主論文の要旨

Indoxyl Sulfate-Induced Activation of (Pro)renin Receptor Promotes Cell Proliferation and Tissue Factor Expression in Vascular Smooth Muscle Cells

（インドキシル硫酸は血管平滑筋の(プロ)レニン受容体を活性化して細胞増殖と組織因子誘導を促進する）

名古屋大学大学院医学系研究科 総合医学専攻
病態内科学講座　循環器内科学分野
（指導：室原 豊明　教授）
买买提・依斯热依力

Background

Chronic kidney diseases (CKD) are associated with an increased risk of cardiovascular diseases (CVD). Activation of (pro)renin receptor (PRR) positively correlated with CKD and CVD. Indoxyl sulfate (IS) is a nephrovascular uremic toxin which promotes the progression of CKD and CVD. The present study aimed to determine the role of IS in PRR activation in human aortic smooth muscle cells (HASMCs).

Method

Seven-week old male rats were used to produce CKD rats by 5/6- nephrectomy. Eleven weeks after subtotal nephrectomy, the rats were randomized into two groups, control CKD rats (n=8), and AST-120-treated CKD rats (n=8). AST-120 (an oral absorbent reduces serum levels of IS in CKD rats and patients) was orally administered to the rats at a dose of 4 g/kg/day with powder chow for 10 weeks, whereas powder chow alone was administered to control CKD rats. Normal rats (n=9) were used to compare the data with CKD rats. After administration of AST-120 for 16 weeks, the rats were anesthetized, and arcuate aortas were excised for immunohistochemical study. In hypertensive rat model IS (200 mg/kg/day in drinking water) was administered to the rats. Briefly, the animal groups consisted of: (1) Dahl normotensive rats (DN, n=8), (2) Dahl normotensive IS-administered rats (DN+IS, n=8), (3) Dahl hypertensive rats (DH, n=8), and (4) Dahl hypertensive IS-administered rats (DH+IS, n=8). At 48 weeks of age (32nd week of the study), the arcuate aortas were excised for immunohistochemical analysis. Serum IS levels were measured by high-performance liquid chromatography. Human aortic smooth muscle cells (HASMCs) were incubated with or without IS. Small interfering RNAs (siRNAs) transfection, real-time polymerase chain reaction (RT-PCR), western blotting and cell proliferation assay were used to investigate the underlying mechanisms.

Result

CKD rats showed significantly increased expression of PRR in the arcuate aorta compared with normal rats. On the other hand, AST-120-treated CKD rats significantly reduced expression of PRR in the arcuate aorta compared to CKD rats (Figure 1A-B). DN+IS, DH and DH+IS rats showed significantly increased expression levels of PRR in arcuate aorta compared with DN rats (Figure 1C-D). Anti-renin/prorenin antibody, which cross reacts with renin and prorenin, was used as a primary antibody. Aortic expression of renin/prorenin was significantly increased in CKD rats compared with normal rats. However, AST-120-treated CKD rats reduced the expression of renin/prorenin compared with CKD rats (Figure 2A-B). Aortic expression of renin/prorenin was significantly increased in DN+IS and DH+IS rats compared with DN and DH rat, respectively (Figure

2C-D). IS stimulated expression of PRR mRNA and protein in a time and dose dependent manner in HASMCs (Figure 3A-D). The molecular weight of PRR protein was 39 kDa.

Serum-starved HASMCs were pre-incubated with 2.5 mmol/L N-acetylcysteine (NAC), an antioxidant, or 10 μmol/L of diphenyleneiodonium (DPI), an inhibitor of NADPH oxidase, and then stimulated with 250 μmol/L IS for 24 h (Figure 4A-B). Both NAC and DPI suppressed IS-induced protein expression of PRR. Therefore, IS up regulated PRR expression in HASMCs through reactive oxygen species (ROS). IS is transported into HASMCs by organic anion transporter 3 (OAT3). OAT3 siRNA suppressed IS-induced expression of PRR in HASMCs (Figure 4C-D). Thus, IS is taken up by OAT3, and induces PRR expression in HASMCs.

IS was identified as an aryl hydrocarbon receptor (AhR) agonist in human hepatocytes, endothelial cells, and vascular smooth muscle cells. IS activates nuclear factor-κB p65 (NF-κB p65) pathway in proximal tubular cells and vascular cells. We hypothesized that IS-induced expression of PRR and prorenin is mediated by activation of AhR and NF-κB p65. Both AhR siRNA and NF-κB p65 siRNA suppressed IS-induced expression of PRR (Figure 4C-F). Thus, IS induced expression of PRR through activation of AhR and NF-κB p65 in HASMCs.

IS increased expression of prorenin mRNA and protein in a time and dose dependent manner in HASMCs (Figure 5A-D). The molecular weight of prorenin was 47 kDa. Both NAC and DPI suppressed stimulatory effects of IS on prorenin expression in HASMCs (Figure 6A-B). OAT3 siRNA, AhR siRNA and NF-κB p65 siRNA inhibited stimulatory effects of IS on prorenin expression. (Figure 6C-F). Therefore, IS induced prorenin expression in HASMCs through ROS, OAT3, AhR and NF-κB p65.

Further, we examined whether IS-induced PRR is involved in proliferation of HASMCs. PRR siRNA suppressed IS-induced proliferation of HASMCs (Figure 7A). Prorenin (20 nmol/L) increased proliferation of HASMCs, whereas PRR siRNA suppressed prorenin-induced proliferation of HASMCs (Figure 7B). Thus, IS induces proliferation of HASMCs via prorenin/PRR pathway. IS is positively associated with tissue factor (TF) expression in CKD. We found that both IS and prorenin enhanced TF protein expression in HASMCs. PRR siRNA suppressed IS-induced and prorenin-induced TF expression in HASMCs (Figure 7C-E).

Discussion

The novel findings of the present study are; 1) Aortic expression of PRR and renin/prorenin was increased in CKD rats, whereas AST-120 reduced their expression; 2) IS increased aortic expression of PRR and renin/prorenin in rats; 3) IS increased expression of PRR and prorenin through OAT3, ROS, AhR and NF-κB in vascular smooth muscle cells; 4) IS-induced PRR activation is involved in vascular smooth muscle cell

proliferation; 5) IS-induced PRR activation is involved in TF expression in vascular smooth muscle cells. Taken together, IS elevated aortic expression of prorenin/PRR in vascular smooth muscle cells through OAT3-mediated uptake, ROS production, and activation of AhR and NF-κB p65. IS-induced activation of PRR is involved in cell proliferation and TF expression in vascular smooth muscle cells.

ROS induced up regulation of PRR in diabetic rat kidneys. IS induces ROS generation by increasing NADPH oxidase NOX-4 and through OAT3-mediated uptake in HASMCs. The present study revealed that NAC, DPI, and OAT3 siRNA suppressed IS-induced expression of PRR and prorenin in HASMCs. Thus, IS induced expression of PRR and prorenin through OAT3-mediated uptake and ROS production. IS was identified as a potent endogenous ligand for AhR. IS induces activation and translocation of AhR in endothelial cells. IS-induced activation of AhR up regulates NF-κB p65 expression in vascular smooth muscle cells. Both AhR siRNA and NF-κB p65 siRNA suppressed IS-induced expression of PRR and prorenin. Thus, IS-induced activation of AhR/NF-κB p65 pathway simulates the expression of PRR and prorenin in vascular smooth muscle cells.

Vascular smooth muscle cell proliferation is a key event in the pathogenesis of vascular complications. Binding of PRR with prorenin induces vascular smooth muscle cell proliferation via activation of ERK1/2, independent of angiotensin II. IS promotes aortic wall thickening and aortic calcification in hypertensive rats. IS stimulated vascular smooth muscle cell proliferation through ROS production, and directly activated ERK1/2. In the present study, PRR siRNA suppressed both IS-induced and prorenin-induced cell proliferation in HASMCs. Thus, PRR is involved in IS-induced vascular smooth muscle cell proliferation.

Tissue factor (TF) is a mediator of injury-related thrombosis, and is elevated in the serum of advanced CKD patients. IS up regulates TF expression in vascular smooth muscle cells and endothelial cells. The present study demonstrated that PRR siRNA suppressed IS-induced up regulation of TF expression in HASMCs. Thus, PRR is involved in IS-induced TF expression in HASMCs. Taken together, IS-induced activation of prorenin-PRR pathway plays an important role in not only vascular smooth muscle cell proliferation but also tissue factor expression.