Urinary biochemistry in experimental septic acute renal failure

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Abstract

Background. Several biochemical urine tests and derived indices are reported as useful in the diagnosis of acute renal failure (ARF) and its classification in prerenal (hypoperfusion) or intrarenal (acute tubular) necrosis. However, they have not been adequately studied in sepsis, the most frequent cause of ARF in ICU.

Methods. In 10 female Merino ewes, we implanted flow probes around the pulmonary and renal arteries to measure cardiac output and renal blood flow (RBF) continuously. Cardiovascular variables were monitored and urine samples collected during a 48 h control period and one week later during a 48 h period of hyperdynamic sepsis induced by an infusion of live Escherichia coli.

Results. Infusion of live E. coli induced systemic hyperdynamic sepsis with renal vasodilatation and increased RBF. Serum creatinine increased from 73.3 ± 15.1 to 276.9 ± 156.3 μmol/l (P < 0.05) and creatinine clearance decreased from 84.6 ± 21.4 to 27.5 ± 21.4 ml/min (P < 0.05). Urine sodium concentration (UNa) decreased significantly from 164.5 ± 50.4 to 14.6 ± 14.3 mmol/l, fractional excretion of sodium (FeNa) from 1.5 ± 0.17 to 0.12 ± 0.11%, fractional excretion of urea nitrogen (FeUn) from 62.7 ± 9.5 to 11.5 ± 15.4%, and urine osmolality from 724.8 ± 277.1 mosmol/l to 329.0 ± 52.1 mosmol/l. The u/p creatinine ratio did not change.

Conclusion. Sustained Gram-negative sepsis induced a hyperdynamic state and hyperaemic ARF. Despite increased renal perfusion, UNa, FeNa and FeUn decreased significantly. Our findings suggest that, in sepsis, these urinary biochemical changes are not reliable markers of renal hypoperfusion.

Keywords: acute renal failure; fractional excretion of sodium; fractional excretion of urea nitrogen; renal blood flow; sepsis; urinary marker

Introduction

Acute renal failure (ARF) affects approximately 6% of patients admitted to intensive care [1]. Sepsis and septic shock are the most common predisposing factors for the development of ARF in this setting [2–6]. Furthermore, among septic patients, the incidence of ARF is high [6,7] as is its mortality [2,3,8–11].

Renal hypoperfusion leading to ischaemia is considered the most likely cause of ARF in sepsis [12]. Accordingly, the diagnosis of ARF in terms of its cause or mechanism has been characterized as prerenal (hypoperfusion) or intrarenal (acute tubular necrosis–ATN). In the absence of a renal biopsy, typically not performed in critically ill patients, the separation between the preceding two conditions is based on urinary biochemistry and derived indices such as urinary sodium concentration (UNa), urine osmolality, fractional excretion of sodium (FeNa), fractional excretion of urea nitrogen (FeUn), renal failure index (RFI) and urine/plasma creatinine ratio (U/P Cr ratio) [13–15]. However, there are limited data concerning the diagnostic robustness of these tests in septic ARF.

Accordingly, we have developed an experimental, reproducible model of septic ARF in large animals, which mimics the hyperdynamic circulation characteristically seen in septic critically ill humans. We used this model to study UNa, urine osmolality, fractional excretion of sodium and urea nitrogen, renal failure index, serum albumin and protein concentration as well as proteinuria during septic ARF and to understand their relationship to systemic and renal haemodynamics.

Materials and methods

Animal preparation

The institutional Animal Ethics Committee approved this study. We procured 10 female Merino ewes (34.2–47.3 kg) for chronic instrumentation. The sheep were held and studied in metabolic cages, with free access to food and water. The animals underwent two separate operative procedures to implant transit-time flow probes (Transonics Systems,
Ithaca, N.Y.) to measure cardiac output and renal blood flow (RBF) as previously described [16].

The animals were allowed to recover at least for 2 weeks. Before the experiment, cannulae were inserted intra-arterially and intravenously as described [16]. A urinary catheter was inserted for measurement of urine volume and for sample collection.

Analogue signals of mean arterial pressure (MAP), central venous pressure (CVP), cardiac output (CO) and RBF were collected using a PC with a customized data acquisition system (Labview, National Instruments, 11500 N Mopac Expwy Austin, TX, USA). Data were recorded at 100 Hz for 10 s at every minute throughout the experimental protocol. Total peripheral conductance (TPC) (CO/MAP) and renal vascular conductance (RVC) (RBF/MAP) were calculated.

Protocol and measurements

We conducted a sequential study with 10 animals. During the experimental periods, MAP, CVP, CO, RBF and heart rate were measured continuously. Initially, all animals were examined during the baseline period. This consisted of a 48 h period with a fluid administration of only normal saline 1 ml/kg bodyweight (BW)/h. After a week, the sepsis study was performed. Sepsis was induced by the administration of a bolus of live *E. coli* (3.9 x 10^8 colony forming units in 15 ml saline) followed by a continuous infusion of 3.5 x 10^8 colony-forming units per hour for 48 h. During the sepsis period, normal saline was administered at the same rate as in the baseline period to prevent hypovolaemia.

Urinary output was measured and urine sampled every 90 min. Arterial blood samples were obtained for analysis of haematological variables, electrolytes, creatinine (SYNCHRON LX® System Beckmann Coulter Inc., Fullerton, CA, USA) and urea nitrogen (SYNCHRON LX® System Beckmann Coulter Inc.) every 12 h. The urine collected for the corresponding 12 h periods was used to measure electrolytes, creatinine and urea nitrogen (SYNCHRON LX® System Beckmann Coulter Inc.). The osmolality was measured by the freezing point depression technique (Advanced Cryomatic Osmometer Model 3C2, Advanced Instruments, Norwood, Massachusetts, USA). The creatinine clearance (CrCl) (Creatinine_{Urine}/Creatinine_{Plasma} x Urine_{Volume/time}), the fractional excretion of sodium (Sodium_{Urine}/Sodium_{Plasma} x Creatinine_{Plasma}/Creatinine_{Urine} x 100), the fractional excretion of urea nitrogen (Urea nitrogen_{Urine}/Urea nitrogen_{Plasma} x Creatinine_{Plasma}/Creatinine_{Urine} x 100) and the renal failure index (RFI) (Sodium_{Urine} x Creatinine_{Plasma}/Creatinine_{Urine}) were calculated.

Statistical analysis

Data are presented as mean ± SD. The 12 h mean values for each variable for the control and the sepsis period were compared using the Wilcoxon ranked-sign test. A P < 0.05 was considered statistically significant. A least squares linear regression analysis was used to correlate urinary markers to CrCl or RBF. Finally, CrCl and RBF were entered as independent variables into three different models of multivariate linear regression analyses with UNa, FeUn or FeNa. A stepwise back approach was chosen and variables with a probability P < 0.1 were removed from the model.

Results

Continuous *E. coli* infusion was maintained for 48 h in eight of 10 sheep. Two animals died from sequelae of septic shock within 12 h after commencing the infusion (mortality of 20%).

Systemic haemodynamics

Shortly after starting the *E. coli* infusion, heart rate and CO markedly increased and remained elevated (Figure 1). Due to peripheral vasodilatation with an increase of TPC, the MAP decreased (Figure 1; P < 0.05) (hyperdynamic sepsis) significantly in sepsis compared with controls. In all animals, a hyperdynamic septic state developed.

Renal haemodynamic parameters

In parallel to the increase of TPC, we observed an increase in RVC (Figure 1). This marked renal vasodilatation was followed by an increase of RBF in sepsis compared with controls (Figure 1).

Measures of renal function and urine biochemistry

Serum creatinine increased from 73.3 ± 15.1 μmol/l in the control period to 276.9 ± 156.3 μmol/l (P < 0.05) during sepsis and CrCl decreased from 84.6 ± 21.4 to 27.5 ± 21.4 ml/min (Figure 2a). Similarly, blood urea nitrogen (BUN) levels increased from 2.44 ± 0.42 to 15.1 ± 4.44 mmol/l (Figure 2a). The sheep became progressively oliguric with urinary output decreasing from 1.69 ± 0.59 ml/h/kg BW to 0.34 ± 0.22 ml/h/kg BW during the final 12 h of the experiment (Figure 2a).

The UNa decreased from 164.5 ± 50.4 mmol/l in the control period to 14.6 ± 14.3 mmol/l during sepsis (Figure 2b). In addition, the pattern of UNa showed an early and persistent reduction compared with control. In comparison, the FeNa was initially increased in sepsis, and then decreased from 1.5 ± 0.17% in the control period to 0.12 ± 0.11% throughout the experiment in the sepsis period (Figure 2b).

The FeUn fell from 62.7 ± 9.5% in the control period to 11.5 ± 15.4% in the sepsis period (Figure 2b) and was persistently reduced for the duration of the experiment. In contrast, the U/P Cr failed to show a significant difference (Figure 3).

The RFI showed a similar pattern and to the FeNa. Initially, the RFI was increased then reached a significant decrease within the sepsis period with a minimum of 0.18 ± 0.18% compared with 2.11 ± 0.26% in the control period (Figure 3).

The urine osmolality decreased from 724.8 ± 277.1 mosmol/l at baseline to 329.0 ± 52.1 mosmol/l (Figure 3). Urinary protein excretion was elevated in the sepsis period (0.60 ± 0.50 g/l) compared with controls (0.32 ± 0.25 g/l; Figure 3) but this change did not reach statistical significance. The serum
protein decreased from $64.9 \pm 6.2$ to $47.1 \pm 5.2$ and the serum albumin fell from $27.8 \pm 2.6$ to $22.5 \pm 3.2$ g/l.

In addition, there was a significant correlation between urine sodium concentration ($R^2 = 0.476$), FeNa ($R^2 = 0.142$) and FeUn ($R^2 = 0.522$) and creatinine clearance (Figure 4). There was also an inverse correlation between these three variables and RBF: UNa ($R^2 = 0.358$), FeNa ($R^2 = 0.371$) and FeUn ($R^2 = 0.516$) (Figure 4). Accordingly, an increased RBF predicted decreased indices. Finally, using CrCl and RBF as independent variables, in three separate multivariate regression analyses with UNa, FeUn or
FeNa as dependent variables, we found that CrCl was a better predictor of UNa than RBF (Table 1), while for FeNa, only RBF was a significant predictor.

Discussion

We conducted a prospective observational animal study to examine urine biochemistry and derived indices during experimental septic ARF in order to understand their relationship with renal function and RBF. We observed that all animals developed oliguric ARF with a nearly 4-fold increase of serum creatinine, the highest level of renal injury in the recently proposed RIFLE classification of ARF [17] and that such ARF developed in the systemic context of hyperdynamic sepsis and the regional context of renal hyperaemia (increased RBF).

We also found that the decrease in CrCl was accompanied by oliguria and sodium retention. The UNa decreased early and remained persistently reduced. In addition, FeNa was decreased during the sepsis period. However, their decreases occurred in the setting of increased RBF and were inversely correlated with RBF such that as flow increased both UNa and FeNa decreased.

Apart from our study, only three studies of sustained septic ARF have been performed in large animals. One found unchanged renal plasma flow and decreased urinary output, UNa and FeNa [18]. The other two studies reported an increased RBF despite a significantly decreased CrCl once a hyperdynamic state developed [19,20]. Again, as seen in our model, there was a decrease in UNa and FeNa [19,20]. This sodium retention has also been observed in septic humans, with a FeNa <1% reported in septic patients with ARF [21]. However, a FeNa <1% is usually taken to indicate a prerenal ARF (hypoperfusion) [14]. This view is contradicted by our findings and as highlighted earlier, by those of others. Thus, UNa concentration and FeNa cannot be
used in mammalian sepsis to draw conclusions about renal perfusion.

The FeUn is considered a marker of proximal tubular function and to indicate renal hypoperfusion when <35% [13]. However, contrary to this view, FeUn also decreased during septic hyperaemic ARF. Our findings contradict the ability of this test to classify prerenal or intrarenal causes of ARF in sepsis and suggest relatively preserved tubular function.

Although the ratio of urine and serum creatinine did not reach statistical difference, it was higher than 40 at every time point in the sepsis group. This ratio is considered a useful index to differentiate between prerenal (decreased RBF) and intrinsic (ATN) ARF (>40 prerenal, <20 intrinsic ARF) [15]. The renal failure index decreased in the sepsis period compared with controls. This decrease has also been described as a marker in 'pre renal' ARF in humans [15].

The urine osmolality, another marker used to classify ARF as prerenal (>500 mosmol/l) or intrarenal (<350 mosmol/l) [15] was significantly lower in the sepsis group. However, only the earliest measurement was <350 mosmol/l, indicating an intrarenal cause of ARF.

Urine protein tended to increase in the sepsis period without reaching significance. We did not distinguish between glomerular proteins like albumin and tubular proteins e.g. α-1-microglobulin [22]. Hence, we cannot report the origin of proteinuria.

Serum protein (g/l) and albumin both decreased within sepsis compared with controls. This is typically seen in critically ill patients, in particular in sepsis [23]. As proteinuria tended to increase but did not reach significance, one can exclude renal loss of protein as the underlying cause. We did not measure the interstitial loss of protein or albumin synthesis but assume that the hypoproteinaemia is mainly due to an increased transcapillary escape rate in our animals as seen in septic humans [23]. In addition, the central venous pressure tended to increase in sepsis (data not shown). Therefore, a dilution effect of slight hypervolaemia might have decreased protein and albumin further.

We calculated the correlation of CrCl and all measured urinary markers. We found that a lower CrCl corresponded to a lower FeUn, UNa and FeNa. In addition, FeUn and UNa were already significantly decreased within the first 12h of septic ARF. Thus, these two biochemical tests were the only two apart from CrCl and serum creatinine to indicate the development of ARF from the beginning. In order to understand the relationship between RBF and urinary
biochemistry, we studied the correlation between RBF and UNa and FeNa and FeUn. We found that the higher the RBF, the lower UNa, FeNa and FeUn. Low values of these markers are usually taken to indicate prerenal ARF [24] caused by hypoperfusion with intact parenchymal function [25]. Our findings directly contradict this view.

Finally, in a multivariate linear regression analysis, we examined the relationship between CrCl and RBF and urine biochemistry. We found that CrCl was a stronger predictor of UNa, while FeUn, RBF and CrCl had the same predictive ability. Only RBF was a significant predictor of FeNa. These observations highlight the interdependence between renal function (glomerular and tubular) and RBF. As renal blood is increased because of renal vasodilatation in this model, we hypothesize that the loss of clearance and the sodium retentive state associated with it might, in fact, be secondary to loss of glomerular filtration pressure. This would, in turn, be due to efferent arteriolar vasodilatation. Such change in filtration pressure would likely mimic the effect of decreased perfusion on urinary markers.

Our study has several strengths and limitations. It is a controlled study of a reproducible model that realistically simulates the systemic haemodynamic features and mortality seen in critically ill septic patients. In particular, it mimics the hyperdynamic

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Fig. 3. Renal markers in baseline and septic period in eight conscious sheep (only six sheep in urine osmolality). Asterisk indicates a $P < 0.05$; U/P ratio, the urine/plasma creatinine ratio and RFI, the renal failure index.
state commonly seen in humans [26–28]. In addition, a similar haemodynamic pattern was described in a case report after a patient self-administered Salmonella endotoxin [29]. Our model also allows for simultaneous assessment of blood flow and urinary indices, making it possible to study their relationship. However, it is neither randomized nor double-blinded. On the other hand, it is prospective and the changes are clear making it unlikely that the findings represent false positive results. As we used transit-time flow probes to measure RBF, we have only recorded the total blood supply to the kidney. Therefore, we cannot provide any information about the distribution of blood flow within the kidney. However, prior studies using Doppler probes or microspheres have failed to demonstrate significant intra-renal shunting [30–33].

Fig. 4. Correlation of UNa vs CrCl, FeUn vs CrCl; FeNa vs CrCl, UNa vs RBF FeNa vs RBF, and FeUn vs RBF. Data of baseline and sepsis of eight sheep is shown.
in sepsis. No antibiotic treatment was administered as would have been the case in humans. Furthermore, conditions with a constant bacteraemia (as in this experiment) are rare in humans apart from endocarditis. In most of other septic cases, bacteraemia is only episodic. All these differences between human sepsis and our model must be taken into account when interpreting the results.

We did not measure any experimental specific renal markers for tubular ischaemia e.g. NGAL [34]. Instead, we concentrated on clinically widely used markers and indices to further investigate their robustness in septic ARF. However, as we observed a hyperaemic ARF with preserved reabsorptive tubular function, we do not assume ischaemia as the underlying cause. The identification of early markers of the development of ARF remains an important research goal.

This paper closely relates to recent work by our group outlining this model of septic ARF [16]. It provides no new information on the systemic and renal haemodynamic changes induced by sepsis and, for ethical reasons, uses the same control population, but takes our analysis further by providing novel insights into the urinary findings in hyperdynamic sepsis and the relationship between such findings and RBF. It also further validates the robustness and reproducibility of the model. This model may depend in part on the infusion of fluid during the experiment. We administered 1 ml/kg/h of saline during the experiment to avoid fluid depletion and replace urinary and insensible losses due to fever (typically > 41°C). The stability of the CVP value suggests this was approximately achieved. Finally, we are unable to present histopathological evidence to confirm or refute the presence of acute tubular necrosis (ATN). We are performing further experiments to clearly define the histopathology and immunohistochemistry of renal tissue in this model.

In conclusion, we have studied a model of septic ARF. Despite increased RBF, ARF developed, serum creatinine increased nearly 4-fold, and creatinine clearance decreased accordingly. In this setting, we studied changes in UNa, FeNa and FeUn. We found that these markers decreased in our model suggesting a prerenal (hypoperfusion-induced) ARF despite a RBF three times greater than normal. In addition, RFI, serum protein, serum albumin and urine osmolality all decreased in the sepsis period compared with controls. Our findings suggest that, in severe sepsis, urine biochemistry and derived indices cannot be used to make inferences on RBF and thus reliably separate hypoperfusion-induced ARF from ATN. We now plan to conduct further experiments to study the relationship between urinary biochemistry and renal histopathology in sepsis.

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Conflict of interest statement. None declared.

References


Table 1. Three multivariate regression analyses in different with UNa, FeNa and FeUn as dependent and creatinine clearance and RBF as independent variables

<table>
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<th>Dependent variable</th>
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<th>Independent variable</th>
<th>Standard coefficient β</th>
<th>P</th>
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