Chapter 5

Production of endothelin-1 and reduced blood flow in the rat kidney during lung-injurious mechanical ventilation

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Published in *Anesthesia & Analgesia* 2008, 107:1276-1283
Abstract

Introduction: The mechanisms by which mechanical ventilation (MV) can injure remote organs, such as the kidney, remain poorly understood. We hypothesized that upregulation of systemic inflammation, as reflected by plasma interleukin-6 (IL-6) levels, in response to a lung-injurious ventilatory strategy, ultimately results in kidney dysfunction mediated by local endothelin-1 (ET-1) production and renal vasoconstriction.

Methods: Healthy, male Wistar rats were randomized to 1 of 2 MV settings (n = 9 per group) and ventilated for 4 h. One group had a lung-protective setting using peak inspiratory pressure of 14 cm H2O and a positive end-expiratory pressure of 5 cm H2O; the other had a lung-injurious strategy using a peak inspiratory pressure of 20 cm H2O and positive end-expiratory pressure of 2 cm H2O. Nine randomly assigned rats served as nonventilated controls. We measured venous and arterial blood pressure and cardiac output (thermodilution method), renal blood flow (RBF) using fluorescent microspheres, and calculated creatinine clearance, urine flow, and fractional sodium excretion. Histological lung damage was assessed using hematoxylin-eosin staining. Renal ET-1 and plasma ET-1 and IL-6 concentrations were measured using enzyme-linked immunosorbent assays.

Results: Lung injury scores were higher after lung-injurious MV than after lung-protective ventilation or in sham controls. Lung-injurious MV resulted in significant production of renal ET-1 compared with the lung-protective and control groups. Simultaneously, RBF in the lung-injurious MV group was approximately 40% lower (P < 0.05) than in the control group and 28% lower (P < 0.05) than in the lung-protective group. Plasma ET-1 and IL-6 levels did not differ among the groups and systemic hemodynamics, such as cardiac output, were comparable. There was no effect on creatinine clearance, fractional sodium excretion, urine output, or kidney histology.

Conclusions: Lung-injurious MV for 4 h in healthy rats results in significant production of renal ET-1 and in decreased RBF, independent of IL-6. Decreased RBF has no observable effect on kidney function or histology.

Implications: Lung-injurious mechanical ventilation of healthy lungs causes an increase in the renal endothelin-1 level that is associated with decreased renal blood flow, without alterations in systemic hemodynamics.
Introduction

Acute kidney injury (AKI) is common in critically ill patients.\(^1\) It has become clear that mechanical ventilation (MV) is an independent risk factor for AKI and attributable mortality.\(^2,3\) The exact mechanism by which MV may injure the kidney remains poorly understood, but a number of mechanisms have been proposed.\(^2,3\) MV per se can promote the release of pulmonary and systemic inflammatory mediators even after a short period of ventilation.\(^4-8\) This is supported by evidence from experimental models using healthy animals,\(^4,7,9\) preinjured animals,\(^10,11\) healthy humans,\(^6,8\) and patients with acute respiratory distress syndrome (ARDS).\(^12\) It seems, therefore, that inflammatory mediators play a pivotal role in MV-induced AKI, just as they do in sepsis-induced AKI.\(^13\) This is supported for example, by the work of Ranieri et al., who observed in ARDS patients that increases in plasma interleukin-6 (IL-6) concentrations were related to the development of AKI.\(^14\)

Indeed, IL-6 is considered an important marker of proinflammatory cytokine activation during MV.\(^15,16\) How IL-6 exerts its detrimental effect on the kidney remains unclear. One possibility is that IL-6 has vasoactive properties, which allow it to enhance the production of renal endothelin-1 (ET-1).\(^17\) ET-1 is regarded as one of the most potent endogenous vasoconstrictors.\(^18-20\) Furthermore, increased systemic and/or local production of ET-1 has been implicated in renal dysfunction under a variety of circumstances.\(^21,22\) These observations suggest that MV may result in IL-6-dependent induction of renal ET-1 production, leading to a significant decrease in renal blood flow (RBF) and thus causing kidney dysfunction. Furthermore, MV may further compromise renal hemodynamics by decreasing cardiac output (CO) due to the increase in thoracic pressure.\(^2\)

There are only a few experimental studies addressing the role of MV in the development of AKI.\(^5,23\) In both studies, the “multiple-hit” model was used, with MV applied in already injured animals. From these studies, it is not possible to distinguish between the effects of MV per se, the underlying disease, or their combination on the development of AKI. To better understand the relevant pathophysiologic mechanisms leading to AKI, it is important to study the effects of MV in the healthy lung in vivo, using ventilatory protocols analogous to those currently used in the intensive care unit. We studied the effects of MV in healthy rats, maintaining normal gas exchange and carefully avoiding systemic hemodynamic effects. We hypothesized that upregulation of systemic inflammation (as reflected by plasma IL-6 levels) in response to a lung-
injurious ventilatory strategy ultimately results in kidney dysfunction mediated by local ET-1 production and renal vasoconstriction.

Methods

Animal Preparation
Animals were treated according to national guidelines and with permission of the Institutional Animal Care and Use Committee. A pilot study was undertaken to establish the protocol. All animals survived the final experimental protocol.

Twenty-seven male Wistar rats (Harlan CPB, Zeist, The Netherlands) weighing approximately 300g were anesthetized with 60 mg · kg\(^{-1}\) · h\(^{-1}\) pentobarbital sodium (Nembutal; CEVA Santa Animale BV, Maassluis, The Netherlands) given intraperitoneally and 70 mg/kg ketamine (Alfasan, Woerden, The Netherlands) IM. Anesthesia was maintained with pentobarbital (15 mg/kg) administered every 30 min through an intraperitoneal catheter. Rats were placed in the supine position on a heating pad; body temperature was maintained at 37°C. A tracheostomy was performed and a cannula (14-gauge) was inserted into the trachea. The bladder was catheterized for urine sampling using a transabdominal approach. For blood sampling and arterial blood pressure measurements, catheters were inserted into the right carotid artery and left femoral artery. Central venous pressure was measured using a catheter inserted in the right jugular vein. Intravascular catheters were connected to pressure transducers and data were analyzed using NewXLC software. Equipment was calibrated before the experiment by applying known pressures to the pressure transducers using a manometer. Readings of the pressure transducers were adjusted when necessary. CO was measured as described previously.\(^{24}\) Briefly, a thermistor from a pulmonary artery catheter was inserted through the right femoral artery into the aortic arch and a catheter was inserted into the right subclavian vein. CO was obtained by averaging three successive thermodilution determinations (CO Computer, 9520A, Edwards Laboratory, Santa Ana, CA). Blood gas analysis was performed using a pH/blood–gas analyzer (ABL 50; Radiometer, Copenhagen, Denmark).

Experimental Protocol
The experimental setup is shown in Figure 1. After a stabilization period of 60 min, blood and urine samples were taken and rats were randomized to one of three groups (1): nonventilated,
sham-operated controls (n = 9) (2); a lung-protective MV setting (positive inspiratory pressure 14 cm H2O, resulting in tidal volumes of 6–8 mL/kg, PEEP 5 cm H2O, respiratory rate 40/min) (n = 9); or (3) a lung-injurious MV setting (positive inspiratory pressure 20 cm H2O, resulting in tidal volumes of 15–18 mL/kg, PEEP 2 cm H2O, respiratory rate 40/min) (n = 9). The ventilator settings were designed to maintain comparable mean airway pressures among groups. In the lung-injurious group, a piece of silicone tubing was added between the Y-piece of the ventilator circuit and the tracheostomy cannula to increase dead-space to maintain normocapnia. The fraction of inspired oxygen was set at 1.0 in both ventilated groups.

**Figure 1.** Timeline of the protocol.

Animals were anesthetized, a tracheotomy was performed, and animals were connected to a ventilator or served as sham-operated controls. Insertion of arterial and venous catheters was followed by a 20-min stabilization period. At t = 0 min, randomization and subsequently mechanical ventilation (MV) started. At t = 120 min, blood samples were taken. At the end of the experiment (t = 240 min), blood samples were taken, animals were killed, and organs were harvested. LP-MV = lung-protective mechanical ventilation. LI-MV = lung-injurious mechanical ventilation.

In all three groups, urine samples were collected in the hour before randomization and in the last hour of the experiment; blood samples were taken after urine collection (Fig. 1). The CO was measured every 2 h. Arterial blood pressure was measured continuously. Arterial blood samples were taken for blood gas analysis (100 μL every hour) and cytokine assays (0.5 mL at t = 0 and 240 min). Blood was replaced by equal volumes of normal saline. Creatinine clearance and fractional sodium excretion were calculated before randomization and after 4 h of MV (Fig. 1) using the formulas \( \text{UCr} \times \frac{V}{\text{PCr}} \) and \( \text{UNa} \times \frac{\text{PCr}}{\text{PNa}} \times \text{UCr} \), respectively. In these formulas, \( \text{UCr} \) represents the urine creatinine concentration (mg/mL), \( V \) is the urine flow (mL/min), \( \text{PCr} \) is the plasma creatinine concentration, \( \text{UNa} \) is the urine sodium concentration, and \( \text{PNa} \) is the plasma sodium concentration. After 4 h, RBF was determined, as described below, by using fluorescent
microspheres. At the end of the experiment, animals were killed with an overdose of pentobarbital. Lungs and kidneys were harvested for histological examination. Parts of the organs were snap frozen and stored at −80°C for further analysis. Blood samples and urine were centrifuged (4 degree, 3000g, 10 min), and serum was stored at −80°C. Administration of fluids was kept at a minimum, and did not differ among the groups. Approximately 4.5 mL normal saline per animal was infused to replace blood samples and flush intravascular catheters.

**RBF**

At the end of the experiment, approximately $0.25 \times 10^6$ fluorescent microspheres of one color were injected into the left ventricle and a reference sample was withdrawn from the left femoral artery using a Genie Infusion/Withdrawal syringe pump (Kent Scientific Corporation, Torrington, CT). Pressure recordings of the left ventricle catheter were made to verify intraventricular catheter position at the time of injection. After death, a part of the right kidney was removed, and left and right trapezius muscle samples were used as reference samples. Fluorescent microspheres were extracted from the tissue, as has been described by Raab et al., and fluorescence was measured using a Luminescence Spectrophotometer (Perkin Elmer LS 50B, Boston, USA) at the appropriate excitation/emission frequencies. RBF in kidneys was calculated as described in detail previously.

**Histology**

After the animals were killed, the heart and lungs were removed from the thorax en bloc. The lungs were isolated from the heart, the trachea was canulated, and 2 mL 4% formalin was instilled in the upper lobe left for fixation over 24 h. Thereafter, the left upper lobe of the lung was serially sliced from apex to base, embedded in paraffin, cut at 5-μm thickness, stained with hematoxylin-eosin, and examined under a 40× objective lens. A quantitative morphometric analysis of lung injury was performed for four variables: septal thickening, the presence of alveolar macrophages, the presence of alveolar erythrocytes, and occurrence of alveolar hemorrhage. A total lung injury score per group was obtained (minimum score 0, maximum score 11).

After fixation, kidneys were embedded in paraffin and 5-μm tissue was cut and stained with hematoxylin-eosin. Tubular dilation, presence of intratubular debris, vacuolization of tubular epithelium cells, and loss of brush border membrane integrity were assessed.
IL-6
Analysis of IL-6 in plasma was performed in a blinded fashion using rat-specific enzyme-linked immunoabsorbent assay kits (CLB, Amsterdam, The Netherlands). The lower detection limit was 1 pg/mL.

ET-1
The concentration of ET-1 in kidney homogenates and in plasma was determined using an ET-1 Enzyme Immuno Assay Kit (Immuno-Biological Laboratories, Fujioka, Japan). This kit is a solid-phase sandwich enzyme-linked immunoabsorbent assay that uses two highly specific antibodies. Tetraethyl benzidine was used as a coloring agent, the strength of which is in proportion to the quantity of ET-1. The reported cross-reactivity of the antibody for the former was ≤0.1% for all big ETs (ET-1 precursors) and ET-2, and 0.1% for ET-3. Results from kidney homogenates were corrected for protein levels.

Statistics
All values are expressed as mean ± se of the mean (sem). All data were tested for homogeneity of variance (Levene’s test). Between-group comparisons were performed using one-way analysis of variance with the Bonferroni adjustment for multiple comparisons, or using a nonparametric test (Kruskal–Wallis) with post hoc analysis, and the Mann–Whitney U-test was used for variables without homogeneity of variances. The analysis was performed with the Statistical Package for Social Sciences (SPSS) 12 (Chicago, IL). A P < 0.05 was considered significant.

Results
Hemodynamics
At the start of the experiment, mean arterial blood pressure, central venous pressure, and CO were comparable among the groups (Fig. 2). After 2 and 4 h, the mean arterial blood pressure was slightly but significantly higher in the lung-protective group compared with the control and lung-injurious groups. After 4 h of MV, central venous pressure was significantly higher in both ventilated groups because of the applied PEEP (4.4 ± 1.4 mm Hg in the lung-injurious group and
4.4 ± 1.3 mm Hg in the lung-protective group) compared with the spontaneously breathing animals (2.6 ± 0.6 mm Hg; P < 0.01). We observed that, after 2 h of MV, the CO temporarily decreased in both ventilated groups, but toward the end of the experiment, no differences were observed among the groups. After 4 h, CO was 393 ± 23 mL · kg⁻¹ · min⁻¹ in the control group, 327 ± 85 mL · kg⁻¹ · min⁻¹ in the lung-protective group, and 367 ± 45 mL/kg body weight/min in the lung-injurious group. During the entire experiment, heart rate remained constant and did not change significantly between the groups.
not differ among the groups. The heart rate did not differ among groups at the start or at the end of the experiment. After 4 h of MV, the heart rate was 357 ± 8 bpm in the control group, 377 ± 11 bpm in the lung-protective group, and 355 ± 12 bpm in the lung-injurious group.

**Mean Airway Pressure and Blood–Gas Analysis**

Mean airway pressure was 8 cm H2O in all groups. In both ventilated groups, the PaO2 did not change, whereas the PaO2 values in the control group were lower (Table 1). The PaCO2 and pH were comparable among the three groups.

<table>
<thead>
<tr>
<th>Table 1. Arterial Blood Gas Analysis</th>
<th>Control</th>
<th>LP-MV</th>
<th>LI-MV</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO2 (torr) t = 0</td>
<td>455.6 ± 11.0</td>
<td>441.3 ± 48.2</td>
<td>487.3 ± 48.5</td>
</tr>
<tr>
<td>PaCO2 (torr)</td>
<td>39.7 ± 1.7</td>
<td>38.7 ± 1.9</td>
<td>41.2 ± 2.1</td>
</tr>
<tr>
<td>pH</td>
<td>7.39 ± 0.02</td>
<td>7.40 ± 0.02</td>
<td>7.39 ± 0.02</td>
</tr>
<tr>
<td>PaO2 (torr) t = 60</td>
<td>78.0 ± 3.2</td>
<td>449.3 ± 32.3*</td>
<td>436.1 ± 43.9*</td>
</tr>
<tr>
<td>PaCO2 (torr)</td>
<td>42.8 ± 1.6</td>
<td>41.6 ± 2.2</td>
<td>39.1 ± 3.5</td>
</tr>
<tr>
<td>pH</td>
<td>7.35 ± 0.01</td>
<td>7.36 ± 0.02</td>
<td>7.38 ± 0.02</td>
</tr>
<tr>
<td>PaO2 (torr) t = 120</td>
<td>76.0 ± 2.7</td>
<td>453.8 ± 29.5*</td>
<td>468.2 ± 43.9*</td>
</tr>
<tr>
<td>PaCO2 (torr)</td>
<td>42.7 ± 1.4</td>
<td>44.1 ± 1.5</td>
<td>41.6 ± 3.5</td>
</tr>
<tr>
<td>pH</td>
<td>7.37 ± 0.01</td>
<td>7.35 ± 0.01</td>
<td>7.38 ± 0.03</td>
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<tr>
<td>PaO2 (torr) t = 180</td>
<td>78.4 ± 1.7</td>
<td>436.2 ± 33.2*</td>
<td>447.5 ± 40.9*</td>
</tr>
<tr>
<td>PaCO2 (torr)</td>
<td>41.8 ± 1.1</td>
<td>41.3 ± 2.2</td>
<td>36.9 ± 1.3</td>
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<tr>
<td>pH</td>
<td>7.38 ± 0.01</td>
<td>7.37 ± 0.02</td>
<td>7.42 ± 0.01*</td>
</tr>
<tr>
<td>PaO2 (torr) t = 240</td>
<td>79.0 ± 3.1</td>
<td>480.1 ± 18.4*</td>
<td>451.1 ± 37.1*</td>
</tr>
<tr>
<td>PaCO2 (torr)</td>
<td>42.1 ± 1.2</td>
<td>40.5 ± 2.9</td>
<td>39.4 ± 3.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.38 ± 0.01</td>
<td>7.38 ± 0.02</td>
<td>7.41 ± 0.03</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM.
* p<0.05 vs. control group.
LP-MV = lung protective mechanical ventilation, LI-MV = lung injurious mechanical ventilation

**RBF**

In the lung-injurious group, the mean RBF was 4.2 mL-min−1-g−1 tissue, which was approximately 40% lower (P < 0.05) than in the control group (mean 7.2 mL-min−1-g−1 tissue) and 28% lower (P < 0.05) than in the lung-protective group (mean 5.8 mL-min−1-g−1 tissue) (Fig. 3).
Renal Function
Creatinine clearance and fractional excretion of sodium did not differ among the groups and remained unchanged throughout the experiment (Table 2). No difference in urine output among the groups was observed during the experiment (Table 2).

Table 2. Kidney Function

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>LP-MV</th>
<th>LI-MV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine Clearance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ml/min)</td>
<td>t = 0</td>
<td>1.22 ± 0.1</td>
<td>1.31 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>t = 240</td>
<td>1.42 ± 0.1</td>
<td>1.88 ± 0.4</td>
</tr>
<tr>
<td>FE sodium (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(T = 0)</td>
<td>0.44 ± 0.1</td>
<td>0.54 ± 0.2</td>
<td>0.43 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>T = 240</td>
<td>0.36 ± 0.1</td>
<td>0.22 ± 0.1</td>
</tr>
<tr>
<td>Diuresis (ml/kg/hr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(t = 0)</td>
<td>1.10 ± 0.2</td>
<td>1.23 ± 0.2</td>
<td>1.16 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>t = 240</td>
<td>1.60 ± 0.2</td>
<td>1.17 ± 0.1</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM. LP-MV = lung-protective mechanical ventilation, LI-MV = lung-injurious mechanical ventilation

Histology
Cross-sections of both lungs and kidneys were assessed for organ damage. The lung-injurious ventilation caused a significantly higher lung injury score (6.8 ± 0.5) than spontaneous breathing.
(4.5 ± 0.5) or lung-protective ventilation (4.8 ± 0.4; P < 0.05) (Fig. 4). No histopathological kidney damage was observed in any of the groups.

**IL-6**

At the start of the experiment, IL-6 plasma levels were comparable among the three groups. After 4 h, there was a trend for plasma IL-6 levels to increase in all three groups (Table 3).

**Figure 4. Lung histology**

Photomicrograph of pulmonary left upper lobe slices from spontaneously breathing rats (upper left frame) and rats subjected to lung-protective mechanical ventilation (upper right frame) or lung-injurious mechanical ventilation (lower left frame) (hematoxylin and eosin, 40x objective lens). Lungs ventilated with the lung-injurious approach showed increased numbers of infiltrated macrophages, erythrocytes, and thickened septa, resulting in a significantly increased lung injury score. Values represent mean ± sem.

**ET-1**

Lung-injurious MV for 4 h resulted in a five-fold increase in production of renal ET-1 compared with lung-protective ventilation (P < 0.05) or to the control group (P < 0.05) (Fig. 5). In contrast to renal ET-1, we observed no difference in plasma ET-1 levels between the lung-injurious MV group and the control group, whereas plasma ET-1 levels were lower than both of these in the lung-protective ventilation group.
Table 3. Plasma Interleukin-6 levels

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>LP-MV</th>
<th>LI-MV</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 t = 0</td>
<td>7.3 ± 2.5</td>
<td>6.0 ± 0.9</td>
<td>5.2 ± 0.6</td>
</tr>
<tr>
<td>IL-6 t = 240</td>
<td>13.5 ± 2.7</td>
<td>10.7 ± 2.4</td>
<td>9.9 ± 2.9</td>
</tr>
</tbody>
</table>

Concentrations of plasma IL-6 (pg/mL). Values represent mean ± SEM. LP-MV = lung protective mechanical ventilation, LI-MV = lung injurious mechanical ventilation

Discussion

Lung-injurious ventilation of healthy rat lungs resulted in production of renal ET-1 and in decreased RBF compared with lung-protective ventilation or to spontaneous breathing (the control). In contrast to our expectations, these effects occurred in the absence of a systemic inflammatory response, since IL-6 plasma concentrations did not increase. The decreased RBF had no observable effect on kidney function or histology. A lung-protective strategy revealed results comparable to those seen in healthy sham-operated control animals.

We demonstrated that production of renal ET-1 is indeed affected by the applied ventilation strategy. ET-1 is a peptide with potent vasoconstrictor effects on renal microcirculation, thereby reducing RBF and glomerular filtration rate. ET-1 is not stored and released, but instead generated in response to a range of stimuli. In general, the effects of ET-1 in the kidney are primarily mediated in an autocrine/paracrine fashion and to a lesser extent to ET-1 derived from the systemic circulation. Thus, the local tissue concentrations may be several-fold higher than the levels measured in the systemic circulation. Indeed, we also observed that local production of renal ET-1 was approximately five-fold higher in the lung-injurious ventilation group. Even more relevant, it also correlated with the observed decrease in RBF.

Interpretation of ET-1 plasma concentrations, like interpretation of the observed differences between the ventilated groups, requires some caution, however. First, the circulating level of ET-1 might not directly reflect the full physiological impact of ET-1. Second, it is important to remember that ET-1 plasma concentration is dependent not only on generation, but also on renal and receptor-mediated clearance and enzyme-mediated metabolism of the peptide. Third, the plasma half-life, approximately 2 min, is very short, and ET-1 is rapidly cleared from the plasma and degraded.
The stimuli that may induce production of ET-1 include various mediators that are increased during MV. Instead of measuring a variety of mediators, we chose to measure IL-6. Previously, Ranieri et al. revealed that an increase in IL-6 plasma concentrations of ARDS patients correlated with the development of AKI, although the mechanism behind this observation was unclear. Recently, the ARDS Network trial showed that IL-6 plasma levels were independently associated with AKI in patients with acute lung injury.

In contrast to our hypothesis, the production of renal ET-1 occurred independently of IL-6. It is therefore unlikely that IL-6 plays an important role in production of renal ET-1. The absence of an IL-6 response in the present study may have been due to the applied ventilatory pressures, which may have been too low or of too short duration, although injurious MV caused significant histological damage to the lungs. In addition, the ventilatory strategy alone may not have been
sufficiently harmful to release IL-6 into the systemic circulation. The occurrence and magnitude of systemic inflammation may require preexisting (lung) injury.\textsuperscript{32-34}

The precise mechanisms by which lung-injurious ventilation increases the production of renal ET-1 remain to be elucidated. Other mediators that may increase during MV may play a role. For example, the proinflammatory mediators tumor necrosis factor-α and IL-1β have been shown to be upregulated during MV.\textsuperscript{19} Also, catecholamines and thrombin are known stimulators of ET-1 production by endothelial cells.\textsuperscript{35} One may argue that the increased production of ET-1 is secondary to the decrease in RBF. Although we cannot exclude this, it is less likely. Several experiments in animal models of sepsis have clearly demonstrated that decreased RBF is the result of increased ET-1 production, and that this effect can be prevented by the administration of ET-receptor antagonists.\textsuperscript{13} For example, infusion pretreatment with a nonselective ET receptor-antagonist increased RBF but did not improve hypotension in canine endotoxemia.\textsuperscript{36,37}

In our study, the vasoconstrictory properties of ET-1 correlated with the observed decrease in RBF in the lung-injurious ventilation group. Other mechanisms by which MV can compromise RBF were minimized.\textsuperscript{2} First, we kept the Paco2 levels equal, and hypoxia was avoided among the groups. Therefore, it is unlikely that minimal gas exchange abnormalities contributed to the differences in RBF. Second, to avoid the hemodynamic consequences of increasing thoracic pressures, we applied the same mean airway pressures in the ventilated groups. As a result, the mean arterial and central venous blood pressures were similar among the groups. Although we observed that the CO temporarily decreased after two hours, the decrease was similar in both ventilated groups; CO normalized towards the end of the experiment. It is therefore unlikely that the decrease in RBF was due to the effect of MV on systemic hemodynamics, i.e., due to alterations in venous return and a decrease in CO. From our experiments, it seems, therefore, that the decrease in RBF is indeed more a ventilator-dependent than a thoracic pressure-dependent mechanism.

The increased renal production of ET-1 and decreased RBF after 4 h of MV was not associated with observable effects on renal histology or kidney function, the latter represented by creatinine clearance, fractional sodium excretion and urine output. Creatinine clearance increased in all three groups over time. This likely represents an increased hydration status due to fluid that was injected for anesthesia and to maintain patency of the arterial catheters during the experiment. Also, the urine output did not differ among the groups throughout the
experiment. The absence of effects on renal function and histology may have been due to the relatively short period of MV. Further research is therefore needed to delineate more precisely the time course of changes in RBF beyond 4 h of MV, as well as to evaluate other biomarkers of early AKI to detect renal injury. The results of these studies may also elucidate the potential role of antagonizing mediators stimulating ET-1 production or the role of ET-1 receptor antagonists in restoring RBF and preventing MV-induced AKI.

We conclude that lung-injurious ventilation results in renal production of ET-1 that is associated with diminished RBF independently of IL-6. Further research is needed to delineate more precisely the mechanisms by which MV is involved in AKI, possibly providing new potential targets for therapeutic interventions.
References


