The role of serum C-reactive protein in acute ischemic-reperfusion injury of kidney

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Abstract
Objective: Blocking early C-reactive protein-mediated inflammatory reaction may have therapeutic implications in improving the prognosis of acute renal failure with severe ischemic-reperfusion injury. Therefore, the role of serum C-reactive protein in acute renal ischemic-reperfusion injury was investigated.

Methods: Fourteen New Zealand albino rabbits were selected and divided into a treated and a control group at random. An acute renal ischemia-reperfusion injury model was induced by clamping the right renal artery for 45 minutes with simultaneous contralateral nephrectomy, followed by right renal reperfusion. The treated group was injected with dexamethasone (1 mg/kg) 2 minutes before renal reperfusion. Serum C-reactive protein, blood urea nitrogen, creatinine, and urine volume were recorded at designed time phases in both groups. Data were expressed as mean ± standard deviation and analyzed using the Student’s t test.

Results: In the control group, there was a steady increase of serum C-reactive protein that reached its peak at 6-hour reperfusion, and a positive correlation between C-reactive protein and blood urea nitrogen and creatinine (r = 0.62 and 0.53, respectively); there was a negative correlation between C-reactive protein and urine volume (r = –0.52). Compared with the control group, C-reactive protein values in the treated group remained mainly in the baseline levels after reperfusion, with C-reactive protein peaking at 4-hour reperfusion (p<0.01), whereas urine volume increased significantly (p<0.01).

Conclusions: This study indicates that C-reactive protein is involved in the pathogenesis of acute renal ischemic-reperfusion injury; blocking early C-reactive protein-mediated inflammatory reaction may have therapeutic implications in improving the prognosis of acute renal failure with severe ischemic-reperfusion injury.

Key words: C-reactive protein, Creatinine/urea, Dexamethasone, Kidney failure, Acute

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INTRODUCTION

Acute renal failure (ARF) is one of most common and severe complications in clinical medicine, affecting up to 5% of all hospitalized patients, with a higher prevalence of 10% to 30% in patients in critical care units. Despite advances in the management of critically ill patients and technological advances in renal replacement therapy, the high mortality of patients with ARF has not changed over the past decades and remains more than 50% (1). Moreover, as a consequence of more advanced medical therapy and more complicated surgical interventions in older and multimorbid patients, the number of patients with ARF is increasing. In the past two decades, it has been postulated that ischemia-reperfusion injury has an important pathogenic role in ARF. Intracellular calcium overload and oxidative stress were key deleterious agents (2-4). More recent research demonstrated that acute phase response, an innate biological response to a disturbance in homeostasis, usually causes severe tissue injuries in the early phase of ischemia until up to 30 hours of reperfusion. This endogenous protective mechanism may be important in renal ischemia-reperfusion injury (5-8). We designed an animal model of acute renal ischemia-reperfusion injury by clamping the right renal artery for 45 minutes with simultaneous contralateral nephrectomy, followed by right renal reperfusion. The role of serum C-reactive protein (CRP) in acute renal ischemia-reperfusion injury was explored through analysis of the relationships among CRP, blood urea nitrogen (BUN), creatinine (Cr), and urine volume. Dexamethasone was given in the treated group as an important modulator of CRP synthesis (8-11).

METHODS

Fourteen New Zealand albino rabbits (provided by the animal office of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei) were selected and randomized into a treated and a control group. Their body weights ranged between 1.6 and 2.4 kg. General anesthesia was achieved by pentobarbital injection (30 mg/kg) through the aural marginal vein. An incision was made through the abdominal midline; renal pedicles were exposed fully and dissected free. A small urine collecting tube was inserted into the upper portion of the right ureter. Baseline serum and urine samples were obtained before artery clamping. Left nephrectomy was performed, and the right renal artery was clamped for 45 minutes and then released. Urine volume at designed time phases were recorded, specifically at the end of 45 minutes of ischemia (0 hour), and after 2, 4, 6, 8, and 10 hours of reperfusion. Serum CRP, BUN, and Cr were measured respectively at the same time point. In the treated group, dexamethasone (1 mg/kg) was given intravenously just 2 minutes before reperfusion. Serum BUN and Cr levels were measured by using automated analysis of serum biochemicals (Hitachi CL7200, Japan) and CRP was measured by using automated immuno-turbidimetry (America Sigma Beckman Assay 360, America Sigma, New York). Data were expressed as mean ± standard deviation and analyzed using the Student’s t test.

RESULTS

C-reactive protein levels at different time phases increased significantly in the control group compared with the treated group (p<0.01) (Table 1). There was a

<table>
<thead>
<tr>
<th>Group</th>
<th>N 0 hour</th>
<th>2 hours</th>
<th>4 hours</th>
<th>6 hours</th>
<th>8 hours</th>
<th>10 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.50 ± 0.31</td>
<td>6.34 ± 0.85</td>
<td>10.21 ± 3.91</td>
<td>12.26 ± 2.67</td>
<td>14.27 ± 3.52</td>
<td>10.33 ± 3.76</td>
</tr>
<tr>
<td>Treated</td>
<td>5.43 ± 0.90</td>
<td>6.42 ± 1.78</td>
<td>7.96 ± 1.86†</td>
<td>8.59 ± 3.23†</td>
<td>7.15 ± 3.58†</td>
<td>5.61 ± 1.59†</td>
</tr>
</tbody>
</table>

N = serum CRP in normal white rabbits; 0 hour = CRP level at the end of ischemia; 2 hours = CRP level at 2 hours after renal reperfusion, etc.

*Data are mean ± standard deviation; n = 7.
†CRP comparison between control and treated groups, p<0.01.
positive correlation between CRP and BUN and Cr ($r = 0.62$ and $0.53$, respectively), and a negative correlation between CRP and urine volume ($r = -0.52$). In the treated group, CRP correlated with BUN and Cr positively ($r = 0.72$ and $0.52$, respectively), and negatively with urine volume ($r = -0.29$). Compared with controls, there was a statistical significance among CRP, BUN, Cr, and urine volume ($p<0.01$) (Tables 2 and 3).

### DISCUSSION

Acute phase response is an innate biological response to a disturbance in homeostasis. C-reactive protein, fibrinogen, and interleukin-6 are the major inflammation factors involved in this response (11-14). Of the three, CRP features a homopentameric structure and calcium-binding specificity for phosphocholine, and its expression is regulated mainly at the transcription level with interleukin-6 being the principal inducer of the gene during the acute phase (11,12). Recent research demonstrated that acute phase response, an endogenous protective mechanism, may be important in tissue ischemia-reperfusion injury (10-14). Although there has been some research concerning the relationship between CRP and acute myocardial infarction and acute hepatic ischemic-reperfusion injury, limited research has been done on the relationship between CRP and acute renal ischemic-reperfusion injury. In this study, we explored the role of CRP in the early phase of reperfusion. The results shown in Table 1 demonstrate that there was a steady increase of CRP in the control group and reached its peak after 6 hours of reperfusion, which is in accordance with published results (5-8). A massive production of CRP by innate immune response was induced by tissue ischemia, and CRP was detected in damaged tissues (11-14). By binding to apoptotic and necrotic cells in a calcium-dependent manner, CRP bound to a multivalent ligand can efficiently initiate the assembly of a C3 convertase through the classical pathway and decorate the surface of the ligand with opsonic complement fragments. Furthermore, CRP activates neutrophils and generates large amounts of oxidative stress. This process deprives tissue of important nutrients such as vitamins A, C, E, B6, and carotenoids together with trace elements, such as zinc and selenium, resulting in tissue malnutrition. Tables 2 and 3 reveal a marked dynamic change in serum BUN, Cr concentration, and urine volume in controls, reaching a peak at the sixth hour of reperfusion. In the treated group, serum BUN and Cr showed only a slight increase, reaching their peak at the fourth hour of reperfusion. Compared with the control group, there was a statistical significance ($p<0.01$). Furthermore, in the control group, a positive correlation between CRP and BUN and Cr was demonstrated ($r = 0.62$ and $0.53$, respectively), together with a negative correlation between CRP and urine volume ($r = -0.52$). In the treated group, CRP correlated with BUN and Cr positively ($r = 0.72$ and $0.52$, respectively), and negatively with urine volume ($r = -0.29$). This suggests that CRP might have a deleterious role in renal ischemia-reperfusion injury. Previous studies have demonstrated that dexamethasone is an important modulator of CRP synthesis and has little effect on other inflammatory factors (8,15). In this study, CRP production was suppressed with dexamethasone (Table
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1). After 60 minutes of reperfusion in the control group, three of seven rabbits remained anuria. However, in the treated group, three of seven rabbits had urine flow after 30 minutes of reperfusion, and after 60 minutes, all seven had urine flow, suggesting that an appropriate dose of dexamethasone could mitigate tissue injury. It was inferred that CRP was involved in the pathogenesis of acute renal ischemic-reperfusion injury. Blocking early CRP-mediated inflammatory reaction may have therapeutic implications in improving the prognosis of ARF with severe ischemic-reperfusion injury. There are reports showing that dexamethasone has a renal protective effect (15), but its specific mechanism remains obscure. This study demonstrates that dexamethasone could decrease CRP production significantly, which may indicate that decreased production of CRP is one of its renal protective mechanisms. However, after 10 hours of reperfusion, urine volume diminished despite an unremarkable increase of CRP. Serum BUN and Cr also worsened. The explanation for this phenomenon deserves further investigation.

REFERENCES