Chapter 9

Summary, discussion and future perspectives.
Summary and Discussion

Mechanical ventilation contributes to acute kidney injury. Only hypotheses?

A Medline database and references from identified articles were used to perform a literature search relating to mechanical ventilation, systemically released mediators and acute kidney injury (AKI). We found that AKI may be initiated or aggravated by MV through three different mechanisms. First, strategies such as permissive hypercapnia or permissive hypoxemia may compromise renal blood flow. Second, mechanical ventilation affects systemic and renal hemodynamics by reducing cardiac output, by redistribution of intrarenal blood flow and through stimulation of hormonal and sympathetic pathways. Third, mechanical ventilation may cause biotrauma - a pulmonary inflammatory reaction that may generate systemic release of inflammatory mediators. These mediators may have effects on the kidney (Chapter 2, Table 1).

The amount of research studying the effects of MV on AKI remains rather limited (Chapter 3, Table 1). Although limited in number, these studies showed a variety of different effects on the kidney which are not limited to inflammatory reactions. Effects on renal apoptosis, vasoactivity and vascular permeability have also been described. Nearly twenty mediators have been described which increase was solely contributable to MV. These mediators are not all pro-inflammatory by nature, but also include anti-inflammatory mediators, mediators involved in coagulation, fibrinolysis, cell adhesion, apoptosis and cell growth (Chapter 3, Table 2). The potential effects of these mediators are pleiotropic and include effects on inflammation, inflammatory cell recruitment, adhesion and infiltration of inflammatory cells into the kidney. Effects on apoptosis and necrosis, vasoactivity, cell proliferation, coagulation and fibrinolysis, transporter regulation, lipid metabolism and cell signaling have also been described (Chapter 3, Table 3). Most research has focused on inflammatory and chemotactic mediators, especially tumor necrosis factor (TNF)-α, interleukin (IL)-6 and macrophage inflammatory protein-2. Despite the fact that IL-10, IL-8 and active plasminogen activator inhibitor -1 all have predictive value on the development of AKI or AKI related mortality, surprisingly little is known about their potential role in the pathophysiology of AKI during MV.

The evidence supporting the effects of the various mediators, released by MV, on the kidney is scarce. In addition, there is a great disparity of knowledge of potential effects on the kidney between different mediators. No studies were identified that used specific blocking of mediators in-vivo or specific knock out models which could establish a causal relationship between MV
induced mediator release and AKI. Therefore, from a theoretical point of view, the systemic release of several mediators induced by MV may play an important role in the pathophysiology of AKI, but evidence supporting a causal relationship is lacking.

To further complicate matters, the hypothesized effects of MV on the development of AKI and the potential effects of mediators released during MV may become more significant in the presence of comorbidity. In the presence of, for example, acute lung injury, it is more difficult to maintain normal gas exchange, and moderate arterial hypoxemia and hypercapnia are often accepted. In addition, during sepsis for example, renal blood flow is compromised due to a decreased cardiac output as a consequence of high intrathoracic pressures and both the immune system and the balance between coagulation and fibrinolysis are severely altered (Chapter 2).

The development of AKI during mechanical ventilation likely represents a multifactorial process. This process includes effects on gas exchange, systemic and renal hemodynamics and systemically released mediators on top of underlying morbidity that is usually present in the critically ill (Chapter 2, Figure 1).

**Effects of mechanical ventilation in different animal models**

As discussed briefly in the previous paragraph, the effects of MV on the kidney can be affected by underlying morbidity. In addition, the effects of MV on lung injury and mediator release per se may depend on underlying lung injury. The different effects of MV may be most pronounced when these different diseases are primarily originated in the lung (direct lung injury), compared to systemically (indirect lung injury). In clinical and experimental studies direct and indirect lung injury differed in terms of respiratory mechanics, response to positive end-expiratory pressure, pulmonary inflammation, ultra-structural characteristics and pulmonary apoptosis. However, the effects of different ventilation strategies during direct and indirect lung injury on lung injury and systemic mediator release are unknown, but are important in the light of MV induced or aggravated AKI.

As described in chapter 4, rats were ventilated with LV or HV after acid instillation or during sepsis. Oxygenation and lung compliance decreased after acid instillation as compared to sepsis. Additionally, wet to dry (W/D) weight ratios and histological lung injury scores increased after acid instillation as compared to sepsis. MV increased W/D weight ratio and lung injury score; however
this effect was mainly attributable to HVt ventilation after acid instillation. Similarly, effects of HVt on oxygenation were only observed after acid instillation. HVt during sepsis did not further affect oxygenation, compliance, W/D weight ratio or lung injury score. Plasma IL-6 and TNF-α concentrations were increased after acid instillation as compared to sepsis, but plasma intercellular adhesion molecule-1 concentration increased after sepsis. In contrast to lung injury parameters, no additional effects of HVt MV after acid instillation on plasma mediator concentrations were observed (Chapter 4).

During MV more severe lung injury develops after acid instillation as compared to sepsis. HVt causes VILI after acid instillation, but not during sepsis. In direct lung injury alveolar filling by edema, fibrin, collagen, neutrophilic aggregates and/or blood predominates. Therefore, during direct lung injury, HVt ventilation likely leads to repeated alveolar overdistension with a tidal volume distributed over a smaller aerated lung volume, and subsequent aggravation of lung injury. During indirect lung injury, in contrast, interstitial edema and collapse may predominate and HVt does therefore not further injure the lungs. However, this differential effect between direct and indirect lung injury was not observed in the systemic release of mediators. Therefore, the adverse effects of MV differ between different types of lung injury. Additionally, systemic mediator levels are not indicative for the severity of lung injury. This may also affect the potential effect of MV induced release of mediators in the development or aggravation of AKI.

**Mechanical ventilation in health and disease**

The precise mechanisms by which MV can injure the kidney remain poorly understood. In the clinical setting mechanically ventilated patients on the intensive care unit are critically ill and various pathologic processes and pre-existing conditions can contribute to the development of AKI in addition to MV. In chapters 5 and 6 the effects of MV in healthy rats and rats suffering from sepsis are described. We hypothesized that MV would initiate or aggravate a pulmonary inflammatory response causing systemic inflammation. Subsequently, systemic inflammation leads to endothelial activation, renal vasoactivity, apoptosis and decreased kidney function.

In chapter 5 healthy, male Wistar rats were randomized to a lung-protective ventilation strategy or a lung-injurious strategy. 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 with a simultaneous 40% lower renal blood flow. 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. In chapter 6 sepsis was induced by cecal ligation and puncture and 24 hrs later rats were randomized to LV T or HV T or served as time matched, non-ventilated controls. Parameters of lung injury were assessed and kidney function was measured. Immunohistological analysis was used to assess apoptosis. No significant lung injury was observed, but kidney function decreased after HV T MV, which was associated with increased renal apoptosis.

Surprisingly, in both studies effects on the kidneys were observed that occurred in the absence of systemic inflammation, i.e. systemic IL-6 and TNF-α levels were similar between groups. During sepsis significant lung injury was not observed in contrast to MV of healthy lungs. This is likely contributable to different types of ventilator, ventilator settings, and rat strains. Herrera et al. previously showed that tidal volumes of minimal 20 ml/kg were necessary to induce easily identifiable lung injury. Although lung injury developed in previously healthy rats, the ventilation strategy alone may not have been harmful enough to release IL-6 into the systemic circulation. It is therefore unlikely, that TNF-α activated the death receptor pathway causing apoptosis.

Although histology revealed lung injury after MV in healthy lungs, during sepsis we found that without significant differences in lung injury, MV induced renal apoptosis and HV T decreased glomerular filtration rate by 40% compared to LV T. A recent post-mortem study of patients suffering from septic shock-induced AKI showed increased apoptosis. In our study, activation of both the death receptor pathway and the mitochondrial pathway led to apoptosis. Apoptosis was more pronounced in the renal medulla than in the renal cortex. Since this is in close concordance with known regional differences in renal blood flow, local ischemia may play an important role in inducing apoptosis by activating the mitochondrial pathway. Chapters 5 and 8 also show that MV decreases renal blood flow and vasoreactivity in healthy rats and in rats with LPS induced pneumonia, respectively. Activation of the death-receptor pathway requires death-receptor-ligand interaction, of which TNF-α is the most important ligand. However, differences in TNF-α levels were not observed.

Both chapter 5 and 6 argue for an important role for the endothelium. In chapter 5 increased ET-1 was associated with decreased renal blood flow; the main source of ET-1 being the endothelium. The histology data in chapter 6 indicate that in the renal cortex endothelial cells
were specifically susceptible to undergo apoptosis. Additionally, markers of endothelial activation, such as plasminogen activator inhibitor-1 and intercellular adhesion molecule-1, were increased during sepsis. PAI-1 levels have shown to correlate with the development of AKI in patients ventilated during lung injury. Both chapters warrant careful ventilator management, even when lung injury is not apparent, and emphasizes the necessity of close monitoring of kidney function.

Interventions during mechanical ventilation

Inflammation plays an important role in multiple organ dysfunction syndrome, including pulmonary and renal failure. In chapters 7 and 8 the role of inflammation in ventilator induced lung injury and AKI is further explored. Inflammation was attenuated by pharmacological inhibition of poly(adenosine diphosphate ribose)(PARP)-1. PARP-1 plays an important role in sensing DNA damage, DNA repair and genomic stability. By interacting with nuclear factor (NF)-κB, PARP-1 also has pro-inflammatory actions. PARP-1 activation has been implicated in the pathophysiology of lung injury and AKI.

In chapters 7 and 8, rats were subjected to intratracheal instillation of LPS and were then randomly assigned to receive mechanical ventilation at either low tidal volume or high tidal volume. The activation of PARP-1 was inhibited by intravenous administration of either PJ-34 or WW85. PJ-34 is a pharmacological inhibitor of or PARP independent of the activating stimuli. WW85, in contrast, is a novel metalloporphyrinic peroxynitrite decomposition catalyst. By blocking peroxynitrite, WW85 prevents peroxynitrite induced oxidative DNA damage thereby reducing PARP-1 activation. Inhibition of PARP-1 resulted in less pulmonary and systemic inflammation measured by IL-6 and TNF-α. But more importantly, attenuated the detrimental effects of HV ventilation on the kidney (Chapter 7 and 8). Inhibition preserved renal function and prevented kidney apoptosis and leukocyte infiltration into the kidney. HV ventilation also impaired endothelial function of arcuate renal arteries and decreased renal blood flow. These effects were also decreased by the administration of PJ-34 and WW85 (Chapter 8).

Inhibition of PARP-1 activity decreased pulmonary and systemic inflammation likely through inhibition of NF-κB. Pharmacological inhibition of PARP-1 attenuated the DNA binding capacity and subsequent reduction of NF-κB transcriptional activity. Moreover, it has been reported that neither enzymatic activity nor the DNA binding activity of PARP-1 is required for NF-κB-dependent transcriptional activation. In contrast to the studies described in chapters 7 and 8, in
the preceding chapters we did not observe systemic inflammation. First, intra-tracheal LPS likely induced an overwhelming pulmonary inflammatory reaction that HV₁ MV induced significant spill-over. In contrast, during sepsis, it has been proposed that HV₁ of at least 20 ml/kg is necessary to induce easily identifiable lung injury. Therefore, in chapter 6, systemic inflammation from pulmonary origin is unlikely to occur considering the used tidal volume of 15 ml/kg. Also, type of ventilator, ventilator settings and models may account for these differences between studies, see also chapter 4.

As observed during sepsis (Chapter 6), HV₁ MV causes renal apoptosis after intratracheal LPS. Prevention of apoptosis by PJ-34 was associated with preserved renal function (Chapter 7). In chapters 5 and 6 a role for renal hemodynamics has already been proposed, suggesting that renal local blood flow may have induced mitochondrial pathway mediated apoptosis. Chapter 8 provides more insight into these mechanisms. HV₁ MV reduced renal perfusion, without an effect on cardiac output, and impaired endothelium dependent vasodilation. Establishing cause and effect relations is precarious in these complex models of inter-organ cross talk. Global systemic vasodilation, maybe involving the release of (inflammatory) mediators, may have directly contributed to the fall in renal blood flow. The data presented in chapter 8 provide evidence that impaired endothelium dependent vasodilation also plays a role. The effect of the inhibitors may therefore be directly on the renal endothelium and indirectly by decreasing systemic inflammation. Multivariable analysis revealed an important role for direct effects. These effects support the findings of chapters 5 and 6, indicating important roles for the renal endothelium, local renal blood flow and impaired vasoactivity in apoptosis induction during VILI.

Future perspectives

Although this study gives more insights in the pathophysiology of MV induced AKI. Several important issues deserve future attention.

Mechanical Ventilation

The first part of the biotrauma hypothesis has been firmly established. However, little is known about the origin of harmful and protective mediators released during MV, the second part of the hypothesis. Whether mediators are released in the alveoli and then spill over into the systemic circulation or are released from the pulmonary endothelium directly into the systemic circulation
remains unknown. Additionally, mediators may even be generated locally in distant organs following, for example, ischemia induced endothelial activation and/or inflammation. Transplantation studies in knock-out animals may provide further clues to the origin of mediators released during MV. Additionally, these studies may also provide more insight into the biological activities of these mediators. To this end, studies using specific blocking agents, such as the biologicals anakinra and infliximab, blocking IL-1β and TNF-α respectively, or the use of specific knock-out models may be used. These subjects deserve attention in future research on VILI.

**Acute Kidney Injury**

AKI is a complex disorder that occurs in a variety of settings, ranging from post-traumatic to septic AKI, with clinical manifestations ranging from a minimal elevation in serum creatinine to anuric renal failure. On a pathophysiologic level the questions why and how AKI develops remain unanswered. Despite the complexity, the most important issue in determining the effects of MV on the induction of AKI, however, remains the lack of proper renal end-points defining AKI.

In the past decade several important changes in nephrology have taken place that affect AKI research. Important for research purposes, the classification of AKI has changed. Today two major classifications are used. First the definition and criteria defined by the Acute Kidney Injury Network and, second, the Risk-Injury-Failure-Loss-End stage renal disease (RIFLE) criteria defined by the Acute Dialysis Quality Initiative. The latter is intended to establish the presence or absence of the clinical syndrome of AKI in a given patient or situation and to describe the severity of this syndrome. In both classification systems blood creatinine and urine output are major criteria. These classification systems provide more guidance to the establishment of proper end-points in clinical AKI research. With the recent progress made in biomarker research, it is likely to predict the development of AKI more accurately in the future. Additionally, sensitive and specific biomarkers may aid the development of improved management strategies for AKI. However, for basic research purposes, there is a clear need for more specific end-points that go beyond newly developed biomarkers, serum creatinine levels and urine production, such as, for example, histological alterations. This immediately leads to the second major change in AKI research. Previously, AKI would have been histologically described as acute tubular necrosis, but recent studies, reviews and meta-analysis have shown that necrosis is very rarely found in post-mortem studies of patients dying with or from AKI in the intensive care unit. We have shown that apoptosis may play a role in AKI, findings that have also been shown in human
However, more studies are needed to establish a clear role for apoptosis in the development of AKI. Subsequently, research should focus on how apoptosis leads to clinical AKI. This is a critically important issue that remains to be elucidated. Other end-points, besides histological end-points, may include loss of specific cellular functions or transporters or hemodynamic alterations, including regulation of vascular tone. The last paradigm shift involves the role of local and systemic hemodynamics, especially during sepsis-induced AKI. Previously, oliguria during sepsis was thought mainly due global renal hypoperfusion. Most critically ill patients with sepsis have a high cardiac output state. Mimicking this hyperdynamic sepsis state, Langenberg and colleagues showed that renal blood flow was markedly increased and renal vascular resistance was markedly decreased. Glomerular filtration rate in these experiments was decreased. These observations suggest an important role for changes in renal vascular activity in sepsis induced AKI. However, no unequivocal scientific evidence can be referenced in order to define the role of renal blood flow in humans. Consequently, animal research may not provide further insights into the human situation. Similar to research on systemic and renal hemodynamics, studies on intrarenal hemodynamics may provide further insights in the quest for proper renal end-points. Conflicting reports exist on preferentially directing renal blood flow to the cortex during sepsis, causing relative hypoperfusion of the medulla. Hyperdynamic sepsis caused renal vasodilation but had limited effects on regional blood flow, but norepinephrine increased global and medullary renal blood flow and restored vascular tone. These observations challenge the view that the medulla is ischemic during sepsis, but emphasize that intrarenal hemodynamic factors are important and deserve future attention. These recent important changes in AKI research emphasize the need for future research to focus on the development of proper renal end-points for basic research.

Lastly, we have shown in the two previous chapters that pharmacologic intervention in MV induced AKI is possible. There is a clear role for inhibition of inflammation and apoptosis, but considering the complexity of VILI and AKI several more pathways may be suitable for intervention. Pharmacologic interventions should be a topic of “bench to bedside research”. Not only should new pharmacologic agents be developed, but also existing agents, as we have shown, can be used to intervene.
Summary, discussion and future perspectives

References


