MMP9-mediated endothelial glycocalyx shedding as a potential mechanism in renal microvascular damage in sepsis-associated acute kidney injury (sAKI)

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Background

Sepsis remains the leading cause of acute kidney injury (AKI), found in 40-50% of patients with AKI in intensive care units. Sepsis-AKI (sAKI) is associated with a high mortality rate of 41%. Amongst the survivors, 20% will progress to develop chronic kidney disease and cardiovascular disease. Therefore, sAKI is a huge health problem and a considerable economic burden. Currently, there are no treatments for sAKI and therapies are supportive, so effective drugs targeting the pathobiology of sAKI are urgently required. Endothelial glycocalyx (eGLX), a carbohydrate-rich lining of the vascular endothelium, is critical in maintaining vascular homeostasis and its disruption contributes to several vascular diseases including sAKI. We have identified matrix metalloproteinase (MMP)-mediated eGLX shedding, as a major contributor to endothelial cell and kidney damage in diabetic kidney disease. We hypothesise that MMP-mediated eGLX loss underlies vascular endothelial and kidney damage in sAKI.

Aims

To investigate renal microvascular eGLX loss and its underlying mechanisms of damage in sAKI.

Methods

The endotoxemia model was induced in mice by giving 10 mg/kg lipopolysaccharides (LPS) injection. Blood urea, plasma creatinine, plasma eGLX syndecan 4, glomerular and total kidney syndecan 4 and MMP9 levels were determined by ELISA, qPCR and Western Blot. Lectin MAL 1 staining and membrane marker R18 were used on confocal images to quantify eGLX depth in both the endotoxemia model and tissue inhibitor of metalloproteinases (TIMP)3 knockout mice. Conditionally immortalised glomerular endothelial cells (ciGEnC) were pretreated with 10μM SB3CT, a potent MMP9 inhibitor, for 2 h followed by stimulation with LPS for 4h and qPCR analysis was performed.

Results

LPS-induced sAKI increased plasma creatinine and urea, confirming kidney dysfunction in the model. This was associated with a decrease in glomerular and peritubular vascular eGLX depth and a corresponding increase in syndecan 4 mRNA expression, suggesting a compensatory response to renal eGLX shedding. An increase in plasma syndecan 4 levels alludes to systemic syndecan 4 shedding. Moreover, an increase in renal MMP9 protein and mRNA expression suggests MMP9 could be mediating syndecan 4 shedding in sAKI. This was also associated with an increase in endothelial damage markers ICAM and VCAM. Study on human ciGEnC confirms LPS-induced reduction in syndecan 4 cell surface expression and a corresponding increase in syndecan 4 mRNA expression. MMP9 inhibitor attenuated syndecan 4 shedding and increased syndecan 4 and MMP9 mRNA, confirming MMP9 mediated LPS-induced GEnC GLX damage. Our study also shows that the equilibrium between MMP9 and its endogenous inhibitor TIMP3 is greatly disturbed, with TIMP3 to MMP9 ratio significantly reduced in sAKI. Crucially, TIMP3KO mice have significantly reduced glomerular endothelial glycocalyx depth, suggesting that TIMP3 restoration could be a potential therapy in sAKI.

Conclusions

Our study shows that renal microvascular eGLX is impaired and this is associated with enhanced MMP9 expression and kidney damage in sAKI. MMP9 mediates eGLX damage in LPS treated ciGEnC. EGLX protection by enhancing MMP9 endogenous inhibitor, TIMP3, represents a potential novel therapeutic target for sAKI.