Elucidation of the Molecular and Toxico-pathological Role of Indoxyl Sulfate in Unilateral Ureter Obstruction-induced Renal Fibrosis

Department of Clinical Pharmaceutical Sciences, Graduate School of Pharmaceutical Sciences, Kumamoto University

HUIXIAN HOU

Background: Chronic kidney disease (CKD) is a progressive condition that affects over 800 million people worldwide and represents an especially large burden in low- and middle-income countries. Increased efforts for better prevention and treatment should be made because of the large number of affected people and the serious negative effects of CKD. Renal fibrosis is considered the final manifestation in patients with CKD, and its prevention is vital for controlling CKD progression. Currently, many therapeutic interventions have been done in animal models and appeared to be effective, however, it is difficult to translate these therapies into CKD patients. Thus, new insights into the molecular mechanisms of renal fibrosis and therapeutic strategies are urgently needed. Our laboratory has reported that indoxyl sulfate (IS) accumulates significantly in serum and kidney of acute kidney injury (AKI) and CKD animal models. However, the effect of IS on progressive renal fibrosis remains unknown. IS is a typical sulfate-conjugated uremic solute and is produced in the liver via CYP2A6/2E1-dependent oxidative metabolism of gut-derived indole, followed by sulfotransferase (SULT) 1A1-mediated sulfate transfer to indoxyl. Therefore, I investigated the toxico-pathological role of IS in renal fibrosis using *Sult1a1*-KO mice and the underlying mechanisms.

Methods: I established 2-week unilateral ureter obstruction (UUO) model on WT and *Sult1a1*-knock out (KO) mice. To confirm whether IS could accumulate in this model, serum and kidney IS concentrations were assessed by LC-MS/MS. Renal fibrosis was assessed by Sirius red staining and the expression of related markers. Inflammation was assessed by the expression of cytokines. Oxidative stress was assessed by the expression of oxidative stress markers and DHE, 4-HNE, 8-OHdG staining. In addition, macrophage polarization and Wnt/β-catenin signaling activation, which may be in-

process interactions in IS-induced renal fibrosis, were examined. Furthermore, to confirm the impact of erythropoietin (EPO) on renal fibrosis, the time-dependent expression of EPO was evaluated, and recombinant human erythropoietin (rhEPO) was administered to the UUO model.

Results: BUN had no increase after UUO surgery in WT and *Sult1a1*-KO mice. While UUO surgery induced significant upregulation in hepatic *Sult1a1*. IS was successfully accumulated in the serum and obstructed kidney. Inflammation and renal fibrosis were exacerbated in WT mice, with an accumulation of IS in the kidney; however, they were significantly suppressed in *Sult1a1*-KO mice. Oxidative stress increased in the WT UUO model but did not significantly differ from it in the *Sult1a1*-KO mice. In addition, although F4/80 was observed no changes, $CD80^+$ expression was downregulated and $CD206^+$ expression was upregulated in *Sult1a1*-KO mice. Sfrp5, an antagonist of Wnt protein, its expression was upregulated, β-catenin expression was downregulated in *Sult1a1*-KO mice. Moreover, EPO mRNA expression was improved considerably in *Sult1a1-KO* mice. The administration of rhEPO further attenuated UUO-induced renal fibrosis in *Sult1a1*-KO mice.

Conclusions: This study demonstrated that UUO-induced renal fibrosis was alleviated in *Sult1a1*-KO mice with a decreased accumulation of IS. Inactivated Wnt/β-catenin signal, infiltrated $CD206^+$ macrophages and improved EPO produce capacity were corelated with the attenuated renal fibrosis in *Sult1a1*-KO mice. Our findings confirmed the pathological role of IS in renal fibrosis and identified SULT1A1 as a new therapeutic target enzyme for the prevention and attenuation of renal fibrosis.