercapnia (n=10)	0.9 (0.5-1.7)	0.03* (0.02-0.04)	3.5 (3.1-4.1)	72.5 (60.3-87.9)	14.5 (9.2-16.9)	2.3 (0.2-6.0)	3.7 (3.1-4.7)

Data are expressed as group medians (interquartile range, IQR). Experimental groups consisted of: control group: exposed to fraction of inspired oxygen (FiO_2) 0.4, positive end-expiratory pressure 5 cmH_2O (PEEP 5), and normocapnia using respiratory rate of 90 min^{-1}; PEEP 1 group: PEEP 1 cmH_2O, rest of settings unchanged; PEEP 9 group: PEEP 9 cmH_2O, RR 120 min^{-1}, rest of settings unchanged, RR in the PEEP 9 group was set at 120 min^{-1} in order to achieve standard blood gas values; FiO_2 0.21 and FiO_2 1.0 groups: inspired fractions of oxygen 0.21 and 1.0 (respectively) with the rest of settings unchanged; hypocapnia and hypercapnia groups: RR of 180 min^{-1} and 60 min^{-1} (respectively) with the rest of ventilator settings unchanged. *P<0.05 was considered as significant when compared to control. Abbreviations: IL-6, Interleukin-6, TNF-α, tumor necrosis factor-α; t-PAI-1, tissue plasminogen activator inhibitor type-1; TIMP-1, tissue inhibitor of metalloproteinase-1; CINC-1, cytokine-induced neutrophil chemoattractant 1; CTGF, connective tissue growth factor; MCP-1, and monocyte chemoattractant protein-1; IQR, interquartile range.

the experiment [15]. After 2 h, the animals were euthanized through exsanguination under full analgosedation. Plasma was stored in EDTA tubes at -80°C until analysis.

Analysis of inflammatory biomarkers

Inflammatory biomarkers in stored plasma samples were assayed between November 2014 and January 2015 in triplets in a blinded fashion according to manufacturer's instructions. Determination of plasma concentrations of IL-6, TNF-α, tPAI-1, TIMP-1, CINC-1, CTGF, and MCP-1 were performed using Rat Vascular Injury Magnetic Bead Panel 1 (RV1MAG-26K) along with Luminex® xMAP® technology by EMD Millipore MILLIPLEX® system. Plasma samples were diluted 1:4 in the serum matrix provided in the kit.

Statistical analysis

Statistical analyses were performed using the software package IBM SPSS Statistics for Windows, Version 25.0 (Armonk, NY, USA). Biomarker values below the limit of detection (LOD) were replaced by LOD/√2 to avoid left-censoring of data. Shapiro-Wilk test was used for assessment of normality. One-way ANOVA was used for group comparison of parametric data. If group means were significantly different, Holm-Sidak post hoc test was used for pairwise comparison of means. Kruskal-Wallis test was used for group comparison of non-parametric data; Dunn-Bonferroni test was used for post-hoc pairwise comparison in case of significant differences within non-parametric groups. Results were Bonferroni-corrected for multiple testing. Values are reported as mean ± standard deviation (SD), unless otherwise stated. Statistically significant data are additionally expressed as vertical box plots with median, 25th and 75th quantile and whiskers equal 25th quantile -1.5 IQR and 75th quantile +1.5 IQR. SpO_2 progression with FiO_2 0.21 was formulated as a scatter plot with a regression line. Statistical significance was set at P<0.05.

Results

Baseline values

At the age of 14 days, animals weighed 36.2 g (3.6 g) (mean (SD)). Infant rats in the FiO_2 0.21 group displayed significantly lower mean SpO_2 77.6% (12.6) than control animals ventilated with FiO_2 0.4 (98.5 % (0.5); P<0.05). All other animals showed mean SpO_2 values in the range of 98.2-98.9%. Animals in the hypocapnia group displayed significantly lower and animals in the hypercapnia group significantly higher mean partial pressures of carbon dioxide (pCO_2) than control animals (mean (SD) kPa; 3.5 (0.7); 8.0 (1.4); 5.2 (0.3); P<0.05, respectively). Values of SpO_2, HR, and pCO_2 for all experimental groups can be seen in Baumann et al., 2018 [15].

Influence of PEEP on inflammatory biomarkers

tPAI-1 was significantly higher in animals ventilated with PEEP 9 cmH_2O in comparison with the control group (P=0.02, **Table 1**; **Figure 1**). PEEP variations revealed no group differences with the other biomarkers.

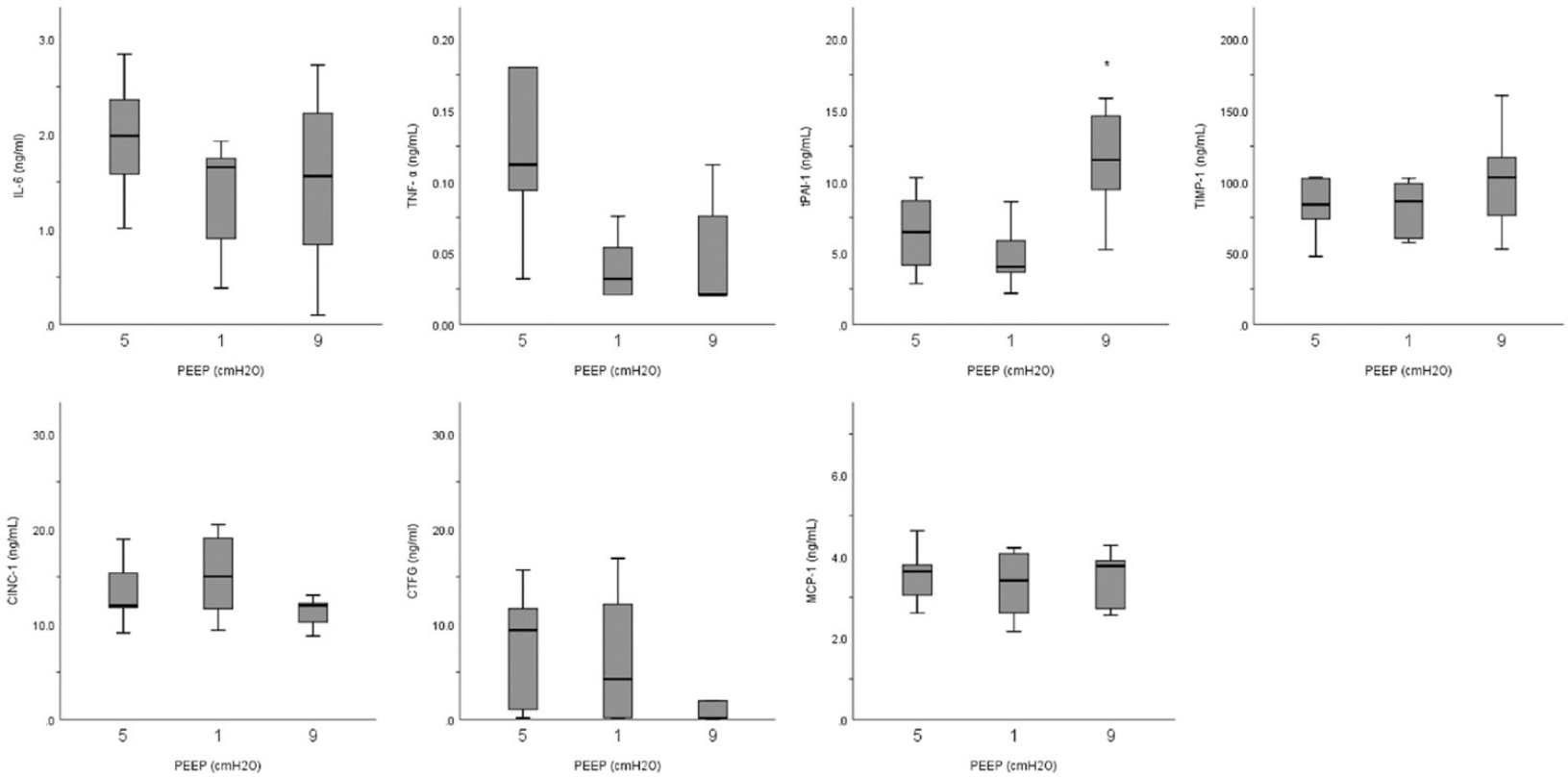

Figure 1. Influence of different PEEP levels on plasma biomarkers. Biomarker concentrations in plasma of two-week old Wistar rats after exposure to mechanical ventilation for two hours with different PEEP levels. Data are expressed as vertical box plots with median, 25th, and 75th quantile and whiskers equal 25th quantile -1.5 IQR and 75th quantile +1.5 IQR. *P<0.05 was considered significantly different compared to control. Abbreviations: PEEP, positive end-expiratory pressure; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; t-PAI-1, tissue plasminogen activator inhibitor type-1; TIMP-1, tissue inhibitor of metalloproteinase-1; CINC-1, cytokine-induced neutrophil chemoattractant 1; CTGF, connective tissue growth factor; MCP-1, and monocyte chemoattractant protein-1.

Influence of FiO_2 on inflammatory biomarkers

IL-6 and TNF-α were significantly driven by FiO_2 0.4 (control group) and 1.0 in comparison with FiO_2 0.21 (P=0.02, and P<0.01, respectively). Variations in FiO_2 had no statistically relevant effect on the rest of the biomarkers (**Table 1**; **Figure 2**).

Influence of hypocapnia and hypercapnia on inflammatory biomarkers

TNF-α was significantly lower in hypercapnia (P=0.01) and tPAI-1 was significantly higher in hypocapnia in comparison with controls (P=0.01), the rest of the biomarkers remained unchanged (**Table 1**; **Figure 3**).

Biomarkers not affected by ventilator settings

TIMP-1, CINC-1, CTGF, and MCP-1 were not significantly affected by any of the experimental ventilation or oxygen supply strategies (**Table 1**).

Discussion

Mechanical ventilation is indispensable in pediatric general anesthesia and intensive care medicine. Exhaustive research on protective ventilation in both adult human patients and adult animal models led to ventilation standards containing or minimizing ventilator associated lung injury. However, little is known about early effects of clinically unavoidable and repeated bedside ventilator adjustments according to patients' individual and rapidly changing requirements on the immune response in children. We present an experimental study evaluating short-term effects of low and high PEEP, FiO_2, and blood carbon dioxide on plasma inflammatory biomarkers in ventilated healthy infant rats. PEEP level of 9 cmH_2O, supplemental oxygen, and high respiratory frequencies resulting in hypocapnia aggravated the inflammatory response. Thus, our hypothesis that alterations of ventilation settings on the respirator cause significant increases of inflammatory biomarkers in plasma was confirmed by the increase of IL-6, TNF-α, and tPAI-1 within two hours.

Effect of mechanical ventilation on inflammation

Elevated plasma concentrations of IL-6 are found in infectious, traumatic, and inflammatory states, rising within minutes and remaining elevated up to several days [16]. According to data obtained from non-ventilated controls [9], IL-6 plasma concentrations of at least ≈1 ng/mL correspond to >10-fold increase in all groups of this study. Hence, it is conceivable that mechanical ventilation *per se* induced a substantial systemic inflammatory reaction. This finding is in line with previous studies showing that IL-6 concentrations rise in tracheal aspirates of healthy children ventilated for 120 min during routine anesthesia [17] and that IL-6 increased in serum of healthy adult rats ventilated for 120 min [13]. However, it is also possible that the surgical procedure of tracheostomy contributed to the systemic IL-6 release [16].

Effect of mechanical ventilation with different PEEP

PEEP is an important element of modern artificial ventilation strategies in patients with both healthy and pre-injured lungs [4]. PEEP maintains a patent airway throughout the mechanical breath cycle and prevents alveolar collapse with development of detrimental atelectasis. In general, a minimum PEEP of 5 cmH_2O is recommended for the so-called open-lung strategy in healthy human patients, both in adults and children [5, 12]. In sick children, a lower PEEP level might be required in presence of barotrauma, such as pneumothorax or lung emphysema. In our study, we did not detect any significant alterations of biomarkers after PEEP reduction to 1 cmH_2O, most likely due to lack of relevant stress and strain.

A higher PEEP might be necessary in situations with reduced compliance and/or reduced functional residual capacity, such as lung edema, massive postoperative capillary leak syndrome, and acute respiratory distress syndrome (ARDS) [12, 18]. Yet, a higher PEEP raises intrathoracic pressure, reduces venous return to the right heart and thus cardiac output into the systemic circulation [19]. Therefore, peripheral organ perfusion can be diminished as e.g. reflected by reduced urine output [14]. In this study, an elevated PEEP of 9 cmH_2O drove only tPAI-1 plasma concentrations, all other biomarkers remained unaffected. tPAI-1 is an inhibitor of the tissue-type plasminogen activator (tPA) and has its principal function in regulating fibrinolysis by inhibition of plasminogen activation [20]. Moreover, it can cause an

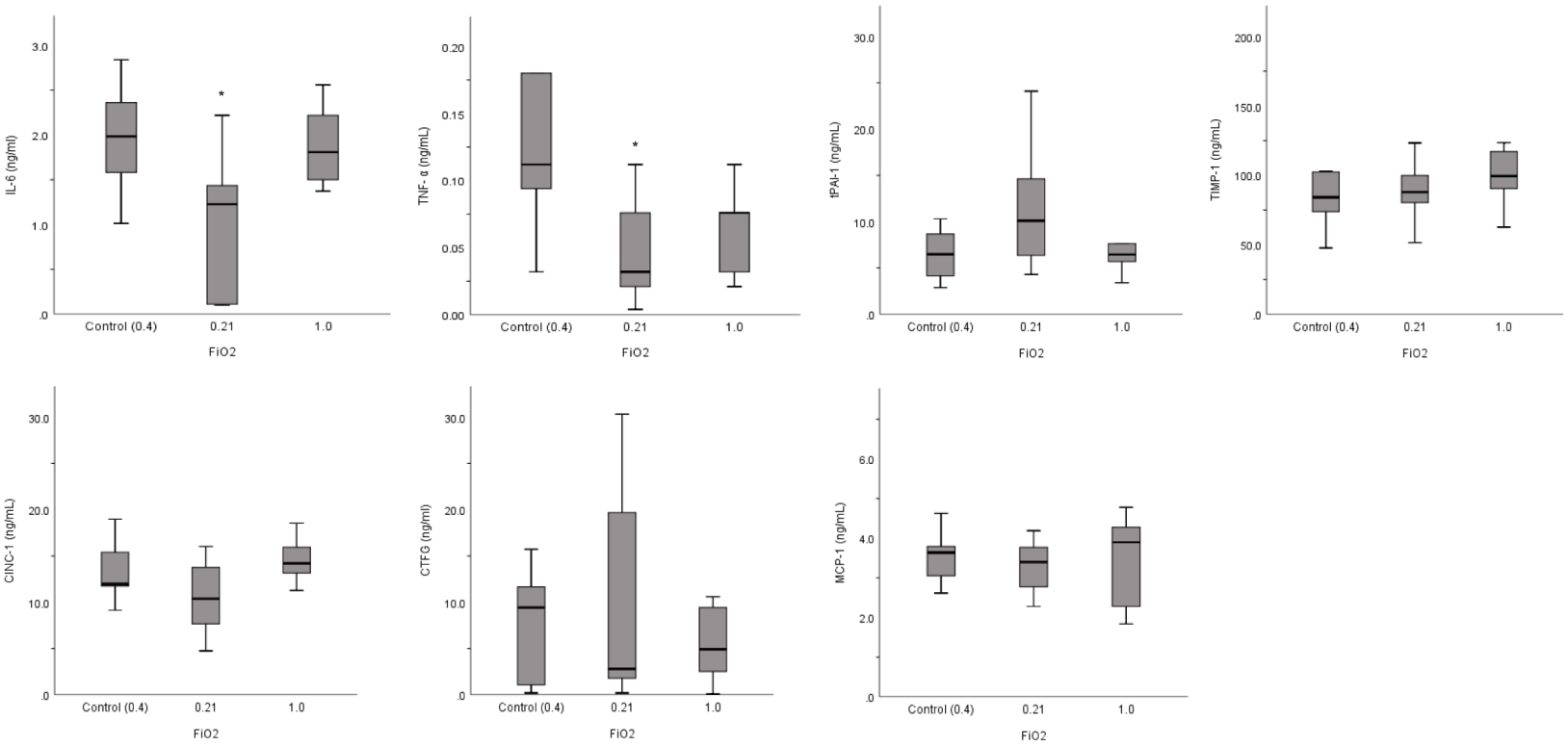

Figure 2. Influence of different FiO_2 on plasma biomarkers. Biomarker concentrations in plasma of two-week old Wistar rats after exposure to mechanical ventilation for two hours with different FiO_2. Normoxemia: FiO_2 0.4; hypoxemia: FiO_2 0.21; hyperoxemia: FiO_2 1.0. Data are expressed as vertical box plots with median, 25th, and 75th quantile and whiskers equal 25th quantile -1.5 IQR and 75th quantile +1.5 IQR. *P<0.05 was considered as significantly different when compared to control. Abbreviations: FiO_2, fraction of inspired oxygen; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; tPAI-1, tissue plasminogen activator inhibitor type-1; TIMP-1, tissue inhibitor of metalloproteinase-1; CINC-1, cytokine-induced neutrophil chemoattractant 1; CTGF, connective tissue growth factor; MCP-1, monocyte chemoattractant protein-1.

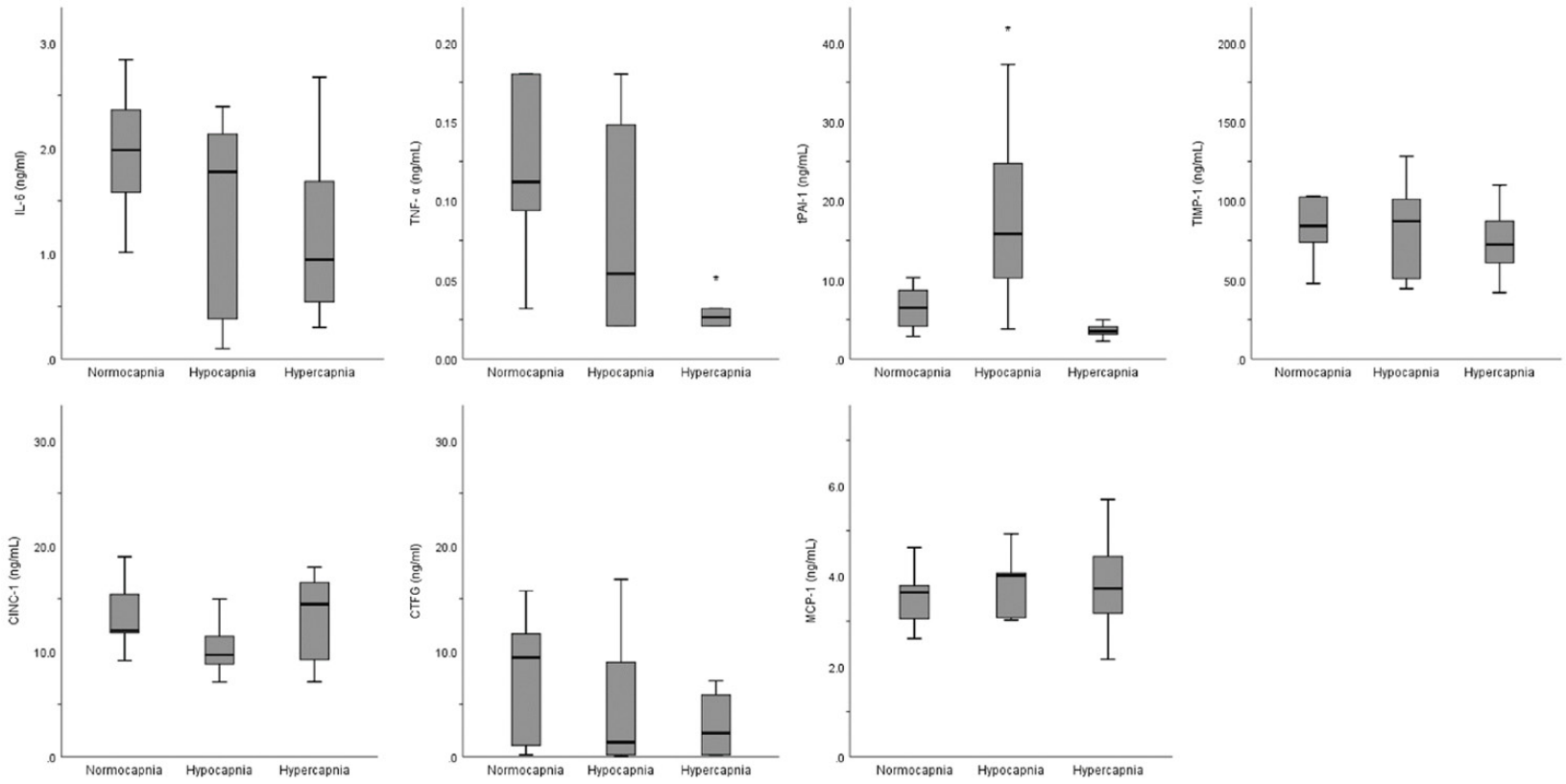

Figure 3. Influence of pCO_2 on plasma biomarkers. Biomarker concentrations in plasma of two-week old Wistar rats after exposure to mechanical ventilation for two hours with different respiratory rates (RR) resulting in respiratory normocapnia (RR 90 min^{-1}), hypocapnia (RR 180 min^{-1}), and hypercapnia (RR 60 min^{-1}). Data are expressed as vertical box plots with median, 25th and 75th quantile and whiskers equal 25th quantile -1.5 IQR and 75th quantile +1.5 IQR. *P<0.05 was considered as significantly different when compared to control. Abbreviations: pCO_2; partial blood carbon dioxide pressures; IL-6, interleukin-6, TNF-α, tumor necrosis factor-α; t-PAI-1, tissue plasminogen activator inhibitor type-1; TIMP-1, tissue inhibitor of metalloproteinase-1; CINC-1, cytokine-induced neutrophil chemoattractant 1; CTGF, connective tissue growth factor; MCP-1, monocyte chemoattractant protein-1.

inflammation related pro-coagulatory state in ventilator associated lung injury (VALI) and is responsible for broncho-alveolar fibrin deposition by a depressed fibrinolysis [21]. Alveolar fibrin deposition is an important feature of pulmonary inflammation [22] and inhibits alveolar fluid clearance. Notably, tPAI-1 correlates with ARDS mortality and is also believed to play a major role in the pathogenesis and prognosis of ARDS [23]. Although we cannot provide a sound explanation of how higher PEEP levels are associated with a rise of tPAI-1, we can only speculate that PEEP-induced alterations of thoracic gas and blood volume possibly affect the complex process of fibrinolysis.

Effect of mechanical ventilation with hyperoxia

Supraphysiological FiO_2 of 0.4 and 1.0 led to significant increases of plasma IL-6 and TNF-α in comparison to FiO_2 0.21, which was in contrast to our previous study where we did not find detectable serum IL-6 levels (detection limit <34.8 pg/mL) in mice after 120 min of mechanical ventilation and exposure to high FiO_2 [24]. Helmerhorst et al. found an increase of IL-6 and TNF-α concentrations in BALF and serum, and an increase of IL-6 and TNF-α mRNA expression in lung homogenate of healthy mice ventilated for 12 hours applying different oxygen concentrations [25]. Further, Dang et al. recently induced an IL-6 and TNF-α increase in blood after three days in a non-ventilated animal model evaluating the antioxidant asiaticoside for the prevention of chronic lung disease in preterm rats exposed to FiO_2 of 0.8 [26]. In the context of hyperoxia-induced IL-6 and TNF-α stimulation, we conclude that the stimulus of mechanical ventilation was aggravated using supplemental oxygen.

Effect of hypocapnic and hypercapnic ventilation

For induction of hypo- or hypercapnia respiratory rates were changed from 90 min^{-1} to 180 min^{-1} and 60 min^{-1}, respectively. We observed significant low TNF-α values and a trend to lower IL-6 values in the hypercapnia group, pointing towards a protective effect of lower respiratory frequencies. At the same time, tPAI-1 was significantly increased in the hypocapnia group, suggesting a more injurious effect of higher respiratory frequencies. This is supported by recent studies that focused on the mechanical power applied by the artificial breath cycle. Elevated respiratory frequencies increased the mechanical power and promoted VALI development in a piglet model, whereas mechanical power itself was linked to mortality in adult intensive care patients [27, 28]. Mechanical power is the energy applied to the respiratory system per artificial breath cycle multiplied by respiratory rate and is expressed in joules per minute (J/min) [29]. It is clear that higher pressures apply higher mechanical power to the system, but mechanical power is influenced by more than that, namely by four components: volume, pressure, flow, and respiratory rate. The higher the respiratory rate, the higher the amount of energy applied to the lung units will be, even though the peak pressures and tidal volume are adjusted to “protective” levels. The pathophysiology behind this is the dissipation of the applied energy into the viscoelastic structure of the lung tissue. As evidenced by lung hysteresis, not all the energy applied to the respiratory system is returned. One part of the energy (pressure x volume) applied by the ventilator for one breath cycle is used to generate gas flow, to expand the chest wall, to overcome alveolar superficial tension, and to unfold the network of elastin- and collagen fibers. If this power is excessive, it may disrupt the fiber and tissue network and cause VILI [30]. Thus, low TNF-α levels after ventilation with low RR of 60 min^{-1} might be explained by the lower mechanical power applied to lung tissue with lower frequencies, as one given tidal volume has more time for entering the airways and expand the alveoli. Further, we think that tPAI-1 was elevated in the hypocapnia group due to hyperventilation with a respiratory rate of 180/min and the resulting mechanical power to the lung tissue. An alternative explanation might be hypocapnic cerebral vasoconstriction and potential consecutive cerebral tissue ischemia [31, 32]. Indeed, we observed a significant higher lactate 2.4 (1.1) mmol/L (mean (SD)) in the hypocapnia group while the peripherally measured oxygen concentration remained in the physiologic range, suggesting actual vasoconstriction [14]. However, even though increased cerebral tPAI-1 mRNA expression has been shown to be driven by cerebral ischemia [33], it seems more likely that tPAI-1 in the present study was induced by high respiratory frequencies as tPAI-1 was elevated as well in the PEEP 9 group without lactate

increase, which might provide a hint for absence of cerebral ischemia [14].

Effect of changes in ventilation settings on other biomarkers

The extracellular matrix remodeling marker tissue inhibitor of metalloproteinase-1 (TIMP-1) is the counterpart of the matrix metalloproteinases (MMPs) which are zinc-binding enzymes responsible for extracellular matrix molecule turnover. TIMP-1 is upregulated in cases of pulmonary fibrosis, and it is proposed that an elevated TIMP-1/MMP ratio leads to fibrotic processes [34]. TIMP-1 has been shown to be elevated in BALF and plasma of adult ARDS patients [35] and correlated with bleomycin induced lung fibrosis in mice [34], but failed to be linked to a lung strain [36]. This is in line with our study, where we could not observe TIMP-1 alterations in systemic circulation between experimental groups.

The mRNA expression in lung tissue of cytokine-induced neutrophil chemoattractant (CINC)-1, a chemokine recognized as acute-phase-reactant and involved in the complex orchestra of inflammatory responses [37], was increased with PEEP 2 cmH_2O compared to PEEP 5 cmH_2O and to non-ventilated controls in an adult rat model evaluating different ventilation modes [38]. Further, CINC-1 concentrations were elevated in BALF of adult Wistar rats ventilated with high tidal volumes [39]. In our study however, we did not observe an influence of PEEP on CINC-1, possibly because the inflammatory pulmonary reaction was not strong enough to be already visible in infant rat plasma. To the best of our knowledge, a transition of CINC-1 from BALF to plasma was not observed in acute lung injury studies performed so far. We thus conclude that the injurious stimuli used in this study were not strong enough to cause ventilation-related differences in plasma CINC-1.

Connective tissue growth factor (CTGF) mRNA overexpression has been linked to VILI in preterm lamb models and correlated with high tidal volumes [40]. In a recent ARDS study involving adult human patients serum CTFG could be correlated to the mechanical power of ventilation and to ARDS-related pulmonary fibrosis [41]. The present study did not reveal any differences in blood plasma, possibly because the time and dose of the ventilation stimuli were too short and too low to induce a CTFG increase in plasma.

Monocyte chemoattractant protein-1 (MCP-1) is a small chemokine that has multiple inflammatory functions and acts as chemotactic signal for neutrophils which contribute to the development of lung inflammation [42, 43]. It has been shown to be elevated in plasma of adult human patients with ventilator-associated pneumonia [43] and was clearly overexpressed in lung homogenates of mice injuriously ventilated with high tidal volumes (34 mL/kg) and an inspiratory/expiratory ratio of 1:1 after 4 hours [44]. In our experiment, however, the variations of PEEP, FiO_2 and respiratory frequencies did not reveal any significant differences between groups, again suggesting that ventilation stimuli were not injurious enough to induce measurable disparities.

Limitations

This study has important limitations that have to be taken into account when interpreting the results. First, our goal was to measure the response of translationally relevant inflammatory markers in plasma. Hence, mRNA expression of the markers and the extent of VALI in lung tissue itself was not assessed. Second, except for the FiO_2 0.21 group, all animals were exposed to at least FiO_2 0.4. This supraphysiologic FiO_2 could have induced an inflammatory reaction by itself. However, as the exposure to those oxygen levels was the same in all PEEP and hypo- and hypercapnia groups, the inflammatory stimuli resulting from ventilator adjustments were applied on top of the oxygen exposure, making results comparable again. Third, all animals were tracheotomized. The tracheotomy is a small and local operation that might induce a systemically measurable inflammatory response. Therefore, the measured inflammatory marker levels cannot be compared to values of non-operated animals. Again, since all animals in this study have been tracheotomized, we presume that our results are comparable. In general, this surgical inflammatory trigger applies to all studies in which animals are tracheotomized for mechanical ventilation and its influence has to be cautiously considered when interpreting results of surrogate markers for inflammatory reactions.

Last, though we included a ventilated control group, we did not design this study with an additional non-ventilated control group. Such a control group would have allowed comparisons between non-ventilated and ventilated animals. However, since the main aim of this translational study was to compare different ventilation and oxygen supply strategies in mechanically ventilated infant rats, we decided to dispense with a second control group.

Conclusion

We found the inflammatory biomarkers IL-6, TNF-α, and tPAI-1 to be driven by clinically relevant alterations of PEEP, supraphysiologic FiO_2, and high respiratory frequencies. We conclude that unavoidable adjustments of PEEP, FiO_2, and respiratory frequency occurring during mechanical ventilation of critically ill patients causes a substantial systemic inflammatory response after a relatively short ventilation period of 120 min. Our study underlines the importance of lung-protective ventilation strategies in clinical use to hamper initiation or propagation of pre-existing pulmonary inflammation. Nonetheless, future translational research needs to reproduce these findings in pediatric studies.

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Disclosure of conflict of interest

None.

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