Intrarenal and urinary oxygenation during norepinephrine resuscitation in ovine septic acute kidney injury

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Norepinephrine is the principal vasopressor used to restore blood pressure in sepsis, but its effects on intrarenal oxygenation are unknown. To clarify this, we examined renal cortical, medullary, and urinary oxygenation in ovine septic acute kidney injury and the response to resuscitation with norepinephrine. A renal artery flow probe and fiberoptic probes were placed in the cortex and medulla of sheep to measure tissue perfusion and oxygenation. A probe in the bladder catheter measured urinary oxygenation. Sepsis was induced in conscious sheep by infusion of *Escherichia coli* for 32 hours. At 24 to 30 hours of sepsis, either norepinephrine, to restore mean arterial pressure to preseptic levels or vehicle-saline was infused (8 sheep per group). Septic acute kidney injury was characterized by a reduction in blood pressure of ~12 mm Hg, renal hyperperfusion, and oliguria. Sepsis reduced medullary perfusion (from an average of 1289 to 628 blood perfusion units), medullary oxygenation (from 32 to 16 mm Hg), and urinary oxygenation (from 36 to 24 mm Hg). Restoring blood pressure with norepinephrine further reduced medullary perfusion to an average of 331 blood perfusion units, medullary oxygenation to 8 mm Hg and urinary oxygenation to 18 mm Hg. Cortical perfusion and oxygenation were preserved. Thus, renal medullary hypoxia caused by intrarenal blood flow redistribution may contribute to the development of septic acute kidney injury, and resuscitation of blood pressure with norepinephrine exacerbates medullary hypoxia. The parallel changes in medullary and urinary oxygenation suggest that urinary oxygenation may be a useful real-time biomarker for risk of acute kidney injury.

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Sepsis is one of the main causes of acute kidney injury (AKI), accounting for nearly 50% of cases of renal failure. Approximately one-third of these intensive care unit patients do not leave the hospital alive.[1,2] Despite the high mortality rate, the pathophysiology of septic AKI remains unclear. Accumulating evidence in models of sepsis with a hyperdynamic circulation, a phenotype similar to human sepsis, indicates that AKI can develop despite increased or maintained renal blood flow (RBF).[3–8] A possible mechanism contributing to septic AKI, in the face of renal hyperperfusion, is redistribution of intrarenal blood flow resulting in localized tissue ischemia and hypoxia. Increased heterogeneity of perfusion in nonrenal tissue is a hallmark of sepsis in patients,[9–11] and there is increasing evidence that hypoxia in the renal medulla may play a critical role in the pathogenesis of AKI.[12–14]

Norepinephrine (NE) is the first-choice vasopressor used to reverse hypotension and maintain renal function in patients with sepsis.[15] We have reported that infusion of NE in ovine hyperdynamic sepsis effectively restored blood pressure and transiently improved renal function, whereas the increase in RBF was unaffected.[3] Given the deleterious effects of hypoxia and its potential role in the development of AKI, it is important to understand the consequences of NE treatment in sepsis on renal oxygenation because NE increases the glomerular filtration rate, renal tubular load, and renal oxygen consumption.

We recently developed a method to permanently implant dual fiberoptic probes in the renal cortex and medulla for the continuous measurement of tissue laser Doppler flux, as a measure of tissue perfusion, and tissue oxygen tension (tPO_2) in conscious sheep.[16,17] We used this technique to examine changes in cortical and medullary perfusion and oxygenation in conscious sheep with hyperdynamic sepsis induced by infusion of live *Escherichia coli* (*E coli*) for 32 hours and the responses to treatment with NE.

We also examined whether renal medullary tPO_2 and urinary PO_2, measured at the tip of a bladder catheter, changed in a similar manner during the development of septic AKI and during treatment with NE. This was based on the observation that the vasa recta run close and parallel to the medullary collecting ducts so that pelvic urinary PO_2 would

be expected to equilibrate with medullary tPO_2.[18] We reasoned that validation of such a technique would provide a scientific basis for using urinary PO_2 as a novel real-time clinical biomarker of risk of AKI.

RESULTS

Development of sepsis with AKI

At 24 hours of sepsis in the vehicle group (N = 8), the mean arterial pressure (MAP) had decreased (from 85 ± 2 to 73 ± 4 mm Hg, $P < 0.001$), and there were increases in heart rate (from 80 ± 11 to 154 ± 18 beats per minute), $P < 0.001$ (Figure 1a and b), RBF (from 6.6 ± 2 to 11 ± 2 ml/min per kilogram, $P < 0.001$), and renal vascular conductance (from 0.08 ± 0.02 to 0.15 ± 0.02 ml/min per kilogram/mm Hg, $P < 0.001$) (Figure 1c and d). Septic AKI was characterized by a 50% reduction in urine flow (from 0.019 ± 0.001 to 0.009 ± 0.001 ml/min per kilogram, $P = 0.001$) and a 65% decrease in creatinine clearance (from 2.2 ± 0.9 to 0.8 ± 0.3 ml/min per kilogram, $P = 0.01$) (Figure 2a and c). Plasma creatinine concentration increased by 70% (from 67 ± 18 to 122 ± 42 μmol/l, $P = 0.009$), and fractional sodium excretion decreased by 40% (from 1.2 ± 0.3 to 0.7 ± 0.3 %, $P = 0.03$) (Figure 2b and d). Similar responses were seen in the NE-treated group (N = 8) at 24 hours of sepsis (Figures 1 and 2).

Septic AKI was characterized by reduced renal medullary perfusion and hypoxia, with parallel reductions in urinary oxygen tension

Urinary PO_2 decreased during the 24-hour period of development of sepsis, in a manner that closely reflected the decrease in medullary tPO_2, but not changes in urine flow (Figure 3a). Regression analysis was performed of 60-minute averages of urinary PO_2 from 8 hours before infusion of *E coli* until just before infusion of NE or saline (Figure 3b). The relationship between medullary tPO_2 and urinary PO_2 had an X-intercept close to 0 (2.4 mm Hg) and a slope that did not differ significantly from unity. Both a simple regression between all observations of medullary and urinary PO_2 and a model that additionally included the categorical variable sheep explained 49% of the variance in urinary PO_2 ($P < 0.001$). Furthermore, although the absolute value of urinary PO_2 does not represent an absolute value of medullary tPO_2 across individual sheep, the reductions in urinary PO_2 observed during development of septic AKI closely reflected changes in medullary tPO_2.

A progressive decline in medullary perfusion (Figure 4b) was associated with the decreases in medullary tPO_2 (Figure 4d) and urinary PO_2 (Figure 4e) over the first 24 hours of sepsis. In contrast, cortical tPO_2 increased (from 29 ± 5 to 37 ± 8, $P = 0.05$) (Figure 4c), and there was a tendency for

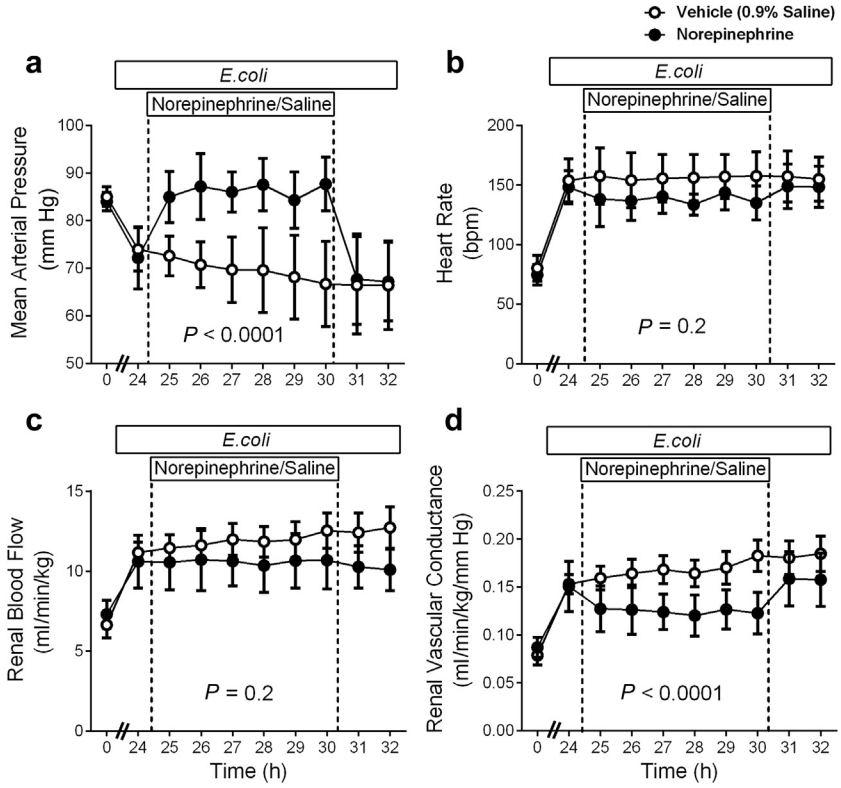

Figure 1 | Mean arterial pressure (a), heart rate (b), renal blood flow (c), and renal vascular conductance (d) during infusion of *Escherichia coli* (*E coli*) from 0 to 32 hours and subsequent treatment with norepinephrine (N = 8) or saline (N = 8) from 24 to 30 hours in conscious sheep. Time 0 is the mean of the 24th hour of the baseline period, and times 24 to 32 hours are means of 1-hour periods. Data are between-animal mean ± SD. Renal variables are presented as absolute values corrected for body weight. *P* values represent treatment-time interactions from a 2-way repeated-measures analysis of variance from 24 to 30 hours of sepsis, with a Bonferroni correction (k = 2). K represents the number of comparisons with the 24th hour of the sepsis time point.