

RESEARCH ARTICLE | *Cardiovascular and Renal Integration*

Furosemide reverses medullary tissue hypoxia in ovine septic acute kidney injury

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Iguchi N, Lankadeva YR, Mori TA, Osawa EA, Cutuli SL, Evans RG, Bellomo R, May CN. Furosemide reverses medullary tissue hypoxia in ovine septic acute kidney injury. *Am J Physiol Regul Integr Comp Physiol* 317: R232–R239, 2019. First published May 29, 2019; doi:10.1152/ajpregu.00371.2018.—In experimental sepsis, the rapid development of renal medullary hypoxia precedes the development of acute kidney injury (AKI) and may contribute to its pathogenesis. We investigated whether inhibiting active sodium transport and oxygen consumption in the medullary thick ascending limb with furosemide attenuates the medullary hypoxia in experimental septic AKI. Sheep were instrumented with flow probes on the pulmonary and renal arteries and fiber optic probes to measure renal cortical and medullary perfusion and oxygen tension (PO₂). Sepsis and AKI were induced by infusion of live *Escherichia coli*. At 24 h of sepsis there were significant decreases in renal medullary tissue perfusion (1,332 ± 233 to 698 ± 159 blood perfusion units) and PO₂ (44 ± 6 to 19 ± 6 mmHg) (both *P* < 0.05). By 5 min after intravenous administration of furosemide (20 mg), renal medullary PO₂ increased to 43 ± 6 mmHg and remained at this normal level for 8 h. Furosemide caused transient increases in fractional excretion of sodium and creatinine clearance, but medullary perfusion, renal blood flow, and renal oxygen delivery were unchanged. Urinary F₂-isoprostanes, an index of oxidative stress, were not significantly changed at 24 h of sepsis but tended to transiently decrease after furosemide treatment. In septic AKI, furosemide rapidly restored medullary PO₂ to pre-septic levels. This effect was not accompanied by changes in medullary perfusion or renal oxygen delivery but was accompanied by a transient increase in fractional sodium excretion, implying decreased oxygen consumption as a mechanism.

acute kidney injury; furosemide; hypoxia; septic shock

INTRODUCTION

Sepsis is one of the main causes of acute kidney injury (AKI). It accounts for nearly 50% of cases of renal failure (35) and has a mortality rate of ~30% (2). There is recent experimental evidence from studies using chronically implanted

fiber-optic probes, to continuously monitor renal cortical and medullary tissue perfusion and oxygen tension (PO₂), that renal tissue hypoperfusion and hypoxia occur during sepsis (6, 19–21). In particular, during the development of ovine septic AKI, there is a rapid decrease in medullary tissue PO₂ that precedes the development of AKI by up to 24 h (6, 21). These findings suggest that medullary hypoxia may contribute to the development of septic AKI and that reducing the level of medullary hypoxia in sepsis would be a logical goal to reduce the incidence and progression of AKI.

Furosemide induces diuresis and natriuresis by inhibition of the Na-K-Cl cotransporters in the thick ascending limb of the loop of Henle, thus reducing sodium transport and the oxygen demand of medullary tubular cells. This change would be expected to improve the intrarenal oxygen supply-to-demand balance and increase tissue oxygenation. In support of this proposition, furosemide has been found to increase renal oxygenation, as measured indirectly by blood oxygen level-dependent magnetic resonance imaging, in both man (10) and experimental animals (11). Although these findings provide theoretical support for the use of furosemide in patients with septic AKI, studies examining the effects of furosemide in such patients have demonstrated mixed benefits and no evidence that they reduce the requirement for renal replacement therapy or mortality (1, 12, 26). Thus, it is possible that although inhibition of Na-K-Cl cotransporters with furosemide may increase renal tubular oxygenation in sepsis, this could lead to increased oxidative stress. Indeed, in septic patients, furosemide increased the levels of urinary F₂-isoprostanes (32), which are considered to be reliable markers of in vivo oxidative stress (27). In view of the increasing interest in the pathological role of oxidative stress in sepsis (3, 25, 40), it is important to determine the effects of furosemide on directly measured medullary PO₂ and urinary F₂-isoprostanes in sepsis. Although probes can be implanted into the renal medulla to measure tissue PO₂ in experimental studies, such a technique is not clinically feasible. Recent findings indicate that medullary tissue PO₂ correlates with bladder urinary PO₂ (21, 22), suggesting that this measure could be used clinically to indirectly assess the level of oxygenation in the renal medulla.

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Thus, we aimed to determine the effects of furosemide treatment on intrarenal tissue perfusion and P_{O_2} and bladder urinary P_{O_2} in an ovine model of septic AKI. In this model, sepsis is induced by infusion of live *E. coli*, which produces a hyperdynamic state similar to that seen in human sepsis, in contrast to the hypodynamic state seen in many rodent models of sepsis (4, 16, 17, 38). In addition, we measured urinary F_2 -isoprostanes to assess whether the hypothesized increase in medullary P_{O_2} following furosemide is associated with increased oxidative stress, as assessed by the levels of F_2 -isoprostanes in the urine.

MATERIALS AND METHODS

Animal preparation. Experiments were conducted on seven healthy adult Merino ewes (38.6 ± 1.8 kg) housed in individual metabolic cages and fed 800 g of oat chaff/day and given water ad libitum. Experimental procedures were approved by the Animal Ethics Committee of the Florey Institute of Neuroscience under guidelines laid down by the National Health and Medical Research Council of Australia.

Sheep underwent two surgical procedures under general anesthesia. In the first, a carotid arterial loop was created and a transit-time flow probe placed around the pulmonary artery, as previously described (18, 23). After ≥ 2 wk, a transit time flow probe was placed around the left renal artery, and the renal vein was cannulated. Fiber-optic probes (CP-004-001; Oxford Optronix, Abingdon, UK) were inserted into the renal cortex and medulla (7). These probes measure cortical and medullary tissue P_{O_2} by fluorescence lifetime oximetry, tissue perfusion by laser Doppler flux, and tissue temperature. In addition, the carotid artery and jugular vein were cannulated, and a Foley bladder catheter (size 12, 30 ml; Euromedical) was inserted for collection of urine (7). After 5 days of recovery, and the day before the experiment commenced, an oxygen probe (LAS-1/O/E; Oxford Optronix) was advanced to the tip of the bladder catheter for measurement of urinary P_{O_2} (21). Analog signals of mean arterial pressure (MAP), heart rate (HR), renal blood flow (RBF), renal cortical and medullary tissue perfusion, P_{O_2} , temperature, and urinary P_{O_2} were recorded at 100 Hz on a computer using a CED micro 1401 interface and Spike 2 software (Cambridge Electronic Design, Cambridge, UK).

Experimental protocol. After a 24-h baseline period, sepsis was induced by intravenous infusion of live *Escherichia coli* (2.8×10^9 colony-forming units over 30 min), followed by a continuous infusion (1.26×10^9 colony-forming units/h for 32 h). Sheep were given a maintenance infusion of intravenous NaCl (0.9% wt/vol) at $1 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ from the start of the baseline period until the end of the intervention period (56 h). We have shown in this experimental model of sepsis that this level of fluid administration maintains central venous pressure over 48 h (18). At 24 h of sepsis, sheep were administered intravenous furosemide (Lasix, 20 mg; Sanofi-Aventis), followed by a bolus of saline (10 ml).

After the injection of furosemide, cardiovascular and renal variables were continuously recorded over an 8-h period. Arterial and renal venous blood samples were obtained at baseline, just before infusion of *E. coli*, at 24 h of sepsis, and then 0.5, 4, and 8 h after furosemide injection for measurement of blood gases and lactate (ABL System 625; Radiometer Medical, Copenhagen, Denmark). Whole body and renal (R) oxygen delivery ($\dot{V}O_2$), oxygen consumption ($\dot{V}O_2$), and oxygen extraction ratio were calculated using standard formulas: $\dot{D}O_2 = CO \times \text{arterial } O_2 \text{ content}$, $R\dot{D}O_2 = RBF \times \text{arterial } O_2 \text{ content}$, $\dot{V}O_2 = CO \times (\text{arterial} - \text{venous } O_2 \text{ content})$, $R\dot{V}O_2 = RBF \times (\text{arterial} - \text{renal venous } O_2 \text{ content})$, and $O_2 \text{ extraction ratio} = 100 \times (\text{arterial} - \text{venous } O_2 \text{ content}) / (\text{arterial } O_2 \text{ content})$. Stroke volume (SV) was calculated as CO/HR , and total peripheral conductance (TPC) was calculated as CO/MAP . Renal vascular conductance (RVC) was calculated as RBF/MAP . Arterial blood

and urine samples were simultaneously obtained for measurement of creatinine and sodium and for quantification of urine F_2 -isoprostanes, markers of in vivo renal oxidative stress (32).

Statistical analysis. One animal died between 12 and 24 h after *E. coli* infusion commenced and was excluded from analyses. The number of observations for each variable is shown in the respective figure legends; the lower number for some variables is due to failure of implanted probes and loss of patency of the renal venous cannulas. Data are reported as means \pm SE. Variables are reported as the average over the 24th hour of the baseline period before infusion of *E. coli* and then as either hourly averages from 24 to 32 h of sepsis or 10-min averages from 20 min before to 60 min after furosemide treatment. Differences between baseline and 24 h of sepsis were compared using Student's paired *t*-test, for which two-tailed $P \leq 0.05$ was considered statistically significant. (GraphPad PRISM 6.0 Software; GraphPad, San Diego, CA). The mean of the pretreatment period was compared with the levels at 24–28 h after furosemide using one-way repeated-measures analysis of variance, for which $P \leq 0.05$ was considered statistically significant.

RESULTS

Development of septic shock. In all sheep, septic shock developed by 24 h after the start of infusion of *E. coli*, as indicated by a MAP < 65 mmHg and arterial lactate > 2 mmol, thus fulfilling the SEPSIS-3 consensus criteria for septic shock (33). In addition, these animals also fulfilled the consensus guidelines for stage 1 AKI according to the Kidney Disease: Improving Global Outcomes criteria, i.e., a decrease in urinary output to $< 50\%$ of its baseline level and a 1.5-fold increase in plasma creatinine (14).

Effects of treatment with furosemide on intrarenal tissue perfusion and P_{O_2} and urinary P_{O_2} in ovine septic AKI. At 24 h after the start of infusion of *E. coli*, when septic AKI had developed, there were selective and significant reductions in medullary tissue perfusion ($1,332 \pm 233$ to 698 ± 159 blood perfusion units) and P_{O_2} (44 ± 6 to 19 ± 6 mmHg) (Fig. 1, A and C). This medullary tissue hypoxia was accompanied by a significant decrease in bladder urinary P_{O_2} (38 ± 4 to 16 ± 2 mmHg) (Fig. 1E). In contrast, cortical perfusion was not significantly changed, and cortical P_{O_2} was significantly increased from baseline values (Table 1).

After an intravenous bolus of furosemide (20 mg), there was a rapid increase in medullary P_{O_2} (19 ± 6 to 43 ± 6 mmHg), reaching a plateau within 5 min (Fig. 1D), which was maintained for the following 8 h (Fig. 1C). Furosemide caused no immediate increase in medullary tissue perfusion (Fig. 1B), although there was a tendency for it to increase over the following 8 h, until 32 h of sepsis (Fig. 1A). In contrast, following furosemide, there were no significant changes in cortical P_{O_2} or cortical perfusion (Table 1). Over the first hour following furosemide, there was a small, nonsignificant increase in urinary P_{O_2} (Fig. 1E), similar to that observed in medullary P_{O_2} (Fig. 1F), which then decreased over the next 7 h to a level comparable with that before furosemide administration.

Effect of furosemide on urinary F_2 -isoprostanes in ovine septic AKI. At 24 h of sepsis, there was no significant change in urinary F_2 -isoprostanes (Fig. 2E). At 30 min after furosemide, there was a decrease in urinary F_2 -isoprostanes, but by 8 h after furosemide the levels were similar to those at baseline or 24 h of sepsis (Fig. 2E). No significant effect of furosemide

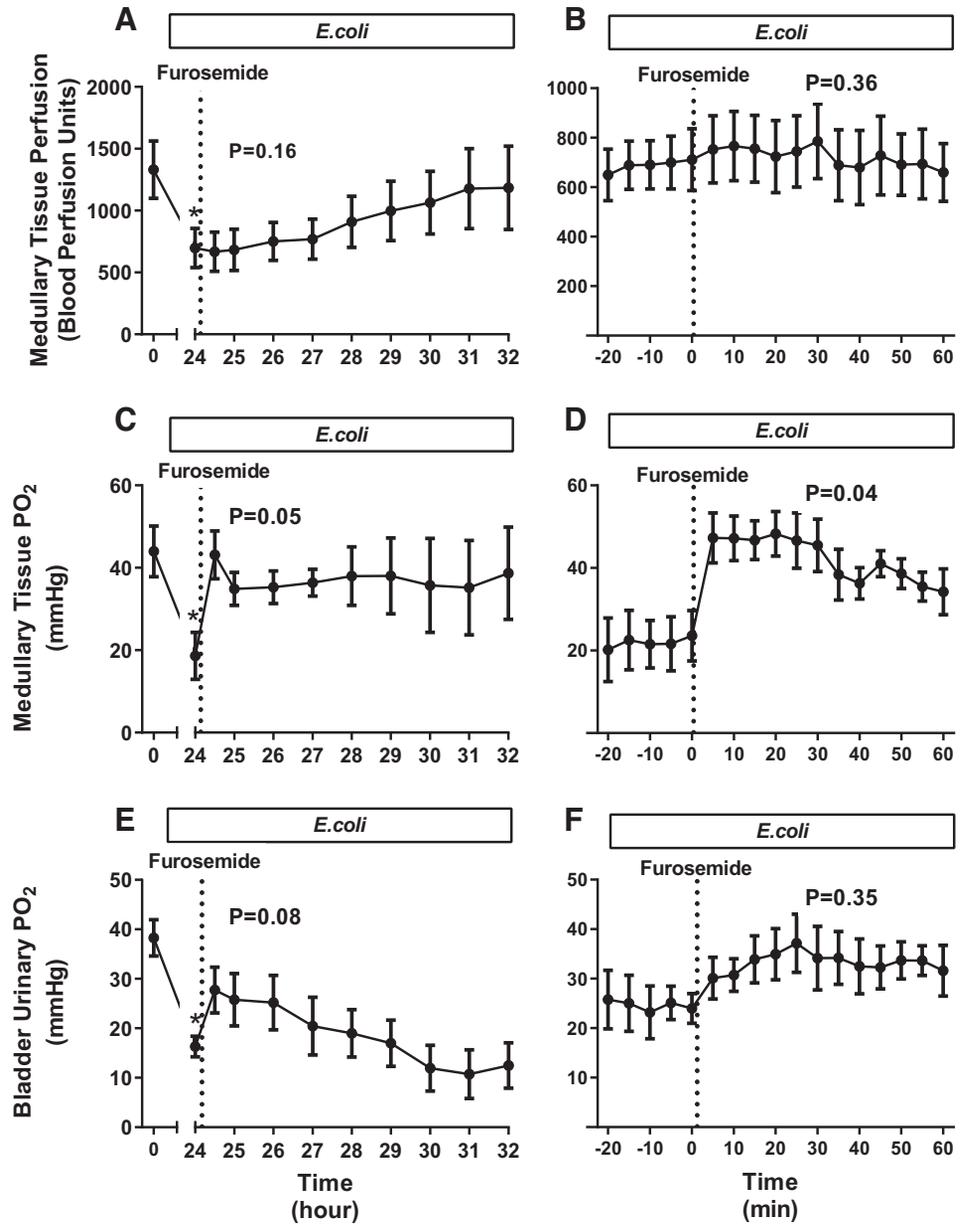


Fig. 1. Effects of furosemide on medullary tissue perfusion and oxygen tension (PO_2) and urinary PO_2 in conscious sheep with septic acute kidney injury (AKI; $n = 6$). After a 24-h baseline period, sepsis was induced by infusion of *Escherichia coli* (*E. coli*) from 0 to 32 h, and furosemide (20 mg) was given at 24 h of sepsis. A, C, and E: time 0 is the mean of the 24th hour of the baseline period, and times 24–32 h are means of 1-h periods. B, D, and F: times are means of 10-min periods. * $P < 0.05$, baseline period (time 0) compared with 24 h of sepsis. Data are means \pm SE. P values are from 1-way repeated-measures ANOVA of data from 24 to 28 h (A, C, and E) and 0–60 min (B, D, and F).

on the level of urinary F_2 -isoprostanes was detected when this was indexed to urinary creatinine concentration (Fig. 2F).

Effects of furosemide on renal function and renal blood flow in ovine septic AKI. At 24 h after the start of infusion of *E. coli*, AKI was indicated by significant decreases in urine flow, creatinine clearance, and fractional excretion of sodium and a significant increase in plasma creatinine concentration (Fig. 2 and Table 1). Furosemide tended to increase urine flow, but the response was transient, variable between animals and did not reach statistical significance. Furosemide caused a transient improvement in creatinine clearance (Fig. 2D), a reduction in urine creatinine (Fig. 2C) and increased the fractional excretion of sodium (Fig. 2B).

Effects of furosemide on systemic and global renal hemodynamics in ovine septic AKI. At 24 h of sepsis there was a significant decrease in MAP (81 ± 3 to 61 ± 4 mmHg) due to peripheral vasodilation, as shown by an increase in TPC (Fig.

3). There were increases in HR, CO, RVC, and RBF (Fig. 3). Following furosemide, all these changes were maintained over the next 8 h in a similar pattern to that seen previously in saline/vehicle-treated sheep with sepsis (6, 21, 22). Sepsis and furosemide had no significant effects on RD_{O_2} , RV_{O_2} , or O_2 extraction ratio (Table 1).

DISCUSSION

The main findings of this study are that, in established ovine sepsis with AKI, treatment with a dose of furosemide as used in septic patients with AKI caused a transient increase in creatinine clearance and fractional excretion of sodium as well as a sustained increase in renal medullary tissue PO_2 to normal levels. There was no significant increase in medullary perfusion, and cortical perfusion and cortical PO_2 were unchanged. There were similar changes in medullary and urinary PO_2

Table 1. Renal oxygenation and function, blood lactate, and body temperature

Variables	Baseline	Sepsis, h			
		24	24.5	28	32
Cortical tissue PO ₂ , mmHg (n = 6)	32.6 ± 3.8	48.6 ± 4.8*	51.8 ± 5.4	44.4 ± 8.1	45.5 ± 6.6
Cortical tissue perfusion, BPU (n = 6)	1,492 ± 124	1,688 ± 378	1,532 ± 205	1,844 ± 458	1,968 ± 625
Medullary tissue PO ₂ , mmHg (n = 6)	44.0 ± 15.1	18.6 ± 14.0*	43.1 ± 14.2	38.0 ± 17.4	38.7 ± 27.4
Medullary tissue perfusion, BPU (n = 6)	1,331.5 ± 569.7	698.0 ± 388.4*	667.4 ± 389.8	909.0 ± 504.6	1,183.4 ± 828.4
Renal O ₂ delivery, ml O ₂ ·min ⁻¹ ·kg ⁻¹ , (n = 7)	0.82 ± 0.08	0.94 ± 0.07	0.93 ± 0.09	0.99 ± 0.09	1.07 ± 0.13
Renal O ₂ consumption, ml O ₂ ·min ⁻¹ ·kg ⁻¹ (n = 4)	0.10 ± 0.02	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01
Renal O ₂ extraction ratio, % (n = 4)	12.2 ± 2.8	8.00 ± 1.5	8.0 ± 0.4	6.3 ± 1.1	7.4 ± 1.2
Plasma creatinine, μmol/l (n = 6)	86.3 ± 9.4	186.0 ± 40.8*	201.7 ± 39.3	205.3 ± 42.5	218.2 ± 44.0
Urinary sodium excretion, mmol/h (n = 7)	5.48 ± 0.84	1.25 ± 0.95*	3.41 ± 2.15	1.06 ± 0.58	0.98 ± 0.55
Blood lactate, mmol/l (n = 6)	0.6 ± 0.2	2.5 ± 0.8*	2.0 ± 0.6	4.3 ± 2.7	2.3 ± 1.4
Core temperature, °C (n = 7)	40.0 ± 0.2	41.5 ± 0.2*	41.3 ± 0.2	41.2 ± 0.2	40.9 ± 0.3

Values are means ± SE; n, number of sheep. BPU, blood perfusion units; PO₂, oxygen tension. Measurements were made at the end of the baseline period, at 24 h of sepsis, and then after a bolus infusion of furosemide given at 24 h of sepsis in conscious sheep. *P < 0.05, significant differences between baseline and 24-h of sepsis from a Student's paired t-test.

during the development of sepsis and during the first 2 h after furosemide, but as oliguria developed this correlation disappeared. There were no significant changes in the urinary levels of F₂-isoprostanes.

Furosemide as a treatment for AKI. Furosemide is a commonly used diuretic that inhibits Na-K-Cl cotransporters at the intraluminal side of the epithelial cells in the ascending limb of the loop of Henle, resulting in natriuresis and diuresis. By

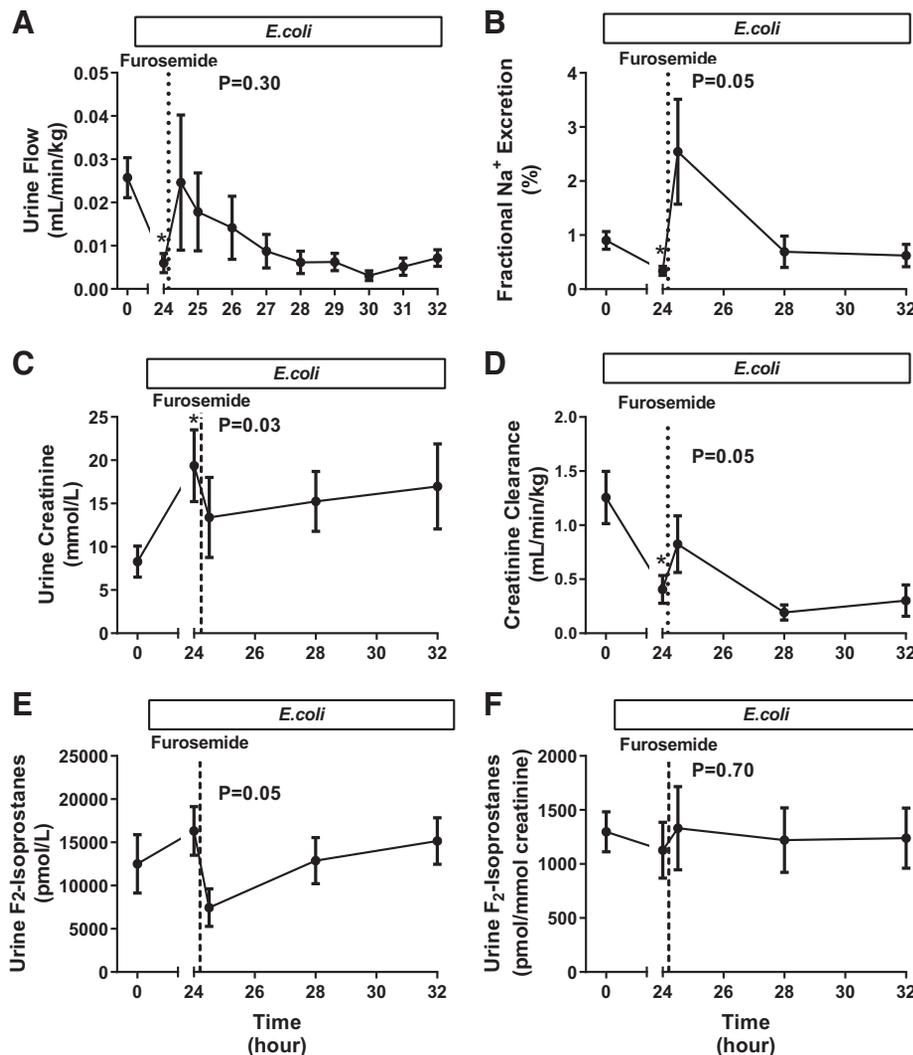


Fig. 2. Effects of furosemide on urine flow, fractional excretion of sodium, urinary creatinine, creatinine clearance, urinary F₂-isoprostanes, and urinary F₂-isoprostanes/creatinine in conscious sheep with septic acute kidney injury (AKI; n = 8). After a 24-h baseline period, sepsis is induced by infusion of *Escherichia coli* (*E. coli*) from 0 to 32 h, and furosemide (20 mg) was given at 24 h of sepsis. Data are means ± SE. For urine flow, time 0 is the mean of the 24th hour of the baseline period, and times 24–32 h are means of 1-h periods. *P < 0.05, baseline period (time 0) compared with 24 h of sepsis. P values are from 1-way repeated-measures ANOVA of data from 24 to 28 h.

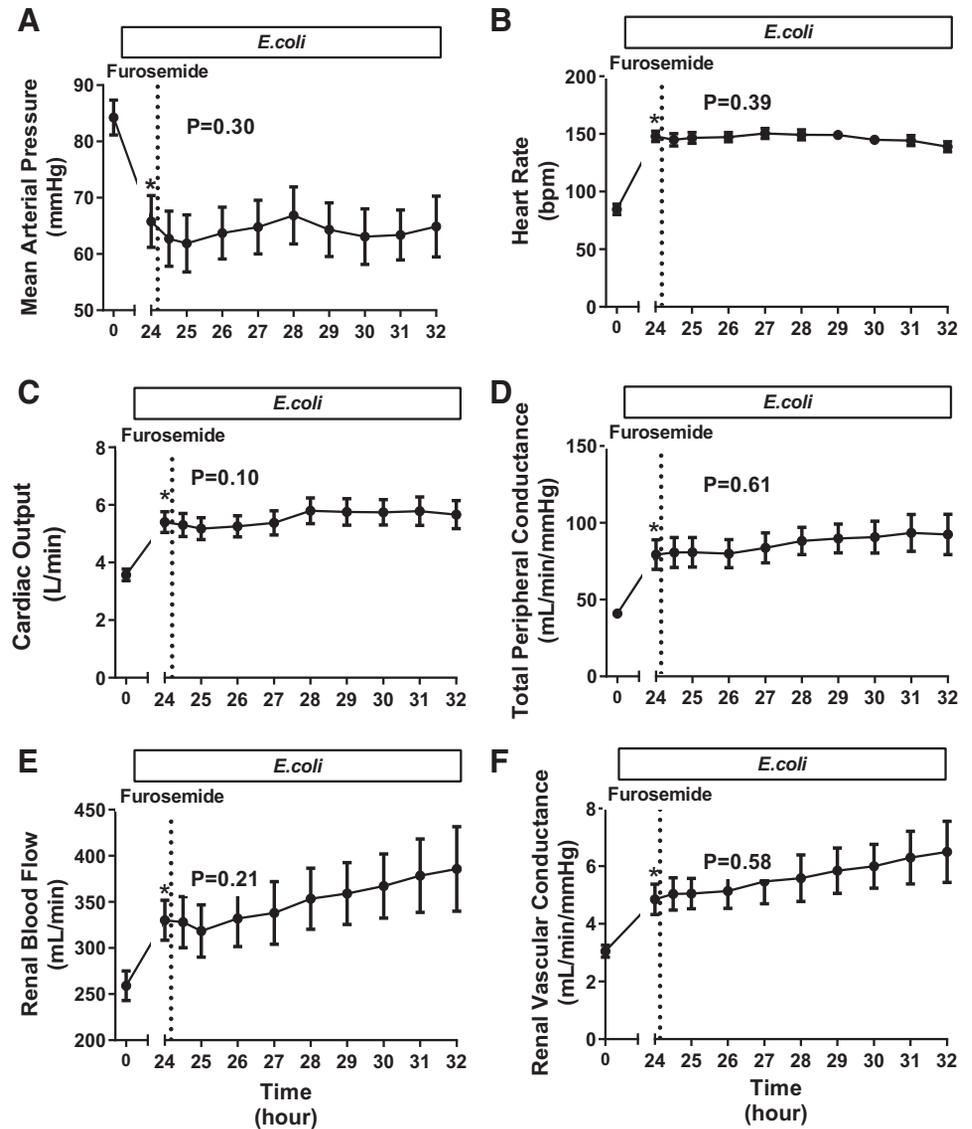


Fig. 3. Effects of furosemide on systemic and renal hemodynamics in conscious sheep with septic acute kidney injury [AKI; $n = 8$, except $n = 7$ for renal blood flow (RBF) and renal vascular conductance (RVC)]. After a 24-h baseline period, sepsis was induced by infusion of *Escherichia coli* (*E. coli*) from 0 to 32 h, and furosemide (20 mg) was given at 24 h of sepsis. Data are means \pm SE. *Time 0* is the mean of the 24th hour of the baseline period, and *times 24–32 h* are means of 1-h periods. $*P < 0.05$, baseline period (*time 0*) compared with 24 h of sepsis. *P* values are from 1-way repeated-measures ANOVA of data from 24 to 28 h.

inhibiting the Na-K-Cl transporter, furosemide reduces energy requirements and improves the oxygen supply balance of the kidney. Therefore, it is possible that loop diuretics might reduce the selective medullary hypoxia that occurs early in septic AKI (6, 19–21). Importantly, in sepsis this hypoxia occurs before the development of AKI, suggesting that it may be an initiating factor. However, seven randomized controlled trials (5, 8, 9, 12, 15, 30, 37) and one systematic review (13) revealed no benefit of furosemide for recovery of AKI. The furosemide doses used in these trials were high (600–3,000 mg/day), only a portion of the patients had sepsis, and the drug was delivered late in the course of AKI in an attempt to convert patients with oliguric to nonoliguric AKI. In contrast, we administered a low dose of furosemide early in the course of septic AKI.

Acute effects of furosemide on intrarenal P_{O_2} . During sepsis there were selective reductions in renal tissue medullary perfusion and P_{O_2} after 24 h, which we have previously shown are maintained until 32 h of sepsis (19, 21, 22). Furosemide increased fractional excretion of sodium within 30 min, and this was accompanied by a rapid increase in medullary P_{O_2} to

levels seen in the healthy state. The increase in medullary P_{O_2} was not accompanied by a change in medullary perfusion or changes in whole kidney oxygen delivery, suggesting reduced oxygen utilization for sodium reabsorption in tubular elements in the renal medulla. There were no significant changes in whole kidney consumption or extraction ratio, but this may reflect the relatively small component of total renal oxygen consumption attributable to the medulla (~20%) (24) and/or the relatively small number of sheep ($n = 4$) in which we determined these variables. However, at 30 min after furosemide, the increases in fractional excretion of sodium were variable, and even in three nonresponsive animals, in which there were minimal changes in fractional excretion of sodium (+0.12, +0.03 and -0.19%), there were similar increases in medullary P_{O_2} (+20.2, +22.9 and +27.8 mmHg) to those seen in responsive animals with large increases in fractional excretion of sodium.

Prolonged effects of furosemide on intrarenal P_{O_2} . The causes of the sustained increase in medullary P_{O_2} induced by furosemide are unclear since the increase in plasma levels of furosemide in patients with septic AKI peaks at 1 h after

administration and declines to very low levels over 4–6 h (31). This study also demonstrated that due to reduced renal clearance of furosemide in AKI, the level of furosemide in urine is only mildly increased. The lack of secretion of furosemide into the lumen of the proximal tubules in AKI, together with the fact that the tubular concentration of furosemide determines its natriuretic effect, accounts for the mild diuretic action of furosemide in sepsis compared with the healthy state. In accord with this, the natriuretic effect of furosemide in ovine sepsis was mild, with only transient increases in urinary sodium excretion and FeNa that dissipated by 4 h after administration of furosemide, whereas medullary Po_2 remained increased. These findings suggest that the mechanism causing the maintained increase in medullary Po_2 over 8 h is not solely dependent on the effect of furosemide to reduce sodium transport. A difference from our previous studies of ovine sepsis, in which medullary perfusion remained decreased (21, 22), is that following furosemide there was a strong tendency for medullary tissue perfusion to progressively increase from 24 to 32 h of sepsis, but whether this was sufficient to account for the maintained increase in medullary Po_2 is unclear.

Effects of furosemide on bladder urinary Po_2 . We have previously shown a close correlation between bladder urinary Po_2 and medullary tissue Po_2 (21, 22). The present study confirmed a similar pattern of change in these two variables during the development of sepsis and in the 2 h following furosemide. However, when urine flow decreased below $0.01 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$, urinary Po_2 decreased despite the furosemide-induced increase in medullary Po_2 being maintained. This finding is in accord with previous studies that have demonstrated a stronger correlation between urinary and medullary Po_2 under conditions with high urine flow. Experimental and computational modeling studies in rabbits indicated that the value of bladder urine Po_2 as an estimate of medullary tissue Po_2 is greatly increased under diuretic conditions (29), and in dogs urinary Po_2 was reduced when urine flow fell below 3 ml/min (28).

Effects of furosemide on urinary F_2 -isoprostanes. In addition to direct measurement of cortical and medullary Po_2 , we measured the effect of furosemide on urinary F_2 -isoprostanes and considered to be *in vivo* biomarkers of oxidative stress and lipid peroxidation (27). In the present study, there was a trend for urinary F_2 -isoprostanes to increase in ovine sepsis, but this was not significant. These data contrast with recent observations in septic patients that renal oxidative stress, as indicated by an increase in F_2 -isoprostanes, was greatest in those patients with the most severe AKI (32). The lack of a significant increase in urinary F_2 -isoprostanes in ovine sepsis at 24 h may be due to the shorter length of AKI than in the septic patients and/or a milder degree of AKI. In septic patients, furosemide increased urine F_2 -isoprostanes, with the greatest increase in those with the lowest creatinine clearance (32). In contrast, in ovine sepsis, there was a tendency for a transient decrease in urinary F_2 -isoprostanes after furosemide, indicating that the significant increase in medullary Po_2 did not result in oxidative stress. The change in urinary F_2 -isoprostanes was absent when indexed for the changes in urine creatinine concentration.

Effects of furosemide on renal function. Furosemide transiently increased creatinine clearance in septic sheep with established AKI and medullary hypoxia, although there was no reduction in plasma creatinine. A possible mechanism under-

lying this increase in creatinine clearance is an effect of furosemide on tubuloglomerular feedback (TGF) (34). Loop diuretics such as furosemide induce their potent diuretic effects not only by inhibiting the Na-K-Cl cotransporters in the thick ascending limb of Henle's loop, but also by inhibiting these cotransporters within the macula densa, thus increasing sodium delivery to the distal portions of the nephron by blocking TGF (36, 39). The transient effect of furosemide to increase creatinine clearance in septic sheep may depend in part on the partial removal of the tonic constrictor effect of TGF on the afferent arterioles, particularly in the face of unchanged systemic and renal hemodynamics.

The mild effect of furosemide on renal function in ovine sepsis may reflect the fact that the action of furosemide depends on its secretion into the lumen of the proximal tubules, which has been shown to be reduced in septic patients with AKI (31). The lack of a sustained improvement in renal function with furosemide in sepsis may account for the lack of clinical benefit of furosemide in septic patients. In addition, the mild and brief natriuretic effect of furosemide in this study likely accounts for the lack of any changes in systemic hemodynamics or total renal blood flow.

Strengths and limitations. Strengths of this study include the measurement of multiple cardiovascular, renal, and biochemical variables in a conscious large animal model of hyperdynamic sepsis with a phenotype similar to human sepsis. Furosemide was administered without general anesthesia or other treatments for septic shock, which may have had confounding effects on systemic and renal hemodynamics. The dose of furosemide used (20-mg bolus in 40-kg sheep) was clinically relevant. Limitations of the study are that the animals did not have comorbidities such as old age, hypertension, or diabetes. Furthermore, we investigated only the effect of furosemide on AKI induced by sepsis, although this is the leading cause of AKI. We investigated only the effects of a low dose of furosemide, but previous clinical trials in patients with AKI showed that multiple high doses of furosemide may have adverse renal outcomes (13).

Perspectives and Significance

There is increasing evidence that in many disease states tissue hypoxia is part of the pathological process causing organ damage and dysfunction. In this regard, in an ovine model of septic AKI, we have demonstrated the occurrence of hypoxia selectively in the renal medulla, but not in the cortex, that precedes the development of AKI. Although it seems likely that this hypoxia is harmful and will contribute to the development and progression of AKI, we have now shown in two different situations, repeated fluid bolus therapy (19) and treatment with furosemide, that restoring medullary tissue oxygenation to healthy levels induces a mild improvement in renal function, but this is not sustained despite medullary Po_2 remaining elevated. Further studies are required to establish whether improvements in medullary Po_2 lead to reduced progression of AKI and a decrease in the incidence of subsequent chronic kidney disease. This study also confirmed previous findings that changes in bladder Po_2 paralleled the changes in medullary Po_2 during the development of sepsis and initially after administration of furosemide and also confirmed previous

findings that a limitation of this technique is that this relationship declines as oliguria develops.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

N.I., Y.R.L., R.G.E., R.B., and C.N.M. conceived and designed research; N.I., Y.R.L., T.A.M., and C.N.M. performed experiments; N.I., Y.R.L., and T.A.M. analyzed data; N.I., Y.R.L., R.G.E., R.B., and C.N.M. interpreted results of experiments; N.I. and C.N.M. prepared figures; N.I., Y.R.L., and C.N.M. drafted manuscript; N.I., Y.R.L., T.A.M., R.G.E., and C.N.M. edited and revised manuscript; N.I., Y.R.L., T.A.M., E.A.O., S.L.C., R.G.E., R.B., and C.N.M. approved final version of manuscript.

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