Pre-treatment with Isoflurane or Sevoflurane is not protective against high tidal volume induced lung injury in rats

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Abstract

Background

Volatile anesthetics (VA) may exert organ-protective effects in various experimental and clinical settings. Mechanical ventilation (MV) induces an inflammatory response and, depending on the ventilator settings chosen, injury in the lungs. It is unclear if prophylactic inhaled VA applied on healthy lungs prior to MV are protective regarding these effects.

Methods

Healthy, spontaneously breathing rats were exposed for 30 minutes to either isoflurane (1.8 Vol %), sevoflurane (3.0 Vol %) or no VA (controls). Animals were allowed to recover and then mechanically ventilated for 4 hours with either high (21 ml/kg body weight) or low (9 ml/kg body weight) tidal volume. Cardiorespiratory parameters and systemic inflammation were assessed at the beginning and during mechanical ventilation. Cellular, non-cellular and histologic markers of pulmonary injury and inflammation were determined.

Results

Irrespective of VA pretreatment, MV with high VT negatively affected markers of lung integrity such as arterial oxygenation and lung wet-to-dry ratio. Regarding the application of VA pretreatment protective effects on lung function were absent but there were changes in some markers of inflammation such as a decrease in blood lymphocyte counts and an increase in interleukin 6 concentration in plasma and in lung lavage fluid. These effects were heterogeneous regarding group allocation and time points.

Conclusions

In this in in vivo animal model, prophylactic administration of inhaled VA was not beneficial or protective regarding ventilation induced lung injury. However, there were effects suggestive of a modulation of inflammatory markers associated with VA prophylaxis. The clinical or biological relevance of these findings so far remain unclear and should be subject to further studies.

Background

Acute lung injury and its most severe form, acute respiratory distress syndrome are devastating clinical conditions, with high mortality rates (1). Mechanical ventilation (MV) is a mainstay of treatment of such patients, but may in itself exert detrimental effects on the lung and worsen the situation (2). In addition, mechanical ventilation of healthy lungs, depending on the tidal volume applied, can cause the clinical findings of ALI (3). The deleterious pulmonary effects of MV are summarized under the term ventilator-induced lung injury (VILI).

Ventilator settings including low tidal volume, positive end-expiratory and limited transpulmonary pressures have been shown to be protective against VILI (4). Beyond that, evidence for preventive or therapeutic measures, in particular of pharmacological nature, is limited.

In recent years, there is a growing body of evidence, showing that volatile anesthetics (VA) have anti-inflammatory and organ protective effects on multiple organ-systems like the heart, the kidney and the brain. (5–7), but proof of effect on outcome is not consistent (8). The effects of volatile anesthetics are mediated via multiple pathways, e.g. excitatory channel activity (9) and they have been shown to alter the expression of multiple genes (10).

Effects of VA on lung injury and inflammation are inconsistent. While beneficial effects were found under various conditions such as during MV of patients with acute respiratory distress syndrome (11), one-lung ventilation and thoracic surgery (12), experimental lipopolysaccharide induced injury (13, 14), cecal ligation and puncture induced sepsis (15), and lung injury induced by bronchial lavage (16), such effects were absent under others, e.g. experimental lung acid injury (17). In addition, there are studies indicating increased oxidative stress (18) and inflammation (19, 20) under anesthesia with volatiles.

Regarding the interaction of VA and MV in healthy lungs, there is only limited experimental data that, by simultaneous application of VA and MV, inflammatory effects of low (8 ml) or moderately increased (12ml) tidal volumes may be attenuated (21–23) and no information is available on the effects of VA application prior to MV.

Hence, using an *in vivo* rat model, we tested the hypothesis, if pretreatment (preconditioning) with a clinically relevant VA (isoflurane or sevoflurane) before a period of MV, is protective regarding development of ventilator-induced lung-injury. To test a possible dependency of any effect on the degree of injuriousity of the MV, two clearly distinct different tidal volumes were applied.

Methods

Animals

Male Wistar rats were obtained from Charles River Laboratories, Germany, Sulzfeld. Rats were 3 to 5 months old and weighed 533 ± 8g. Rats were housed in the animal facility of the university under standard conditions, at constant temperature (21° C) and regular day-night cycle with water and food ad libitum. Animals were handled according to the institutional guidelines for the care of laboratory animals and the laws of the state of Thuringia, Germany. Invasive procedures were performed under anesthesia as described below. Experiments were carried out on weekdays and randomization was done by sealed envelope.

Volatile Anesthetic Treatment

Rats were exposed to inhalation of isoflurane (ISO) or sevoflurane (SEVO) in air plus oxygen (AIR), using a standard Vapor (Ohmeda Isotec 4 or Sevotec 5, resp., Ohmeda Medical Inc, Laurel, USA) in a custom build chamber for 30 minutes. Inflow of air was controlled by a gas regulator (Aalborg Instruments Controls, Monsey, USA), and concentration of oxygen, exhaled carbondioxide and VA-concentration were continuously monitored in the gas outflow using a gas monitor (Datex Compact AS/3, Datex Engstrom / GE Healthcare, Freiburg, Germany). Applied concentrations of VA were sevoflurane 3.0% or isoflurane 1.8%, respectively, in air plus oxygen with a FiO2 of 0.5 at a total gas flow of 2 l per minute. A heating device (E. Zimmermann, Leipzig, Germany) was used to preserve body temperature of the animals in the induction-box. Animals in the control group were placed in the induction-box for the same time period under identical conditions except that they received no volatile anesthetics. After 30 minutes animals were allowed to recover at room air in their cage.

Instrumentation

One hour after the end of exposure, animals were weighed and narcotized by injection of thiopentone 120 mg / kg BW intraperitoneally. After application of an antibiotic prophylaxis (Cefuroxim 12,5 mg subcutaneously) and disinfection of the skin, the right internal jugular vein and right carotid artery were exposed and catheters for fluid application and blood pressure monitoring were introduced. Then the trachea was opened by incision and a tracheostomy-cannula (outer diameter 2mm) was embedded in the lumen and secured by ligature. Continuous intravenous infusion of a balanced electrolyte solution was started at a rate of 10 ml / hr (Deltajonin, DeltaSelect GmbH, Dreieich, Germany).

Mechanical Ventilation

Animals were placed in supine position and volume controlled mechanical ventilation (MV) was started (Animal Respirator CIV 101, Columbus Instruments, Ohio, USA).

After pretreatment with either ISO or SEVO or AIR, rats were randomized to mechanical ventilation with one of two VT regimens, resulting in 6 experimental groups (n = 8 per group).

Ventilator settings were as follows: FiO2 0.4, respiratory rate 40 bpm, I:E-Ratio 1:1, PEEP 5 mmHg. VT was set at either 9 ml per kg (low) or 21 ml per kg body weight (high). Applied to healthy rodents, these are differently harmful MV settings, causing no or only mild (VT 9 ml/kg) versus pronounced (VT 21 ml/kg) detrimental pulmonary effects (24–27).

Pancuronium bromide was injected for muscle relaxation after initiation of MV (0.5 mg) and repeated when necessary. VT was limited to an upper body weight of 550g, according to previous experiments, showing no increase in lung weight beyond this weight (28).

Anesthesia was maintained throughout the experiment by injection of thiopentone and ketamine. Body temperature was kept constant at 38°C by using a closed-loop heating system (HSE Temperaturregler, Hugo Sachs Elektronik, March, Germany). Arterial normocapnia was achieved by adding carbondioxide to the inspiratory gas, if respiratory alkalosis developed (groups with high VT).

In vivo Measurements

Blood pressure and heart rate were measured continuously and recorded every 60 minutes. A sideport of the tracheal cannula was used to determine airway pressure. Arterial blood gases were measured hourly. Blood was drawn for blood cell count and plasma cytokine levels at the beginning, at 2h and at the end of MV. Total white blood cell counts (TWBC) and differentiation of blood cells was done by microscopy. Neutrophils were determined using a cell smear (May-Gruenwald-Giemsa stain; 100 white blood cells counted at a magnification of x500) and absolute neutrophil numbers were calculated according to the TWBC.

Post mortem measurements

After 4 hours of MV, animals were sacrificed by exsanguination by cannulating the inferior vena cava after median laparotomy. Animals surviving less than 4 hours were excluded from data analysis.

Sternotomy was performed, lungs were harvested en bloc and processed for further sampling.

Lung lavage: Left lungs were lavaged as previously described (29). In brief: After instillation of PBS, effluent from the left lung was centrifuged. The cell pellet was resuspendet and white blood cells were counted by microscopy, corrected for the amount of recovered lavage fluid. Protein content in lavage fluid supernantant was determined by turbidimetry (Roche Diagnostics assay; Hitachi 717 analyzer), with a detection threshold of 60 mg / ml.

Lung wet-to-dry-ratio (WD ratio): A specimen of the right upper lung lobe was excised and weighed before and after desiccation at 45°C for 96 hours, and the quotient was calculated (WD ratio).

Lung histology: The remaining lobes of the right lung were perfused and fixated in a formalin solution. Slides were prepared for light microscopy after embedding in paraffin, cutting (5µm slices) and staining (hematoxylin and eosin). Histologic injury effects were quantitatively evaluated: In ten randomly selected high power fields per slide (magnification x400; excluding fields containing conducting airways and blood vessels) the number of macrophages and neutrophils in lung interstitial tissue were recorded. In addition, in the same high power fields the number of alveolar neutrophils and the percentage of alveoli, alveolar sacs and alveolar ducts containing any hyaline membranes / azidophilic material were recorded (30). The investigator (B.S.) was not aware of group allocation of the tissue section.

Cytokine levels in plasma and lung lavage: Cytokine levels were assessed using commercially available ELISA kits for the rat (Rat IL-6 Quantikine ELISA Kit and Rat IL-1 b Quantikine ELISA Kit, R&D Systems GmbH, Wiesbaden, Germany), used according to manufacturer guidelines.

Statistical analysis

Statistical analysis was performed using SPSS Statistics Ver. 21 (IBM Corp., Armonk, New York, United States). Comparison between study groups was done by two-way analysis of variance using a repeated

measures design and post hoc testing where appropriate. Results are presented as mean ± standard error of the mean (SEM). A p-value of ≤ 0.05 was considered statistically significant.

Results

Pre-treatment with VA

Pre-treatment with ISO or SEVO rapidly caused loss of consciousness in the animals. During ISO or SEVO administration animals remained anesthetized while spontaneously breathing. Anesthetic concentrations continuously measured at the gas outflow of the inhalation chamber were sevoflurane 3.0% or isoflurane 1.8%, respectively. All animals pre-treated with ISO or SEVO recovered within few minutes after cessation of VA inhalation and were awake and alert prior to thiopentone anesthesia induction which was given before instrumentation and MV.

Baseline variables

Prior to mechanical ventilation, body weight, heart rate, arterial blood pressure, hemoglobin-concentration and arterial blood gases were comparable, regardless of pre-treatment (AIR / SEVO / ISO, Table 1).

Table 1 Baseline and cardiorespiratory parameters in rats before mechanical ventilation.

Values are mean ± SEM; MAP= mean arterial pressure, HR = heart rate, Hb = Hemoglobin concentration, PaO2 = arterial oxygen tension, PaCO2 = arterial carbondioxide tension,

	AIR	ISO	SEVO
Weight [g]	532±16	547±16	519±7
MAP [mmHg]	122±8	132±9	128±7
HR [bpm]	375±9	377±9	379±8
Hb [mmol/l]	8,0±0,1	8,3±0,1	8,2±0,1
PaO2 [mmHg]	195,4±3,1	192,7±4,1	197,1±2,5
PaCO2 [mmHg]	50,5±2,2	54,3±1,9	55,4±2,2

Hemodynamic and respiratory parameters during MV

Heart rate: During MV there was no change in heart rate, independent of pre-treatment or VT. (Additional file 1).

MAP: During MV there was a decrease in mean arterial pressure in all groups, and significantly more so in animals ventilated with high VT ($p < 0,004$, all groups). There were no differences according to pre-treatment with or without VA (Figure 1).

PaO2: Independent of VT, in most groups oxygenation declined over time. There was no statistically significant effect of pre-treatment, but animals receiving ISO or SEVO in the high VT group tended to lower values at the end of the MV period (Figure 1).

PaCO2: As expected, in animals ventilated with high VT, there was an initial drop in PaCO2. After returning to normal values, there were no differences between groups (Additional file 1).

Paw: Mean airway pressures were higher in animals ventilated with high VT. Paw decreased over time in this group. There were no differences according to pre-treatment with VA (Figure 1).

Markers of lung injury and inflammation

Lung WD ratio: Lung WD ratio was significantly higher in animals ventilated with high VT compared to lower VT but there were no differences according to pre-treatment with or without VA (Figure 2).

Protein content in lung lavage: Irrespective of pre-treatment, there was a significant increase in protein content in groups ventilated with high VT. Protein content in lung lavage was low and comparable between groups ventilated with low VT and between groups ventilated with high VT, respectively (Figure 2).

IL 1β in lung lavage: In general, levels of IL 1β in lung lavage were low. There were no discernible differences according to ventilator-settings or application of VA (Figure 2).

IL 6 in lung lavage: Levels of IL6 in lung lavage were low in groups ventilated with low VT with no significant differences according to pre-treatment. Irrespective of pre-treatment, there was a tendency to higher values in the high VT-groups, but variation in values was high and effects did not reach statistical significance (Figure 2).

Neutrophils in lung lavage: Compared to MV with low VT neutrophil counts were lower in all groups ventilated with high VT and this effect was significant in the absence of VA pretreatment (Figure 2).

Macrophages in lung lavage: There were no significant differences in macrophage counts in lung lavage irrespective of tidal volume applied or of pre-treatment with or without VA. (Additional file 1).

Lymphocytes in lung lavage: There were no significant differences in lymphocyte counts in lung lavage irrespective of tidal volume applied or of pre-treatment with or without VA (Figure 2).

Lung histology (Table 2):

Macrophages in lung interstitial tissue: As compared to animals ventilated with low VT and pre-treated with AIR, macrophage counts in lung interstitial tissue were lower in animals ventilated with low VT and pre-treated with ISO ($p<0.002$) or SEVO ($p<0.1$).

Neutrophils in lung interstitial tissue: Irrespective of pre-treatment animals ventilated with ~~a~~ high VT had significantly less neutrophils in lung interstitial tissue ($p=0.043$), than animals ventilated with low VT.

Intraalveolar neutrophils: There were no differences in intra-alveolar neutrophil counts, irrespective of pre-treatment or VT.

Hyaline membranes: Calculation of the airspace injury score (AIS, number of hyaline membranes in the airspace divided by the number of alveoli in 10 high-power fields) showed no differences between groups, according to pre-treatment with or without ISO or SEVO or according to VT.

Table 2: Inflammatory cell counts and hyaline membranes in lung tissue sections (light microscopy)

Values are mean ± SEM; PML = Neutrophils, Mac = Macrophages, Alv = Alveolar, Tiss = interstitial tissue, AIS = Airway Injury Score

	AIR 9	AIR 21	ISO 9	ISO 21	SEVO 9	SEVO 21
PML Alv	7.4 ±1.9	6.0 ±1.9	12.5 ±4.3	14.5 ±5.8	7.8 ±2.0	6.5 ±2.6
Mac Alv	2.6 ±0.9	5.4 ±2.1	4.8 ±2.8	5.4 ±2.1	5.0 ±1.0	6.0 ±2.5
PML Tiss	518.5 ±41.0	411.0 ±32.8	471.3 ±110.5	414.8 ±55.9	502.0 ±56.8	429.8 ±47.7
Mac Tiss	42.3 ±4.7	33.7 ±6.0	9.0 ±5.1*	30.8 ±8.0	31.0 ±3.2	32.3 ±6.8
AIS	0.5 ±0.2	0.4 ±0.4	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1

Markers of systemic inflammation

TWBC: There was an increase in TWBC in all groups with a peak at 2 hours of MV and persistently elevated values at the end of MV. At the beginning of MV TWBC were lower in groups pre-treated with VA as compared to controls (Figure 3).

Blood lymphocyte counts: During MV there was a significant decrease in lymphocyte counts in all groups regardless of VT and of pre-treatment with or without VA. At the beginning and after 120 minutes of MV lymphocyte counts were lower in groups pre-treated with VA as compared to controls (Figure 3).

Blood neutrophil counts: During MV there was a significant increase in neutrophil counts in all groups regardless of VT and of pre-treatment with or without VA (Figure 3).

IL 6 in plasma: In animals ventilated with low VT there was a significant increase in IL 6 in plasma after pre-treatment with isoflurane and air but not after pre-treatment with sevoflurane. In animals ventilated with high VT there was a significant increase in IL 6 in plasma in both groups pre-treated with VA. This increase was absent in animals without VA pretreatment (Figure 4).

IL 1β in plasma: Irrespective of group allocation and VT there were no significant changes in levels of IL 1β in plasma during MV nor were there significant differences between groups (Figure 4).

Mortality

There was no mortality after pre-treatment and mortality rate during MV did not differ between groups (chi-square-test, p=0.21).

Discussion

This is, to our knowledge, the first study investigating in an in-vivo healthy animal model, the effects of a prophylactic exposure to an inhaled volatile anesthetic on the development of ventilator induced lung injury.

The choice of volatile anesthetics in this study features two substances with similar application mode (i.e. delivered by a vapor in relatively low concentration in inspired gas) and broad use in clinical anesthesia, but with different properties regarding pharmacokinetics, adverse effects and costs (31).

Regarding ventilator settings, VT was used to trigger detrimental pulmonary effects and the VT levels selected were intended to distinguish two differently harmful ventilation strategies. Accordingly, while a systemic inflammatory response was associated with MV regardless of the VT applied, MV with high VT additionally attenuated lung function and provoked lung injury. These effects are consistent with data from the literature (2).

The main result of the study however, as assessed by an array of markers of pulmonary and systemic injury and inflammation, is the absence of a clear beneficial effect of both isoflurane and sevoflurane on the detrimental effects exerted by mechanical ventilation.

These findings are in contrast with other studies in this field reporting protective or beneficial effects of isoflurane or sevoflurane on lung injury.

One reason for the disparate findings may be the diversity of settings and models used to investigate the effect of VA on lung function and integrity (32).

Beneficial or protective effects of VA have repeatedly been shown in injured lungs (e.g. endotoxin induced injury) which were pre-treated, post-treated or treated simultaneously with VA (33, 34). In some studies, the effect of VA was explored in two hit models combining e.g. endotoxin induced lung injury and MV (35, 36). In general, these models reflect the effects of VA on lungs already exhibiting a (pro-) inflammatory response.

In contrast, animals with healthy lungs were used in our experiments and MV was the single detrimental stimulus to the lung.

There is limited data on the interaction between VA and MV applied simultaneously in healthy lungs. In three studies in mice, isoflurane administered during MV attenuated inflammatory changes induced by MV of 8 ml/kg (23) and 12 ml/kg body weight (21, 22), whereas another study found evidence of a proinflammatory pattern at the transcriptional level in rats during MV with VA (20)

Different from these studies and hence representing still another distinct setting, in our study VA was applied prophylactically and a delay was established between administration of VA and the onset of MV.

We speculate that, depending on the kinetics and on the duration of the mechanisms (such as effects on gene expression or on pathways of inflammation) possibly induced by VA and MV, the interaction of effects could be different depending on if both interventions are applied simultaneously or consecutively. It is possible that there are time-dependent windows of preconditioning with different pathways involved in the effects of VA (37).

In addition, duration and dose of VA pretreatment could be confounding factors. Timing and dosing of the two volatiles we applied is in accordance with other publications (33, 34, 38, 39). In these studies, VA applied before a noxious stimulus to the lung, have shown protective effects. However, none of these studies used MV as a noxious stimulus to the lungs.

Another reason for the absence of protective or beneficial effects of isoflurane or sevoflurane in our study could be a misbalance between the relative potencies of the interventions applied. In the setting of MV with low VT producing only minor (if any) changes in most of the variables assessed, detection of a possible beneficial effect of VA seems difficult. On the other hand, in the setting of a more harmful stimulus such as MV with high VT provoking pronounced changes, effects of VA may not be discernable if they are only mild.

Of note, although in our study, clear beneficial effects of prophylactic VA administration were absent, this was not equivalent to absence of any effect of VA application: Decreased blood lymphocyte counts at the beginning of the ventilation period as well as lower macrophage counts in lung interstitial tissue in animals pre-treated with VA may reflect effects of VA on the inflammatory milieu. Further, increases in blood IL 6 levels during MV with high VT were even more pronounced in animals pre-treated with VA. Although from our study we are unable to confirm any biological relevance of such effects, in our opinion these findings deserve some attention.

Firstly, inducing general anesthesia by application of VA may *per se* affect homeostasis and biological integrity. In this regard, associated changes in white blood cell populations (i.e. such as lymphocyte counts in our study), irrespective of being caused by VA directly or by general anesthesia indirectly, are suggestive of some modulation of the immune status or the inflammatory milieu, respectively. This notion is supported by in vitro experiments demonstrating increased apoptosis in lymphocytes exposed to sevoflurane and isoflurane (40).

Such effects in the context of increasing IL 6 levels during MV with high VT after VA pre-treatment may result in a different inflammatory milieu as compared to pre-treatment without VA. However, interpretation of the biological significance of changes in cytokine concentrations is challenged by the fact that huge variations in these values are common (41) expression is time dependent (42) and function is pleiotropic.

Some limitations of our study warrant discussion.

Firstly, we did not investigate potential effects of VA on the level of biochemical or molecular pathways, interactions or cascades. Instead, it was our aim to detect effects translating to more global markers of

injury and inflammation. In this context, the markers used in the present study are well established and appropriate to reflect the downstream effects of many cascades involved in ventilator induced lung injury (4, 32).

However, we are unable to distinguish, if below our radar a response to VA occurred, which may not have translated to quantitative effects on cells, proteins and functional markers (e.g. oxygenation) as assessed in the present study.

Secondly, we cannot exclude that other anesthetics used in the experiment counterbalanced the effects of VA pretreatment. Effects on the immune system and on inflammation have been shown for ketamine and thiopentone (both were used in this study) and might have interfered with effects of VA (17, 43). In theory however, this argument takes effect for any choice of anesthetic applied during MV. On the other hand, abandoning anesthetics during instrumentation and MV seems inappropriate for practical and ethical reasons.

Thirdly, different tidal volumes applied might cause different arterial carbondioxide concentrations between groups, which consecutively may have an independent modulatory effect on mechanical properties and inflammatory response of the lung (44, 45). We corrected for this potential confounder by adding CO2 to the inspiratory gas in the groups treated with high VT resulting in comparable CO2 concentrations in all groups.

Conclusions:

In conclusion, this study failed to demonstrate in healthy rats significant beneficial or protective effects of pre-treatment with isoflurane or sevoflurane on the detrimental pulmonary effects caused by high tidal volume mechanical ventilation. This is in contrast with other studies showing beneficial effects of VA on lung injury albeit in different experimental settings. The findings suggest, that the experimental settings, in which VA are applied as well as the model of lung injury (e.g. endotoxin vs. ventilator-induced) play a role for their effects.

In the present study there were, nevertheless, some effects of VA pre-treatment reflecting changes in immune function and pulmonary inflammatory response. As long as the relevance of such changes remains unclear, based on our data, prophylactic application of VA to ameliorate ventilator induced lung injury seems not appropriate.

To more precisely identify interactions between VA, anesthesia and MV, modulations of the model, e.g. regarding dose, duration or timing of the pre-treatment with VA should be subject to further research.

Abbreviations

AIS Airway Injury Score

ALI acute lung injury

Alv	Alveolar
ELISA	enzyme-linked Immunosorbent assay
Hb	hemoglobin concentration
HR	heart rate
IL 1β	Interleukin-1β
IL 6	Interleukin-6
ISO	isoflurane
Mac	Macrophages
MAP	mean arterial pressure
MV	mechanical ventilation
PaCO2	arterial carbondioxide tension
PaO2	arterial oxygen tension
Paw	mean airway pressure
PML	polymorphonuclear neutrophils
SEM	standard error of the mean
SEVO	sevoflurane
Tiss	interstitial tissue
TWBC	total white blood cell counts
VA	volatile anesthetics
VILI	ventilator-induced lung injury
VT	tidal volume
WD ratio	Lung wet-to-dry-ratio

Declarations

- Ethics approval and consent to participate

The study was approved by the institutional Animal Care Committee of the State of Thuringia, Germany (TLLV, Bad Langensalza, registration number 02-036/09).

- Consent to publish

Not applicable

- Availability of data and materials

  All data generated or analysed during this study are included in this published article and its supplementary information files.

- Competing interests

  The authors declare that they have no competing interests.

- Funding

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- Authors' contributions

  FS investigation, data curation, analysis, validation, visualization, writing, editing

  LH supervision, conceptualization

  BS investigation, acquisition

  KS conceptualization, study design

  TS supervision, conceptualization, data curation and analysis, methodology, writing

  All authors read and approved the final manuscript.

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Figures

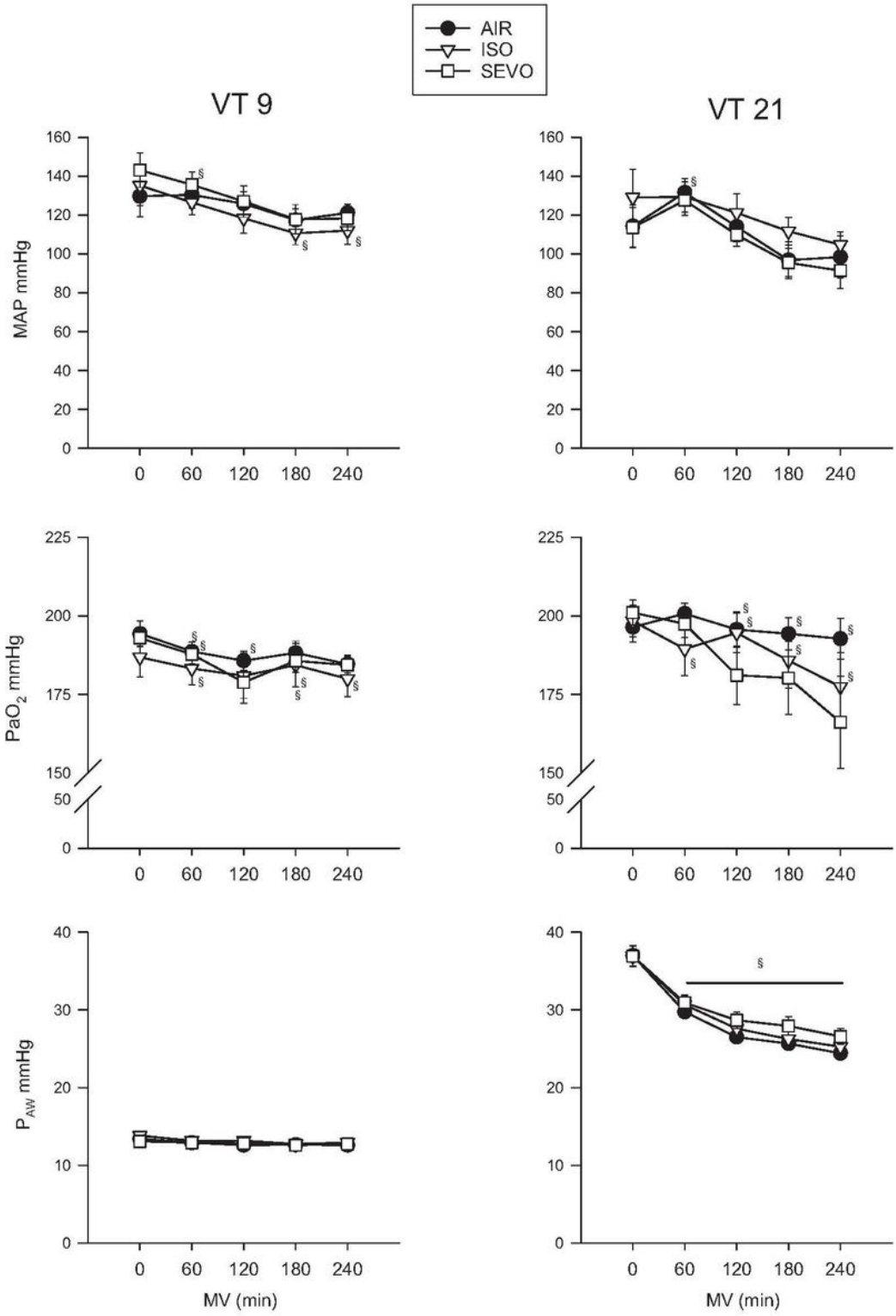

Figure 1

Hemodynamic and respiratory parameters Mean arterial pressure (MAP), arterial oxygen tension (PaO2) and mean airway pressures (Paw) in minutes after initiation of mechanical ventilation (MV) in control-animals (black circles) and rats pre-treated with isoflurane (white triangles) or sevoflurane (white box) ventilated with tidal volumes (VT) of 9 or 21 ml / kg § t = 0 vs time point (horizontal line: all values significant at time point)

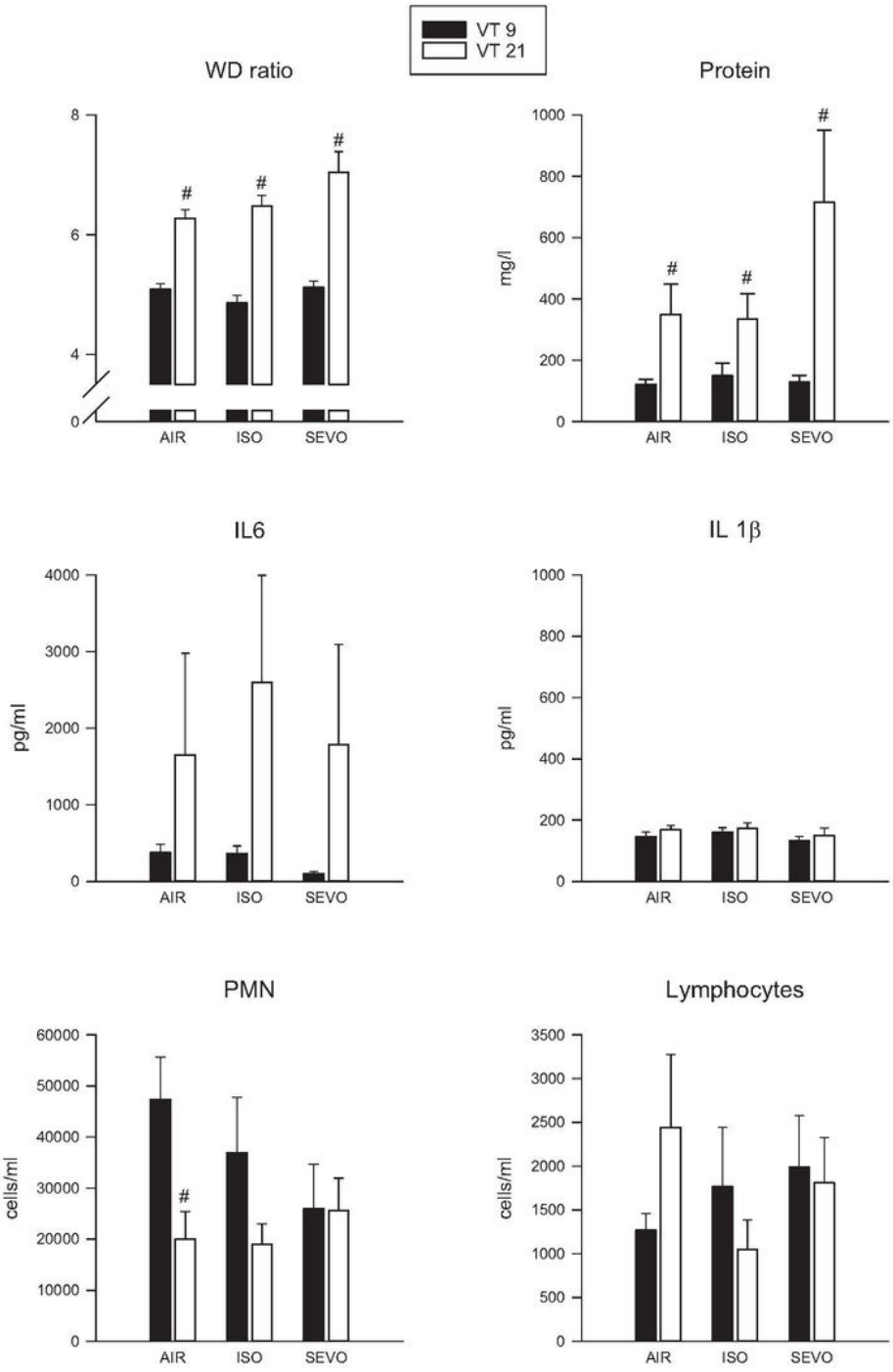

Figure 2

Lung lavage / Markers of lung injury and inflammation Lung wet-to-dry weight ratio (WD ratio), protein content, interleukin-6 (IL 6), interleukin-1β (IL 1β), neutrophil count (PMN) and lymphocyte count in lung lavage fluid in rats ventilated with tidal volume (VT) of 9 or 21 ml per kg body weight. # VT 9 ml / kg vs 21 ml / kg

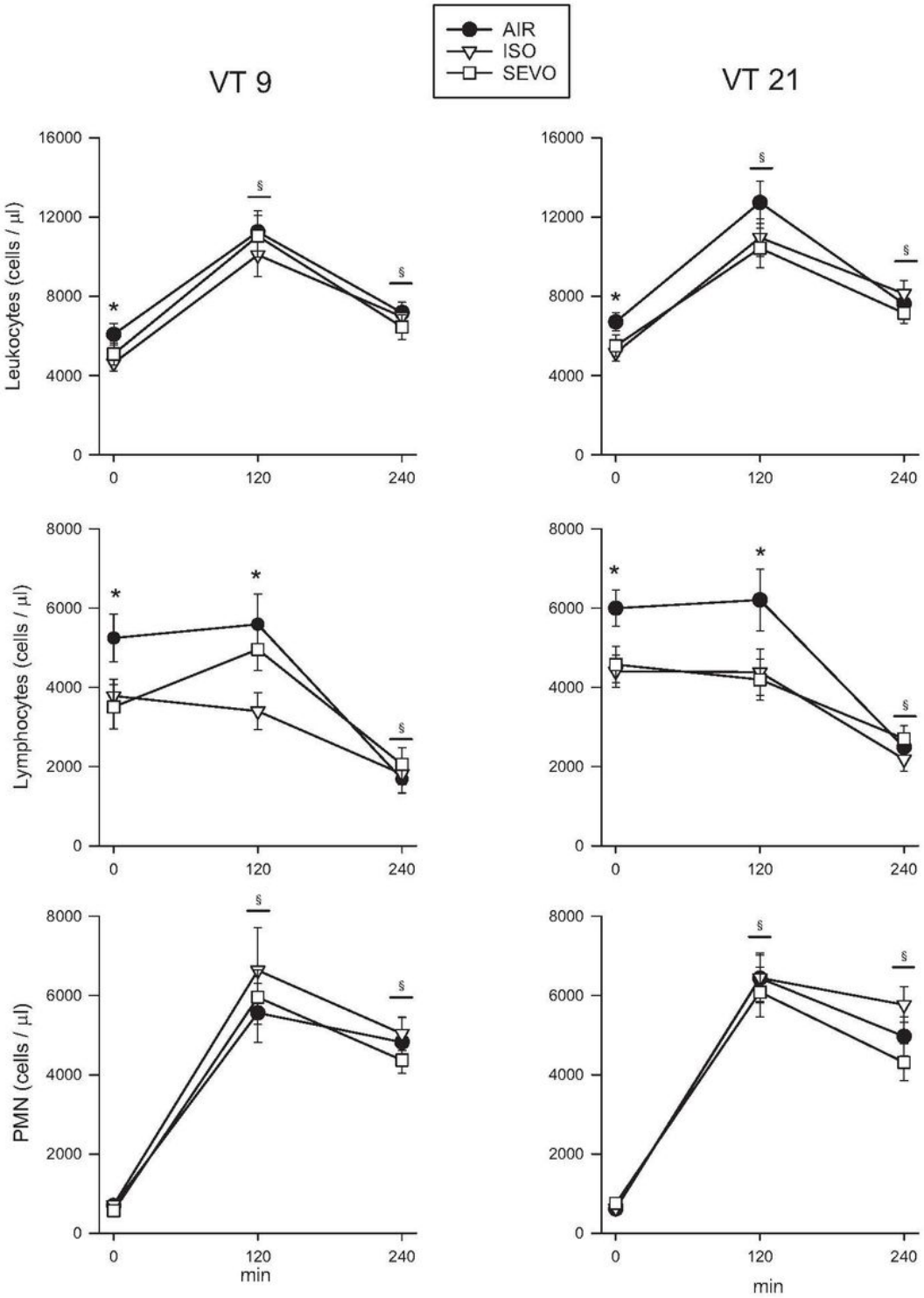

Figure 3

Systemic inflammation Markers of systemic inflammation after start of mechanical ventilation (MV) in control-animals (black circles) and rats pre-treated with isoflurane (white triangles) or sevoflurane (white box) ventilated with tidal volumes (VT) of 9 or 21 ml / kg. Leukocytes = total white blood cell counts, Lymphocytes = Lymphocyte counts, PMN = blood neutrophil counts; IL 1β = Interleukin-1β in serum; IL 6 =

Interleukin-6 in serum. * VA vs. AIR § t = 0 vs time point (horizontal line: all values significant at time point)

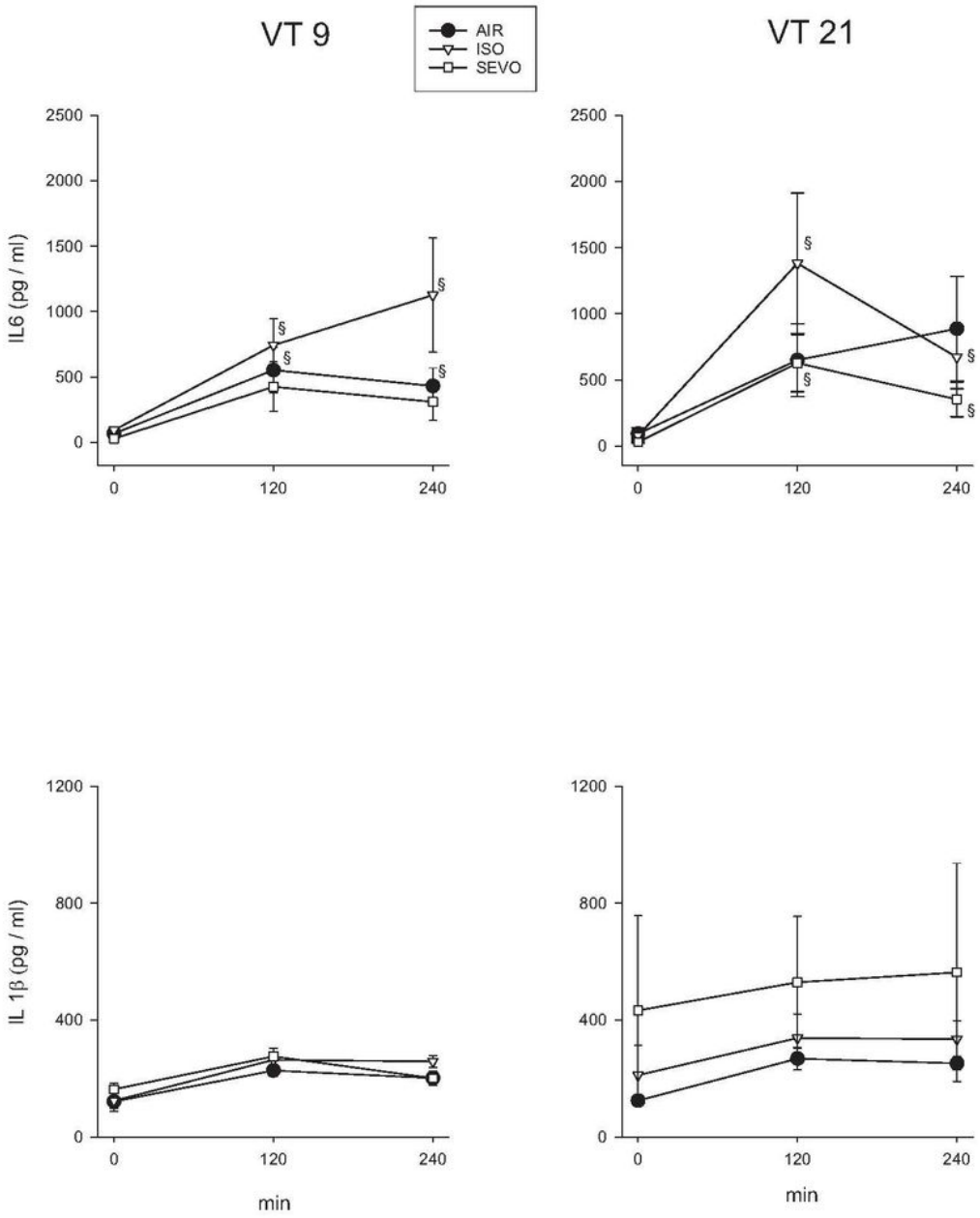

Figure 4

Systemic inflammation Markers of systemic inflammation after start of mechanical ventilation (MV) in control-animals (black circles) and rats pre-treated with isoflurane (white triangles) or sevoflurane (white box) ventilated with tidal volumes (VT) of 9 or 21 ml / kg. Leukocytes = total white blood cell counts,

Lymphocytes = Lymphocyte counts, PMN = blood neutrophil counts; IL 1β = Interleukin-1β in serum; IL 6 = Interleukin-6 in serum. * VA vs. AIR § t = 0 vs time point (horizontal line: all values significant at time point)

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