Simvastatin improves sepsis-induced mortality and acute kidney injury via renal vascular effects

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Acute kidney injury (AKI) occurs in about half of patients in septic shock and the mortality of AKI with sepsis is extremely high. An effective therapeutic intervention is urgently required. Statins are 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors that also have pleiotropic actions. They have been reported to increase the survival of septic or infectious patients. But the effect of simvastatin, a widely used statin, on sepsis-induced AKI is unknown. The effects of simvastatin and tumor necrosis factor (TNF)-α neutralizing antibody were studied in a clinically relevant model of sepsis-induced AKI using cecal ligation and puncture (CLP) in elderly mice. Simvastatin significantly improved CLP-induced mortality and AKI. Simvastatin attenuated CLP-induced tubular damage and reversed CLP-induced reduction of intrarenal microvascular perfusion and renal tubular hypoxia at 24 h. Simvastatin also restored towards normal CLP-induced renal vascular protein leak and serum TNF-α. Neither delayed simvastatin therapy nor TNF-α neutralizing antibody improved CLP-induced AKI. Simvastatin improved sepsis-induced AKI by direct effects on the renal vasculature, reversal of tubular hypoxia, and had a systemic anti-inflammatory effect.

Kidney International (2006) **69,** 1535–1542. doi:10.1038/sj.ki.5000300; published online 22 March 2006

KEYWORDS: inflammation; hypoxia; extravasation; TNF-α; microvascular permeability

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Received 30 September 2005; revised 20 December 2005; accepted 1 March 2006; published online 22 March 2006

Acute kidney injury (AKI) is a common life-threatening disease, whose mortality has remained at about 45% over three decades, despite advances in supportive care. Sepsis is a contributing factor in about half of patients of severe AKI.[1] Septic shock is the most common contributing factor to AKI in intensive care unit.[2] Acute kidney injury occurs in half of septic shock patients whose blood cultures are positive.[3] The mortality is higher in AKI patients with sepsis (75%) than in those without sepsis (45%).[4] Acute kidney injury independently increases the morbidity and mortality, although other organ failures also contribute.[5] Thus, the strategy of treatment for sepsis-induced AKI is urgently required. Activated protein C decreases mortality from severe sepsis,[6] and intensive insulin therapy or early goal-directed therapy, including early resuscitation, is beneficial in patients with severe sepsis or septic shock.[7,8] However, there are no drugs to prevent or treat sepsis-induced AKI.[9,10]

We have recently developed a clinically relevant sepsis-induced AKI model based on a classic cecal ligation and puncture (CLP) model of polymicrobial sepsis that can be used to screen drugs and investigate the pathogenesis of sepsis. CLP differs from endotoxin injection models because there is bacterial infection that mimics human sepsis.[11–13] Serum creatinine starts to increase at 12 h but not 6 h after CLP, although tubular damage can be detected at 6 h by magnetic resonance imaging techniques[14] and renal cyr61 expression, a tubular damage marker.[15] The renal pathophysiology after CLP is currently unknown.

3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) such as simvastatin have pleiotropic effects independent of lipid lowering.[16–18] Statin therapy has clinically beneficial effects on cardiovascular, cerebrovascular and acute and chronic kidney diseases via diverse effects.[17,19–21] The protective effects of statins on both human and animal sepsis have been recently shown. A retrospective study in humans reported that statin therapy reduced both overall and attributable mortality in patients with bacteremia.[22] A controlled study revealed that prior statin therapy was associated with a reduction of severe sepsis and intensive care unit admission.[23] In animals, simvastatin improved survival in a murine CLP model.[24,25] Despite these observations, the possible role of statins on sepsis-induced AKI remains unknown.

In the present study, we investigated whether simvastatin has an effect on sepsis-induced AKI and studied its

mechanism of action. Specifically, we investigated renal vascular permeability, microperfusion, tubular hypoxia and histologic damage. As simvastatin decreased circulating tumor necrosis factor (TNF)-α during sepsis, treatment with anti-TNF-α antibody was examined.

RESULTS

Effect of simvastatin on sepsis-induced mortality and acute kidney injury

To determine whether simvastatin had an effect on CLP-induced mortality and renal dysfunction in aged mice treated with fluid and antibiotics, we measured survival and renal function. The survival for mice treated with saline was 100% at 24 h, 42% at 48 h and 26% at 72 h after CLP. The survival for aged mice treated with simvastatin was 95% at 24 h, 84% at 48 h and 73% at 72 h (Figure 1). Simvastatin significantly improved survival after CLP. This survival advantage is consistent with the previous reports[24,25] of the effect of simvastatin on sepsis in mice. However, previous studies did not evaluate renal function. Serum creatinine and blood urea nitrogen (BUN) were significantly increased at 6 h after CLP compared to sham and further worsened at 24 h. Prior statin treatment significantly prevented the renal dysfunction at 24 h but not 6 h after CLP, as detected by BUN and high-performance liquid chromatography serum creatinine (Figure 2).

Effect of simvastatin on sepsis-induced tubular damage

As reported previously, CLP caused very subtle changes in renal histology consisting of patchy tubular vacuolization but no thrombosis, tubular necrosis or cast formation.[15] The renal histology in both of the cortex and the outer stripe of the outer medulla (OSOM) worsened significantly after CLP (Figure 3). Simvastatin significantly prevented the deterioration of tubular damage induced by CLP in both the cortex and the OSOM (Figure 3).

Effect of simvastatin on sepsis-induced vascular permeability

Changes in vascular permeability are thought to be important in the pathogenesis of sepsis-induced organ injury.[26] Lipopolysaccharide injection, which is another septic model, increases vascular permeability in various organs, including kidney, lung, liver and heart.[24,27–29] However, the renal vascular permeability after CLP is unknown. Therefore, vascular permeability was assessed by Evans blue dye (EBD) leakage. Since CLP did not significantly change vascular permeability at 2 h after CLP (data not shown), we evaluated

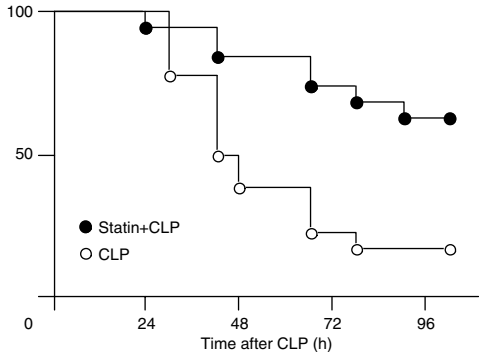

Figure 1 | Effect of simvastatin on survival after CLP. Aged mice were subjected to CLP. Simvastatin (40 mg/kg) or vehicle was administered for 3 days before CLP. Open circles indicate CLP group ($N = 19$). Closed circles indicate statin + CLP group ($N = 19$) ($P < 0.05$).

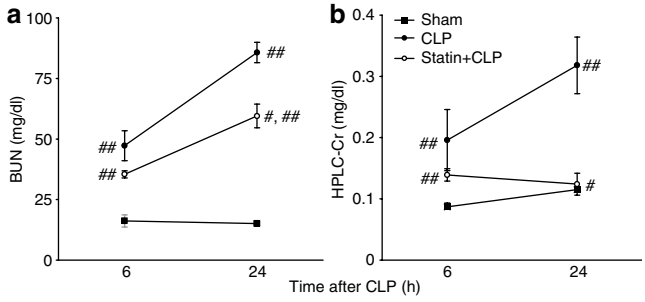

Figure 2 | Effect of simvastatin on renal function following CLP surgery. Mice were treated as in Figure 1. Mice were killed at indicated times for measurement of (**a**) serum BUN and creatinine by (**b**) HPLC serum creatinine. Closed circles indicate CLP group. Open circles indicate statin + CLP group. Closed squares indicate sham group. Values are mean ± s.e. ($N = 6$–16 per group). $^{\#}P < 0.05$ vs CLP. $^{\#\#}P < 0.05$ vs sham.

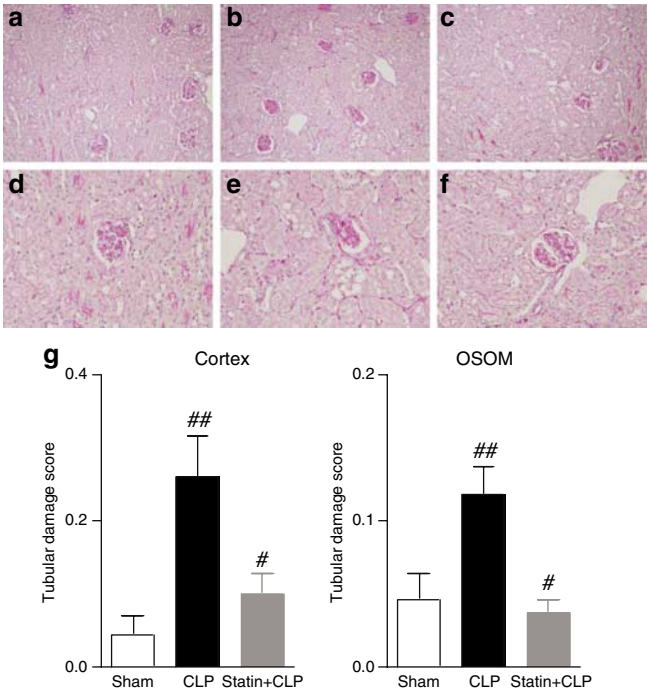

Figure 3 | Effect of simvastatin on renal histology following CLP surgery. Mice were treated as in Figure 1. Mice were killed at 24 h after surgery. Histology of cortex in (**a**, **d**) Sham group, (**b**, **e**) CLP group, (**c**, **f**) statin + CLP group; original magnification: (**a**–**c**) × 200, (**d**–**f**) × 400. (**g**) The tubular damage score (see Materials and Methods section) was measured in the cortex (left panel) and the OSOM (right panel). Values are mean ± s.e. ($N = 6$–16 per group). $^{\#}P < 0.05$ vs CLP. $^{\#\#}P < 0.05$ vs sham.