Beneficial Effects of Vasopressin Compared with Norepinephrine on Renal Perfusion, Oxygenation and Function in Experimental Septic Acute Kidney Injury

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ABSTRACT

Objectives: To compare the effects of restoring mean arterial pressure (MAP) with vasopressin or norepinephrine on systemic hemodynamics, renal blood flow (RBF), intra-renal perfusion and oxygenation, and renal function in ovine septic acute kidney injury (AKI).

Design: Interventional Study

Setting: Research Institute

Subjects: Adult Merino ewes

Interventions: Flow probes were implanted on the pulmonary and renal arteries (and the mesenteric artery in sheep that received vasopressin). Fiber-optic probes were implanted in the renal cortex and medulla to measure tissue perfusion and oxygen tension (PO_2). Conscious sheep were administered *Escherichia coli* to induce septic AKI. Vasopressin (0.03 [0.03-0.05] IU/min; N=7) or norepinephrine (0.60 [0.30-0.70] μg/kg/min; N=7) were infused intravenously and titrated to restore baseline MAP during 24-30 hours of sepsis.

Measurements and Main Results: Ovine septic AKI was characterized by reduced MAP (-16 ± 2%) and creatinine clearance (-65 ± 9%) and increased RBF (+34 ± 7%) but reduced renal medullary perfusion (-44 ± 7%) and PO_2 (-47 ± 10%). Vasopressin infusion did not significantly affect renal medullary perfusion or PO_2 and induced a sustained (6-hours) ~2.5-fold increase in creatinine clearance. Vasopressin reduced sepsis-induced mesenteric hyperemia (+61 ± 13 to +9 ± 6%). Norepinephrine transiently (2-hours) improved creatinine clearance (by ~3.5-fold) but worsened renal medullary ischemia (to -64 ± 7%) and hypoxia (to -71 ± 6%).

Conclusions: In ovine septic AKI, restoration of MAP with vasopressin induced a more sustained improvement in renal function than norepinephrine, without exacerbating renal medullary ischemia and hypoxia or reducing mesenteric blood flow below baseline values.

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INTRODUCTION

Sepsis is associated with acute kidney injury (AKI), which increases mortality (1, 2). Norepinephrine is the recommended first-line vasopressor for sepsis-associated hypotension, with vasopressin being recommended as an adjunct to reduce catecholamine requirements and/or to treat catecholamine-resistant hypotension (3). In the Vasopressin and Septic Shock Trial (VASST), low-dose vasopressin with norepinephrine did not decrease mortality compared with norepinephrine alone (4). However, the combination was associated with a lesser requirement for renal replacement therapy (RRT) in patients with AKI at randomization (5). In the Vasopressin vs. Norepinephrine as Initial Therapy in Septic Shock (VANISH) trial, lower serum creatinine, higher urine output and lower use of RRT were found in patients treated with vasopressin (6). These observations may reflect a differential effect of vasopressin on renal medullary perfusion and oxygen tension (PO_2) compared with norepinephrine.

Medullary ischemia and hypoxia may be key pathophysiological features of AKI secondary to sepsis (7-11). In ovine gram-negative sepsis, early renal medullary ischemia and hypoxia precede the development of AKI by several hours (10, 12-14) and these changes are worsened by norepinephrine infusion (14, 15). In early ovine sepsis, low-dose vasopressin (0.02 IU/min) improved urine output and renal function to a greater extent than norepinephrine (16, 17). We have recently demonstrated that in established ovine septic AKI norepinephrine, but not angiotensin II, significantly reduces renal medullary PO_2, (13, 14). Currently, the effects of vasopressin on renal and intra-renal perfusion and PO_2 in established septic AKI are unknown.

Accordingly, in ovine gram-negative sepsis with AKI, we investigated whether resuscitation with vasopressin reduced renal medullary perfusion and PO_2 less than norepinephrine, whilst improving renal function. To address concerns that vasopressin causes splanchnic ischemia, we tested whether restoring mean arterial pressure (MAP) with vasopressin alone reduced mesenteric blood flow to values below baseline.

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MATERIALS AND METHODS

Animal preparation

The experimental protocols were approved by the Animal Ethics Committee of the Florey Institute of Neuroscience and Mental Health under the guidelines of the National Health and Medical Research Council of Australia. Two aseptic surgical procedures, separated by 3-4 weeks, were performed in twenty sheep (35-45 kg) under general anesthesia. As previously described, sheep had a carotid artery loop constructed and a transit-time flow probe (20-mm, Transonic Systems, Ithaca, NY) implanted on the pulmonary artery (13-19). The day before the second surgery, the carotid arterial loop and jugular vein were cannulated (16-18). In a second surgery, a transit-time probe (4-mm) was placed on the left renal artery, the renal vein was cannulated and fiber-optic probes (Oxford Optronix, Abingdon, United Kingdom) were inserted into the renal cortex and medulla (18, 20). In sheep allocated to vasopressin infusion, a transit-time probe (6-mm) was placed on the superior mesenteric artery (16, 17). Finally, a Foley catheter (size 12; Euromedical, Malaysia) was inserted into the bladder.

Experimental Protocol

In conscious sheep, baseline measurements commenced 4 days after the second surgery. After 20-hours of baseline measurements, gram-negative sepsis was induced by intravenous infusion of an isolate of *Escherichia. coli* (*E. coli*) obtained from a septic patient. Live *E. coli* was administered as a loading infusion of 2.8×10^9 colony-forming units (CFU) over 30-minutes followed by a continuous infusion of 1.26×10^9 CFU/h for 32-hours, as previously described (12-15, 18, 19, 21). Hartmann's solution (2 mL/kg/h, sodium lactate, Baxter, Australia) was infused from 0-32 hours of sepsis; the total fluid volume infused was 2560 mL/40kg sheep. At 24-hours of sepsis, animals we infused with arginine vasopressin (Link Healthcare, Warriewood, NSW, Australia) or norepinephrine (Hospira, Melbourne, VIC, Australia) from 24-30 hours of sepsis. The doses of arginine vasopressin and norepinephrine were titrated to

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restore MAP to baseline (pre-morbid) levels in each sheep. After withdrawal of vasopressor therapy at 30-hours of sepsis, monitoring was continued for an additional 2-hour period while *E. coli* was infused (30-32 hours of sepsis).

Analog signals of MAP, heart rate (HR), cardiac output (CO), renal blood flow (RBF), cortical and medullary tissue perfusion and PO_2, and mesenteric blood flow were continuously recorded at 100 Hz on a computer using a CED Micro 1401 interface with Spike 2 software (Cambridge Electronic Design, Cambridge, United Kingdom).

Arterial and renal venous blood samples were collected at baseline and at pre-defined time intervals during sepsis for measurement of blood gases (ABL Systems-625, Denmark). Arterial blood and urine samples were simultaneously collected for assessment of plasma and urinary creatinine and sodium concentrations (Austin Health Pathology, Heidelberg, Victoria, Australia). At 32-hours of sepsis, animals were euthanized with pentobarbital (100 mg/kg, i.v.) and the positions of the fiber-optic probes within the renal cortex and medulla were confirmed.

Statistical Analysis

Variables are reported as mean ± standard error of mean for parametric data or as median [interquartile range] for non-parametric data (D'Agostino and Pearson Omnibus test, confirmed by Shapiro-Wilk test). Specific-time point comparisons were performed using Student's paired t test (for variables expressed as mean) or a Wilcoxon matched-pairs signed rank test (for variables expressed as median). Variables during 24-30 hours of sepsis, when animals were hypotensive and treated with either vasopressin or norepinephrine, were analyzed using two-way repeated measures analysis of variance (Graphpad Software 6.0, La Jolla, CA). These analyses were performed on absolute values (for variables expressed as mean) or by ranks (for variables expressed as median). p values for within-subjects' factors were conservatively adjusted using the Greenhouse-Geisser method. Mesenteric blood flow and

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vascular conductance, in sheep treated with vasopressin, were analyzed using a Dunnett's test for multiple comparisons against the pre-treatment period (24-hours of sepsis). Two-sided p value of less than or equal to 0.05 was considered statistically significant.

RESULTS

Hyperdynamic sepsis and AKI at 24 hours of *E. coli* infusion

Three sheep from each group reached predefined ethical criteria (14, 15) between 12 and 30 hours of sepsis and were euthanized; data from these sheep were excluded from the analysis. In the remaining 7 animals/group, none of the sepsis-induced changes were different between the groups prior to administration of vasopressin or norepinephrine.

Infusion of live *E. coli* for 24-hours resulted in development of a hyperdynamic circulatory state characterized by increased CO and HR (both $p<0.001$), reduced MAP and stroke volume, increased total peripheral conductance (all $p<0.05$) and increased arterial blood lactate ($p = 0.04$) (Table 1). At 24-hours of sepsis, AKI was characterized by a >1.5-fold increase in plasma creatinine and ~50% reductions in creatinine clearance, urine output and fractional sodium excretion (Table 1). AKI developed despite increased RBF ($p = 0.003$), renal vascular conductance ($p < 0.001$) and renal oxygen delivery ($p = 0.02$) (Table 1).

Renal hyperemia was associated with preserved renal cortical perfusion ($p = 0.5$) and PO_2 ($p = 0.1$) (Table 1). In contrast, there were selective, significant reductions in renal medullary perfusion ($p = 0.001$) and PO_2 ($p < 0.001$) (Table 1).

Systemic hemodynamic effects of vasopressin and norepinephrine

To restore MAP to baseline levels, vasopressin was infused at 0.02 to 0.07 IU/min in 6 of the sheep. In one animal with hyperlactemia (7.6 mmol/L), the infusion rate of vasopressin was increased to 0.22 IU/min (see Figure, Supplemental Digital Content which shows doses of

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vasopressors in individual animals). Vasopressin significantly reduced total peripheral conductance, CO and HR (Fig. 1B, C & D), albeit to values above baseline, but stroke volume was unchanged (Fig. 1E). Norepinephrine, at a median dose of 0.60 [0.30-0.70] μg/kg/min, increased MAP (Fig. 1A), but in contrast to vasopressin, it increased CO and stroke volume and did not reduce total peripheral conductance (Fig. 1C, D & E).

Effects of vasopressin and norepinephrine on arterial blood lactate

At 24-hours of sepsis, prior to either treatment, arterial blood lactate levels were numerically greater, but not significantly different ($p = 0.1$) between the vasopressin and norepinephrine groups. This was due largely to hyperlactemia (0.6 to 7.6 mmol/L) in one sheep in the vasopressin group. During infusion of vasopressin arterial blood lactate tended to decrease, compared with a lack of change in the norepinephrine treatment group, but this apparent effect did not reach statistical significance (Fig. 1F).

Renal functional effects of vasopressin and norepinephrine

Urine output tended to be higher during treatment with vasopressin than with norepinephrine, but this difference did not reach significance (Fig. 2C). The cumulative urine output over the 6-hour treatment period with vasopressin was 730 [312-1371] mL and 418 [253-542] mL with norepinephrine ($p=0.4$ Man-Whitney U test). Vasopressin induced a sustained increase in creatinine clearance that lasted throughout the 6-hour infusion, whereas the increase with norepinephrine was transient, lasting ~2 hours ($P_{TreatmentxTime} = 0.01$; Fig. 2A). Fractional sodium excretion was increased by vasopressin, but not by norepinephrine ($P_{TreatmentxTime} = 0.008$; Fig. 2D).

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Renal and intra-renal hemodynamic effects of vasopressin and norepinephrine

Neither vasopressin nor norepinephrine significantly affected RBF, renal vascular conductance, renal oxygen delivery, renal oxygen consumption (Fig. 3A-D) or cortical tissue perfusion and PO_2 (Fig. 4A & C). During vasopressin treatment renal medullary perfusion ($P = 0.2$) and PO_2 ($p = 0.08$) were maintained or slightly increased (Fig. 4B & D). In contrast, norepinephrine worsened medullary ischemia ($p = 0.03$) and the degree of medullary hypoxia ($p = 0.007$) (Fig. 4B & D). P values were derived from a Dunnett's test for multiple comparisons against the pre-treatment period (24-hours of sepsis).

Effects of vasopressin on mesenteric hemodynamics

At 24-hours of sepsis, prior to vasopressin treatment, mesenteric blood flow ($p = 0.04$) and mesenteric vascular conductance ($p = 0.03$) (Fig. 3E & F) were increased. Vasopressin significantly reduced mesenteric blood flow and vascular conductance, but these levels were still above baseline (Fig. 3E & F).

Systemic and renal effects to withdrawal of vasopressor therapy

From 30-32 h of sepsis, following cessation of infusion of vasopressin or norepinephrine, MAP rapidly declined (Fig. 1A). This hypotension was associated with a rapid dissipation of the improved creatinine clearance in the vasopressin group, with little effect in the norepinephrine group (Fig. 2A). After cessation of vasopressin, urine output, and renal cortical and medullary PO_2 decreased to similar levels to those in the norepinephrine group (Figs. 2 & 4). After withdrawal of vasopressin therapy, there were significant rebound increases in CO, total peripheral and renal vascular conductance, mesenteric blood flow and vascular conductance (Figs. 1 & 3).

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DISCUSSION

In an ovine model of gram-negative sepsis with established AKI, we compared the systemic hemodynamic and renal effects of treatment with either vasopressin or norepinephrine alone at doses targeted to restore baseline MAP. Restoring MAP with vasopressin reduced CO but preserved medullary perfusion and PO_2, whereas norepinephrine treatment worsened sepsis-induced renal medullary ischemia and hypoxia. Vasopressin was associated with sustained increases in creatinine clearance for the 6-hour infusion period, compared with only a transient 2-hour renal functional gain with norepinephrine. Vasopressin reduced the sepsis-induced increases in mesenteric blood flow and vascular conductance, but only to levels at or above those in healthy sheep, without adverse effects on lactate levels.

Relationship to previous studies

In ovine hyperdynamic sepsis, renal medullary hypoxia occurs within one hour of *E. coli* infection, 12 to 24 hours prior to AKI, despite increased or preserved RBF, renal oxygen delivery, and cortical perfusion and PO_2 (12-14). Restoration of blood pressure with norepinephrine can result in transient renal functional gains, but it worsens the underlying renal medullary hypoxia (14, 15). Thus, vasopressor drugs that improve or, at least, do not worsen renal medullary hypoxia may be preferred from a renal point of view in the setting of septic AKI (11). Vasopressin may be such a drug.

The findings of the VASST trial suggest renal benefits from using vasopressin combined with norepinephrine compared with norepinephrine alone in septic patients (4, 5). The VANISH trial compared the effects of early vasopressin with norepinephrine in sepsis and demonstrated numerically reduced use of RRT without altered mortality rate in the vasopressin group (6). The Vasopressin versus Norepinephrine in Patients with Vasoplegic Shock after Cardiac

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Surgery Trial (VANCS) demonstrated a reduced incidence of AKI (10.3% vs. 35.8%) in patients receiving vasopressin alone compared with norepinephrine alone (22). In contrast, in the Vasopressin versus Norepinephrine for the Management of Septic Shock in Cancer Patients (VANCS II) trial, vasopressin as a first-line vasopressor was not superior to norepinephrine in reducing 28-day mortality or AKI (23). However, in the VANCS II trial, vasopressin infusion was only titrated to maintain MAP greater or equal to 65 mmHg in patients with cancer and sepsis (23). Consistent with the VASST and VANCS trials, in our study, vasopressin used in isolation and targeted to restore baseline MAP had a more favorable profile of effects on the kidney than norepinephrine, which worsened the underlying renal medullary ischemia and hypoxia in septic sheep with AKI. The enhanced natriuretic response to vasopressin therapy may have contributed to the preservation of renal medullary PO_2 by reducing renal tubular sodium reabsorption and thus oxygen utilization (24). The greater, more sustained improvement in renal function with vasopressin may be attributed to preferential vasoconstriction of the efferent arterioles and thus increased glomerular hydrostatic pressure and net filtration pressure (25). Consistent with this notion, a recent meta-analysis comparing the renal effects of vasopressin and norepinephrine in patients with distributive shock indicates that vasopressin may be associated with a reduced need for RRT and a lower incidence of AKI (26). Our findings support the notion that achieving a high target blood pressure with vasopressin in sepsis may induce more sustainable renal functional gains than with norepinephrine.

The target blood pressure achieved in sepsis with vasopressor support may also be important for renal functional outcomes. In septic patients with premorbid hypertension, increasing MAP to above 80 mmHg with vasopressor catecholamines reduced the need for RRT without affecting mortality (27). If this higher target blood pressure had been achieved with vasopressin

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instead of catecholamines, our study suggests that the renal improvement may have been greater. In the current study, the ability of vasopressin to raise MAP depended entirely on peripheral vasoconstriction since CO significantly decreased, likely reflecting an action of vasopressin to increase baroreceptor sensitivity and reduce sympathetic nerve activity (28). Despite such a reduced CO, and a higher MAP target than commonly used in human sepsis, vasopressin did not decrease RBF, renal oxygen delivery, or cortical and medullary perfusion and PO_2.

A major clinical concern with the use of high doses of vasopressin in human sepsis is the development of splanchnic ischemia (29). In our study, vasopressin reduced the sepsis-induced increases in mesenteric conductance and blood flow, but to not below pre-sepsis levels. Moreover, the effect of vasopressin to reduce mesenteric hyperemia was associated with a tendency towards a reduction in arterial blood lactate levels. We have reported similar changes with low-dose vasopressin (0.02 IU/min) in early ovine sepsis (17). Consistent with our experimental findings, adverse effects including mesenteric ischemia and hyperlactatemia were not significantly different in patients with cancer and sepsis treated with either vasopressin or norepinephrine alone in the VANCS II trial (23).

Study implications

Our findings show that in a large animal model of hyperdynamic septic AKI, restoration of baseline MAP can be achieved with vasopressin alone. Moreover, they indicate in this model of gram-negative sepsis that such doses of vasopressin decrease CO, but not RBF, and restore mesenteric blood flow to pre-sepsis levels. Finally, they imply that vasopressin therapy is superior to norepinephrine in terms of renal function and maintenance of renal medullary perfusion and PO_2.

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Study Strengths and Limitations

Our study has several strengths. It provides a comprehensive, temporal assessment of cardiovascular and renal function and intra-renal perfusion and PO_2 during primary resuscitation with vasopressin or norepinephrine in ovine sepsis. Animals were treated when signs of stage 1 AKI were evident (i.e. 1.5-fold increase in plasma creatinine), as defined by the Kidney Disease: Improving Global Outcomes Criteria (30). Studies were performed in conscious sheep to remove the confounding effects of general anesthesia (31, 32), although unlike many septic patients, the animals were not sedated, mechanically ventilated or given antibiotics. Vasopressin is usually used as a complementary treatment with a catecholamine, but here we aimed to study the effects of vasopressin and norepinephrine in isolation.

We also acknowledge some limitations. Our studies were performed in young sheep without comorbidities seen in some septic patients, but the clinical phenotype of septic AKI was comparable to that in humans. Six sheep reached ethical endpoint criteria and were euthanized prior to study completion. The remaining animals did not fulfil the Sepsis-3 consensus criteria only because plasma lactate remained below 2 mmol/L. It is important to note that in septic sheep lactate increases less than in humans, so the use of human definitions of sepsis to ovine sepsis is not straightforward. We assessed the effects of vasoactive drugs in gram-negative sepsis, which may not reflect the changes that occur with other pathogenic organisms. We targeted a restoration of MAP toward baseline levels, an approach that differs from current recommendations, but that has been applied to human sepsis without evidence of adverse effects on mortality (27).

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CONCLUSIONS

In an ovine model of hyperdynamic septic AKI, infusion of vasopressin, as the primary and only vasopressor at doses targeted to restore baseline MAP, maintained renal medullary perfusion and PO_2 and achieved sustained renal functional gains over a 6-hour interventional period. In contrast, norepinephrine worsened sepsis-induced medullary ischemia and hypoxia and only transiently improved renal function. Treatment with vasopressin alone had no apparent deleterious effects on the mesenteric macro-circulation or arterial blood lactate levels. These findings support the need to further investigate the effectiveness and safety of vasopressin as a first line vasopressor therapy in septic AKI and to determine if preservation of renal PO_2 with vasopressin treatment is associated with enhanced renal function during prolonged treatment and recovery.

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FIGURE LEGENDS

Figure 1. Systemic hemodynamics during vasopressin or norepinephrine infusion in ovine sepsis. Mean arterial pressure (A), heart rate (B), cardiac output (C), total peripheral conductance (D), stroke volume (E) and arterial blood lactate (F) during infusion of live *Escherichia Coli* from 0 to 32 hours and subsequent treatment with norepinephrine (N=7; open squares) or vasopressin (N=7; closed circles) from 24 to 30 hours in conscious sheep. Data are mean ± sem. Time 0 is the mean of the 20th hour of the baseline period, and times 24-32 are means of 1-hour periods. P values represent treatment-time interaction differences between norepinephrine and vasopressin treatment from a two-way repeated measures analysis of variance from 24 to 30 hours of gram-negative sepsis. A Dunnett's test was performed for multiple comparisons against the pre-treatment time-point (i.e. 24-hours of sepsis))

Figure 2. Renal function during vasopressin or norepinephrine infusion in ovine sepsis. Creatinine clearance (A), plasma creatinine (B), urine output (C) and fractional sodium excretion (D) during infusion of live *Escherichia Coli* from 0 to 32 hours and subsequent treatment with norepinephrine (N=7; open squares) or vasopressin (N=7; closed circles) from 24 to 30 hours in conscious sheep. Data presentation and statistical analysis are as detailed in Figure 1.

Figure 3. Global renal hemodynamics and oxygen delivery/consumption balance during vasopressin or norepinephrine infusion and mesenteric hemodynamics during vasopressin treatment in ovine sepsis. Renal blood flow (A), renal vascular conductance (B), renal oxygen delivery (C), renal oxygen consumption (D) , mesenteric blood flow (E) and mesenteric vascular conductance (F) during infusion of live *Escherichia Coli* from 0 to 32 hours and subsequent treatment with norepinephrine (N=7; open squares) or vasopressin (N=7;

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closed circles) from 24 to 30 hours in conscious sheep. Data are mean ± sem for parametric variables and as median [interquartile range] for non-parametric variables. P values represent treatment-time interaction differences between norepinephrine and vasopressin treatment from a two-way repeated measures analysis of variance performed on absolute values for data expressed as mean and by ranks for data expressed as median from 24 to 30 hours of gram-negative sepsis. A Dunnett's test was performed for multiple comparisons against the pre-treatment time-point (i.e. 24-hours of sepsis))

Figure 4. Regional kidney perfusion and oxygenation during vasopressin or norepinephrine infusion in ovine sepsis. Renal cortical tissue perfusion (A), medullary tissue perfusion (B), cortical tissue oxygen tension (PO_2) (C) and medullary tissue PO_2 (D) during infusion of live *Escherichia Coli* from 0 to 32 hours and subsequent treatment with norepinephrine (N=7; open squares) or vasopressin (N=7; closed circles) from 24 to 30 hours in conscious sheep. Data presentation and statistical analysis are as detailed in Figure 1.

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