ACUTE RENAL FAILURE AND TUBULAR DAMAGE DUE TO SEPSIS IN AN ANIMAL MODEL

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Summary

Generalised sepsis was induced in sheep by caecal perforation. Serial measurement of haemodynamic parameters revealed that the subsequent generalised sepsis induced increased cardiac output and decreased systemic resistance comparable to that known to occur in man. Glomerular filtration rate in these animals fell significantly 48 hours after induction of sepsis and there was evidence of tubular damage in the finding of low molecular weight proteinuria and increased clearance of lysozyme. Pathological examination of the kidney revealed normal glomeruli, no consistent changes in tubular cells on light microscopy, negative immunofluorescence, but structural changes in proximal tubular cells on EM. In this model, non-hypotensive sepsis predictably produces damage to proximal tubular cells accompanied by reduction in GFR.

Introduction

The development of acute renal failure in patients with generalised sepsis has important effects on both morbidity and mortality. Sepsis has been considered an important factor predisposing to the development of acute renal failure in up to one-third of patients in various series [1]. The mechanism whereby sepsis may lead to acute renal failure is unknown. In a recent study of critically ill patients with systemic sepsis we demonstrated the appearance of low molecular weight proteinuria in all, suggesting that some damage to proximal tubular cells was a uniform occurrence in this condition [2]. Laboratory animal studies of the effect of systemic sepsis on renal function have usually involved the infusion of live E. coli or endotoxin; models characterised by the rapid development of oliguria and renal failure [3,4]. However, these animal models do not mimic the early human systemic response to infection [5] which is usually characterised by a high systemic flow, low peripheral vascular resistance state. We have therefore studied a model of caecal ligation-induced sepsis in order to
identify the effects of generalised sepsis in sheep [6]. The purpose of the study was to examine the functional and morphological changes in the kidney during high output non-hypotensive sepsis.

Materials and methods

Full details of the animal model have been described elsewhere [7]. In brief, after preliminary insertion of catheters in the aorta and the pulmonary artery, the sheep were allowed to recover in a metabolic cage with free access to food and water. Control haemodynamic, pulmonary and renal function measurements were obtained. Under general anaesthesia, a lower midline laparotomy was performed, and a control renal biopsy was taken. The caecum was devascularised and then perforated. Over the ensuing 12 hours all sheep showed clinical evidence of systemic infection with an increase in respiratory rate and the development of lethargy and anorexia. Haemodynamic parameters including systemic blood pressure, pulmonary capillary wedge pressure and cardiac output were monitored frequently, and repeat renal biopsies were performed 24 and 48 hours after the induction of sepsis. Urine was collected for a four hour period each day and blood samples obtained as required for evaluation of renal function by standard methods. Renal biopsy material was examined by light microscopy, immunofluorescence, and electron microscopy.

Results

In all sheep the presence of a polymicrobial peritonitis and bacteraemia was confirmed at 24 hours by blood culture. Organisms most frequently grown included Serratia marcescens, Enterobacter cloacae, Pseudomonas, Bacteroides species and various strains of E. coli. Autopsy examination at the end of the protocol demonstrated the presence of peritonitis and an inflammatory mass in the lower right quadrant in all sheep.

Haemodynamic measurements

Mean blood pressure recordings in the group of ten sheep studied did not change significantly from baseline to 48 hours. Heart rate increased in all sheep from a mean of 90 beats/min to a mean of 159 beats/min, and cardiac index rose from a mean of 4.9L/min/M^2 to a mean of 6.9L/min/M^2 at 48 hours. These changes were accompanied by a fall in systemic resistance; the systemic vascular resistance index fell from 1909d.sec.cm-5/m^2 to 1434d.sec.cm-5/m^2 at 48 hours. Pulmonary capillary wedge pressure was maintained at high normal levels throughout the 48 hour period by fluid administration as required. Central venous pressure did not change during the study.

Renal function

Details of renal functional changes are given in Table 1. All sheep exhibited a significant reduction in glomerular filtration rate both at 24 hours and 48 hours.
TABLE I. Renal function changes after the induction of generalised sepsis in sheep

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>24 hours</th>
<th>48 hours</th>
</tr>
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<tbody>
<tr>
<td>Serum creatinine</td>
<td>75 ± 10</td>
<td>106 ± 20</td>
<td>180 ± 75*</td>
</tr>
<tr>
<td>(µmol/L)</td>
<td></td>
<td></td>
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<tr>
<td>Creatinine clearance</td>
<td>2.7 ± 0.8</td>
<td>1.9 ± 0.8</td>
<td>1.3 ± 0.5*</td>
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<tr>
<td>(ml/sec)</td>
<td></td>
<td></td>
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<tr>
<td>U. osmolarity</td>
<td>659 ± 458</td>
<td>830 ± 476</td>
<td>602 ± 159</td>
</tr>
<tr>
<td>(mOsm/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F.E._Na (%)</td>
<td>2.07 ± 0.72</td>
<td>0.50 ± 0.44</td>
<td>1.73 ± 2.77</td>
</tr>
<tr>
<td>U. protein (mg/ml)</td>
<td>0.187 ± 0.15</td>
<td>0.663 ± 0.39*</td>
<td>0.619 ± 0.40*</td>
</tr>
<tr>
<td>Fe lysozyme</td>
<td>1.65 ± 1.24</td>
<td>6.6 ± 6.8</td>
<td>16.6 ± 2.35*</td>
</tr>
</tbody>
</table>

*Change from baseline significant (p<0.05)

after the induction of sepsis. All animals exhibited a significant increase in proteinuria during the experiment and polyacrylamide gel electrophoresis confirmed that the proteinuria was largely due to the appearance of low molecular weight proteins (less than 30,000 Daltons). Further evidence of tubular cell damage was provided by marked increase in fractional clearance of lysozyme. The fractional excretion of sodium fell for 24 hours and then rose again at 48 hours, while the renal concentrating ability was apparently well maintained.

Control renal biopsies done prior to the induction of sepsis revealed no detectable abnormalities on light, fluorescence or electron microscopy. Light microscopic examination of the tissue obtained at 24 or 48 hours after the induction of sepsis likewise showed no consistent abnormality, except in two animals who had developed intrarenal thrombosis. Immunofluorescence studies were likewise entirely negative. The most marked changes found had occurred by 24 hours and were visible only on electron microscopy. The lesions were patchy and of varying severity; the most frequent change seen was proximal tubular cell swelling with mitochondrial swelling and apical bleb formation. The glomeruli were typically normal, although occasional mesangial densities were seen.

Other

All sheep exhibited a marked pyrexia during the study. Total proteins and serum albumin fell significantly from baseline to 48 hours, and there was a progressive fall in haemoglobin and white cell count. Plasma renin activity rose from a mean of 0.9ng/ml/hr at baseline to a mean of 6.1ng/ml/hr at 48 hours.

Discussion

The nature of the relationship between generalised sepsis and renal impairment remains obscure despite their frequent coexistence. Unlike most other animal
models of systemic sepsis the sheep peritonitis model described here reproduces the haemodynamic profile of early systemic sepsis in the human, and offers an opportunity to study renal functional and morphological changes in evolution of sepsis. In this situation the potential confusing effects of hypotension, nephrotoxic antibiotics and contrast media can be avoided.

This model of peritonitis in sheep not only mimics the haemodynamic changes of septic shock in man, but also seems to produce similar renal damage. All sheep demonstrated low molecular weight proteinuria and an increased clearance of lysozyme suggestive of tubular cell damage, as has been demonstrated in the human [2]. The precise nature of the renal function disturbance remains unclear. All sheep demonstrated a fall in glomerular filtration rate with a rise in serum creatinine. The well maintained urinary concentrating power together with the low fractional excretion of sodium and the absence of severe pathological changes in the kidneys all tend to suggest that the renal function impairment might be due to volume contraction (‘pre-renal’). On the other hand, it is difficult to attribute significant reduction in renal function to renal hypoperfusion in face of the well maintained blood pressure, the reduced peripheral resistance, the elevated cardiac output and the well maintained pulmonary capillary wedge pressure. It is possible that the proximal tubular cell damage is a uniform occurrence and is not particularly related to the reduction in glomerular filtration rate. The latter may be associated with the kidney attempting to retain sodium and produce a concentrated urine in response to stimuli as yet unidentified; there are clinical reports in man that the fractional excretion of sodium may be as low as 0.20 per cent despite clearly established ARF requiring haemodialysis in patients with sepsis, myoglobinuric renal failure or renal failure due to burns [8].

Generalised sepsis induced in sheep by caecal perforation predictably produces haemodynamic changes similar to those seen in man. The sepsis also predictably reduced the glomerular filtration rate with urinary indices suggestive of renal hypoperfusion. Simultaneously, the appearance of tubular proteinuria and the increase in the fractional clearance of lysozyme testify to the presence of some proximal tubular damage. This model will permit closer examination of the pathophysiology of renal failure in sepsis and will facilitate the evaluation of possible pharmacological interventions.

References

8. Vaz AJ. Arch Intern Med 1983; 143: 738
Open Discussion

RITZ (Heidelberg) I appreciate that this is a preliminary communication but in order to speculate about the mechanism involved it would be helpful to have information on whether or not there was DIC present. It is known that DIC will increase alpha adrenergic vasoconstriction in the kidney. This might explain the paradoxical presence of acute renal failure in the presence of a hypercirculatory state.

LINTON We think that in the two animals that showed intraglomerular thrombosis that likely there was DIC although there was no other evidence to support that view. In the animals which did not demonstrate glomerular thrombosis, that was nine of 11, there was absolutely no evidence of any haematological or pathological disturbance.

WALLS (Leicester) Have you taken any of the sheep and treated them at 48 hours with antibiotic therapy or corrective surgery to see whether it is going to mimic a human situation?

LINTON No, we haven’t done that. It is obviously the next thing to do. The big question is whether the addition of antibiotics might make it better or worse and we don’t know the answer to that. At the moment we have simply continued with these experiments with pharmacological intervention in an attempt to prevent the development and I can tell you that the calcium channel blockers virtually entirely eliminate the lesion.

Di PAOLO (Chieti, Italy) Is it possible to explain your experiment by a mechanism, like hepato-renal syndrome, with activation of kinins and slow reacting substance?

LINTON Yes, it is entirely probable that this is a situation which is analogous to the renal failure which occurs in the hepato-renal syndrome. It is known that you can have renal failure with a low FENa, it is also known that you can have the same situation in human sepsis and you can have the same situation in burns. We are currently looking at both the prostaglandin system and the kinin system in the model.

IAINI (Israel) Can you give us a haematocrit or blood osmolarity?

LINTON Yes, the serum osmolarity did not change significantly through the experiment, the haematocrit in all the sheep falls significantly.

IAINI There is no dehydration?

LINTON No dehydration so far as we can tell.

CHAIRMAN Isn’t it true that you really don’t have acute renal failure because you prevented it by overloading the sheep, would you agree with my statement?
LINTON It depends what you mean by acute renal failure. I think what we are seeing is an animal with generalised sepsis just as we see in man. If you define acute renal failure as being the development of acute renal impairment in the course of some other disease they have what we like to call acute renal failure and sepsis. You are right in that there is no question that there is no convincing evidence that the tubules as such are damaged in this situation. The tubules undoubtedly shed low molecular weight protein and lysozyme but they can still concentrate and they can still retain sodium.

CHAIRMAN In order to diagnose acute renal failure in humans you still need a low U:P creatinine ratio and U:P osmolarity above unity and you did not meet the criteria.

LINTON No, that's a definition problem. Carl Kjellstrand would contend that you don't need either of these.