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様式１０２

学位論文の内容の要旨

専　攻	分子情報制御医学	部　門	生体情報学
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論文題目	A protease-activated receptor-1 antagonist protects against podocyte injury in a mouse model of nephropathy		

（論文要旨）

Background

Chronic kidney disease is a substantial worldwide burden to patients and society. Albuminuria is a typical characteristic of kidney disease and results from disruption of the glomerular filtration barrier. Podocytes are important cells for maintaining glomerular filtration barrier function. Thus, podocyte loss causes serious nephropathy. For this reason, podocytes have been considered a principal therapeutic target for protecting the glomerular filtration barrier against plasma protein leakage into urine.

The PAR-1 (protease-activated receptor 1), a Gi protein-coupled receptor, is activated by thrombin-induced proteolytic cleavage. The N-terminal extracellular region of PAR-1 is cleaved by thrombin and the cleaved peptide then interacts with the PAR-1 receptor to induce transmembrane signaling. 8 Both human and rodent kidneys express PAR-1. A recent review suggested that PAR-1 plays a functional role in rat glomeruli. In a clinical study, urinary thrombin excretion in patients with glomerulonephritis was higher than in healthy subjects. However, the contribution of PAR-1 to progression of glomerular injury has not been elucidated. In this study, we examined the role of PAR-1 on development of glomerular podocyte injury and its potential value as a therapeutic target. PAR-1 upregulation was confirmed in two glomerular injury models, doxorubicin-induced nephritis and anti-glomerular basement membrane (GBM) antiserum-induced nephritis model. The pharmacological inhibition of PAR-1 in doxorubicin model prevented development of podocyte injury. Moreover, to address biological changes in the podocyte after PAR-1 stimulation, we assessed PAR1-dependent regulation of apoptosis and intracellular Ca2+ concentration ([Ca2+]i) in podocytes. These processes were reported to represent a common pathway driving loss of podocyte foot processes.

Protocol and Results

Mice were divided into 3 groups: control, doxorubicin + vehicle (15 mg/kg doxorubicin and saline) and doxorubicin + Q94 (doxorubicin at 15 mg/kg and the PAR-1 antagonist Q94 at 5 mg/kg/d) groups. Where indicated, doxorubicin was administered intravenously and PAR-1 antagonist or saline vehicle by subcutaneous osmotic mini-pump. PAR-1 expression was increased in glomeruli of mice treated with doxorubicin. Q94 treatment significantly suppressed the increased albuminuria in these nephropathic mice. Pathological analysis showed that Q94 treatment significantly attenuated periodic acid–Schiff and desmin staining, indicators of podocyte injury, and also decreased glomerular levels of podocin and nephrin. Furthermore, thrombin increased intracellular calcium levels in podocytes. This increase was suppressed by Q94 and Rox4560, a transient receptor potential cation channel (TRPC) 3/6 antagonist. In addition, both Q94 and Rox4560 suppressed the doxorubicin-induced increase in activities of caspase-9 and caspase-3 in podocytes. These data suggested that PAR-1 contributes to development of podocyte

and glomerular injury and that PAR-1 antagonists have therapeutic potential.

Discussion

An intact kidney filtration barrier can protect essential proteins in the blood. Podocytes are important components of this barrier. Once podocytes are damaged, plasma albumin can leak into the urinary space, resulting in a pathologic condition known as albuminuria. Treatment options to prevent or attenuate podocyte damage or loss are, therefore, greatly needed. In our study, PAR-1 expression was increased in the glomeruli in two mouse nephropathy models. Furthermore, Q94 treatment attenuated albuminuria and podocyte injury in vivo. In vitro, Q94 suppressed caspase activity and increases in intracellular calcium levels. These data supported PAR-1 inhibition as a potential therapeutic strategy against podocyte damage. These findings were consistent with a recently published study showing increased PAR-1 expression and involvement of PAR-1 in tissue pathophysiology in a mouse model for diabetic nephropathy.

Our data supporting involvement of TRPC on thrombin-induced regulation of [Ca2+]i in podocytes provided further insights into the pathophysiology of podocytopathy in glomerular disease. Previous studies reported that disrupted Ca2+ signaling, such as sustained Ca2+ influx, was involved in development of podocyte injury. We demonstrated that thrombin-induced Ca2+ influx was both PAR-1- and TRPC3/6-dependent. PAR-1 is coupled with Gq/11 and second messengers, such as diacylglycerol, were implicated in activating TRPCs. In addition, podocyte apoptosis was suppressed by Rox-4560, a TRPC3/6 antagonist, and co-treatment with Q94 and Rox-4560 showed no additional inhibition of caspase-9 activation. This suggested that PAR-1 and TRPC shared the same pathway for regulating [Ca2+]i and apoptosis in podocytes.

It is well known that thrombin is a coagulation factor. Importantly, a key feature of glomerulonephritis is formation of a fibrin matrix in Bowman's capsule. Thrombotic disease is a significant factor increasing morbidity in kidney diseases, especially in patients with nephrotic syndrome and undergoing hemodialysis. In addition, it was reported that patients with crescentic glomerulonephritis had high thrombinuria. Moreover, anticoagulants, such as soluble thrombomodulin, can decrease inflammation and prevent microalbuminuria induced by chronic endothelial activation, suggesting a potential link between chronic kidney disease and hypercoagulability. Evidence suggested that PAR-1 inhibition would have long-term therapeutic effectiveness in the glomeruli, although, in our study, we did not find any thrombosis in the kidneys of the doxorubicin treated mice. Doxorubicin was reported to induce both thrombocytopenia and procoagulant activation. Thus, in future research, we should also examine a chronic kidney disease model with both apparent thrombotic features and podocyte injury.

In conclusion, our study showed that PAR-1 levels in glomeruli was closely associated with damage to the glomerular filtration barrier and that PAR-1 upregulation during nephropathy might exacerbate nephropathy. PAR-1 inhibition, not only for anti-coagulation but also for glomerular protection, should be considered as a new therapeutic target.

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