CORRELATION BETWEEN ACCUMULATION OF INDOXYL SULFATE AND RENAL DYSFUNCYION IN RATS WITH 5/6 NEPHRECTOMY

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OBJECTIVES

Indoxyl sulfate (IS), a representative uremic toxin, is known to be increased in the serum of chronic kidney diseases (CKD) patients and promote the progression of the disease. IS is also detected in renal tubular cells of CKD patients and 5/6 nephrectomized rats administrated with IS. It has been suggested that the retention of IS is induced through organic anion transporters, OAT1 and/or OAT3 in the basolateral membrane of renal tubular epithelial cells and this retention leads to nephrotoxicity. However, the mechanism for IS accumulation in renal tubules remains unclear.

To reveal the correlation between accumulation of IS and renal dysfunction, we further investigated the expression of transporters in the apical membrane and of markers for renal injury, and a content of IS in renal tubular epithelial cells using a novel antibody against IS in 5/6 nephrectomized rats.

METHODS

In vivo experimental design
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 prameters
Blood urea nitrogen (BUN) and serum creatinine (sCr) levels were measured using automatic biochemistry analyzer.

Determination of IS concentration
IS conecentration was determined by previously reported high-performance liquid chromatography (HPLC) and by Enzyme-linked immunosorbent assay (ELISA) with our novel antibody for IS.

Immunohistochemical analysis
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 levels of IS in serum (sIS) in the normal and CKD rats (23 weeks old, and 10 weeks after nephrectomy). sIS is determined by HPLC.

	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

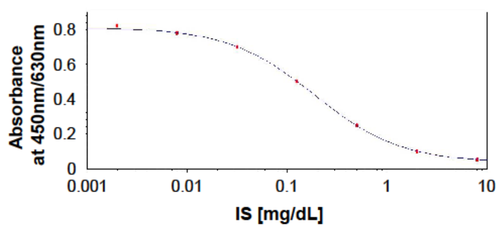

Figure 1. Establishing a standard curve for ELISA.

Concentration (mg/dL) Nominal conc.	Calc. conc.	Accuracy (%)
0	0.0041	-
0.0020	(+)	-
0.0078	0.0083	106.24
0.0313	0.0319	102.08
0.1250	0.1255	100.40
0.5000	0.4939	98.78
2.0000	2.0699	103.50
8.0000	7.9981	99.98

Figure 2. Validation of accuracy of ELISA by comparing the nominal and calculated concentration.

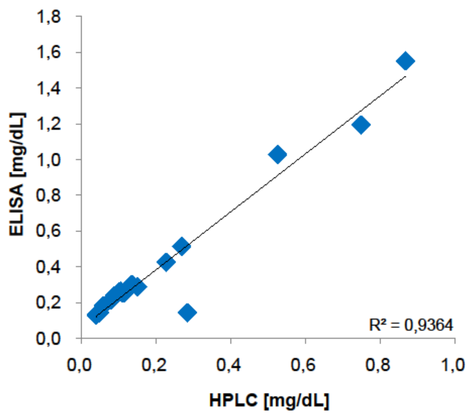

Figure 3. Comparison of ELISA and HPLC on IS concentration determination.

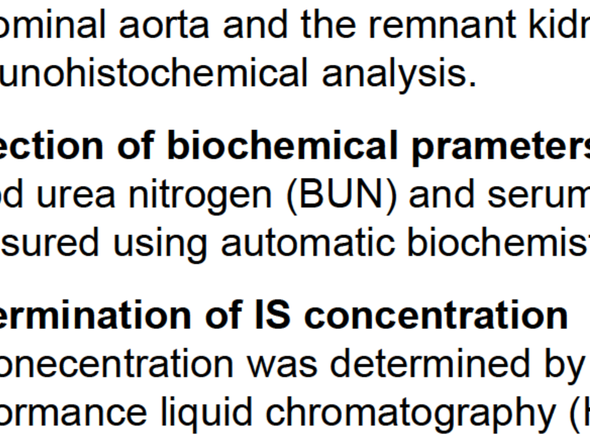

Figure 4. Immunostaining of IS in a normal rat (**A**) and a CKD rat (**B**), and of KIM-1 in a normal rat (**C**) and a CKD rat (**D**) (x20).
KIM-1 is the marker for early kidney injury.

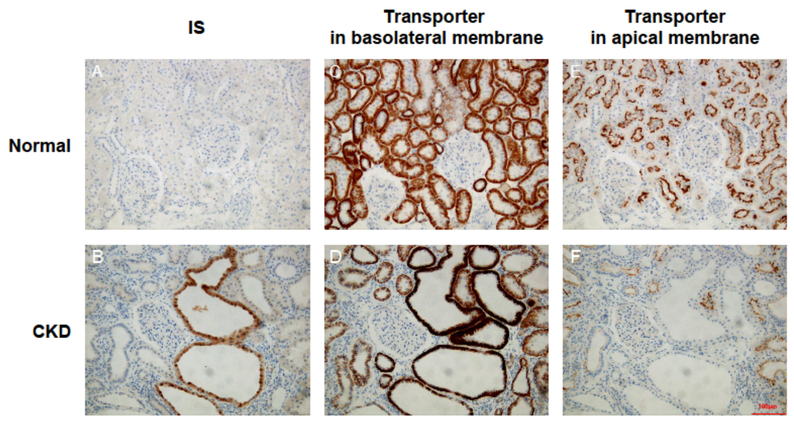

Figure 5. Immunostaining of IS in a normal rat (**A**) and a CKD rat (**B**), of basolateral transporter in a normal rat (**C**) and a CKD rat (**D**), and of apical transporter in a normal rat (**E**) and a CKD rat (**F**), (x200).

- Besides sCr and BUN, the sIS level was increased in CKD rat models as well.
- The sIS value validated by ELISA showed a good correlation with HPLC.
- In the CKD rat model, IS was accumulated in the renal hypertrophic tubular cells, in which KIM-1 was not expressed.
- IS positive cells expressed only basolateral transporter, but not apical transporter.

CONCLUSIONS

Our finding suggests that IS is mainly accumulated in the renal hypertrophic tubular cells at the late stage of CKD rats. This accumulation could be caused by the decreased IS excretion from tubular cells to tubular lumen.

REFERENCES:

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