ASSOCIATION OF INDOXYL SULFATE WITH RENAL LESION IN 5/6 NEPHRECTOMIZED RATS

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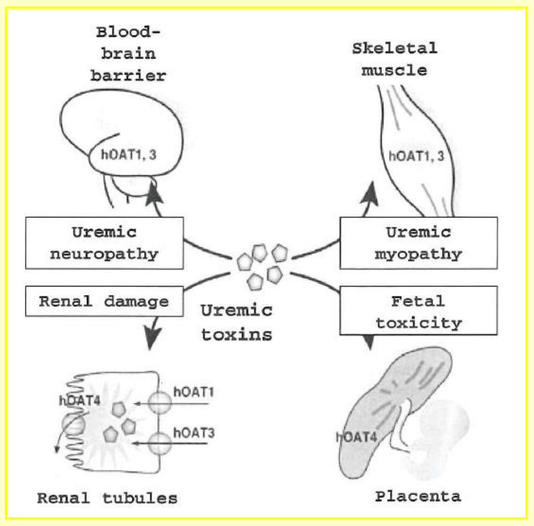
INTRODUCTION

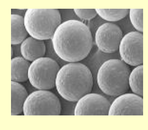
◆ Indoxyl sulfate (IS)

- is a representative protein-bound uremic toxin.
- is present in a high level in the serum of chronic kidney diseases (CKD) patients and hemodialysis patients.
- is related to CKD, several complications such as cardiovascular disease (CVD) progression and mortality.
- is detected in renal tubular cells of CKD patients and 5/6 nephrectomized rats administrated with IS.
- is taken through organic anion transporters, OAT1 and/or OAT3 in the basolateral membrane of renal tubular epithelial cells.

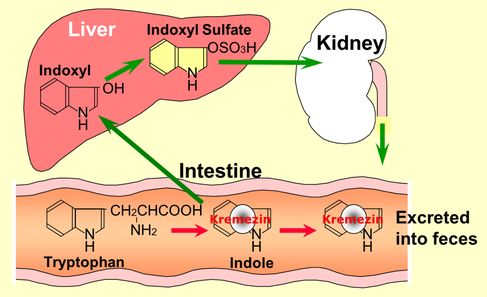


Pathological role of organic transporters (ref.[4])

■ AST-120



- Spherical adsorptive carbons
- Oral drug for chronic kidney disease, which is launched in Japan, Korea, Taiwan and the Philippines
- Indications (in Japan): improvement in uremic symptoms and prolonging the time to initiation of dialysis
- AST-120 could reduce IS level in the body with renal dysfunction .



Putative mechanism of AST-120 for reducing IS level

➢ It is still missing how IS locally accumulate and is involved in renal dysfunction.
To reveal that, we here immunostained renal tissues of 5/6 nephrectomized rats using different antibodies including a novel anti-IS antibody.

METHODS

Experimental design in vivo

Male Sprague-Dawley rats aged 13 weeks were anesthetized and five-sixths of the kidneys was removed. The rats were sacrificed 10 weeks after 5/6 nephrectomy. The blood was collected from the abdominal aorta and the remnant kidneys were removed for immunohistochemical analysis.

Detection of biochemical parameters

Blood urea nitrogen (BUN) and serum creatinine (sCr) levels were measured using automatic biochemistry analyzer.

Measurement of IS

Serum IS concentration was determined by previously reported high-performance liquid chromatography (HPLC).

Generation of monoclonal antibody against IS

IS derivatives conjugated with carrier proteins were injected into mice followed by affinity screening.
*This antibody will be launched from Trans Genic Inc.

Immunohistochemistry

Formalin-fixed, paraffin-embedded renal tissue sections were stained with antibodies against IS, Kidney Injury Molecule-1 (KIM-1), transporters in the balateral or apical membranes following antigen retrieval.

RESULTS

Table 1. Biochemical parameters and serum IS concentration(sIS) in the normal and CKD rats.

	Body weight (g)	sCr (mg/dL)	BUN (mg/dL)	sIS (mg/dL)
Normal (n=10)	613.5±18.7	0.44±0.12	20.8±1.4	0.087±0.031
CKD (n=13)	535.7±57.5	2.21±1.77	101.7±64.5	1.109±0.973

mean±SD

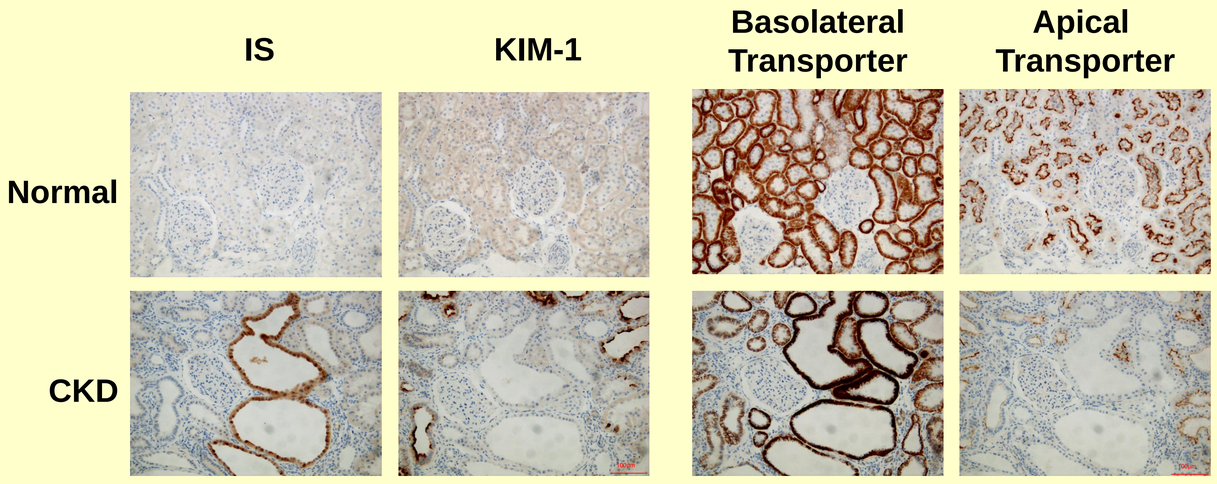


Figure 1. Accumulation of IS and expression of KIM-1 in renal tubular cells.

Figure 2. Expression of transporters in basolateral and apical membranes.

➢ Serum IS concentration was higher in CKD rats than in normal rats.

➢ In CKD rats, IS accumulated in the dilated renal tubular cells, in which KIM-1, a marker for early kidney injury, was not expressed.

➢ In IS positive cells, the expression of basolateral transporter was retained, but that of apical transporter was diminished.

CONCLUSIONS

IS might accumulate in the renal tubules at the later stage of renal injury and this accumulation could be caused by the decreased IS excretion from tubular cells to tubular lumen, though further studies are required.
The present study reveals the recognition of IS in renal tissues using the novel IS-antibody and we provide a new insight into understanding how IS locally associates with histological lesions.

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