



CRO service specialized in fibrosis/inflammation research

- Pharmacology study using UUO model mice -

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Renal fibrosis

Renal fibrosis complicates various forms of chronic kidney diseases (CKD) as a manifestation of renal injury. Of particular importance is interstitial fibrosis (fibrosis in the tubulointerstitium) because of its association with tubular functional impairment and poor prognosis. Interstitial fibrosis is thought to be a common pathological pathway to end-stage renal disease.

UUO model

Unilateral ureteral obstruction (UUO) is a well-characterized disease model for renal fibrosis. The UUO model encompasses key pathophysiological features of CKD; interstitial fibrosis, tubular atrophy and inflammatory cell infiltration within a relatively short period, which makes this model attractive for in vivo high-throughput screening.

Day 0 → Surgery and treatment:

- 1) Anesthetize and shave off the hair
- 2) Cut open the abdominal region
- 3) Ligate the left ureter with 4-0 thread
- 4) Suture the skin and transfer mice to a clean cage
- 5) Treat mice with candidate drugs from Day 0

Day 14 → Sacrifice and sample collection:

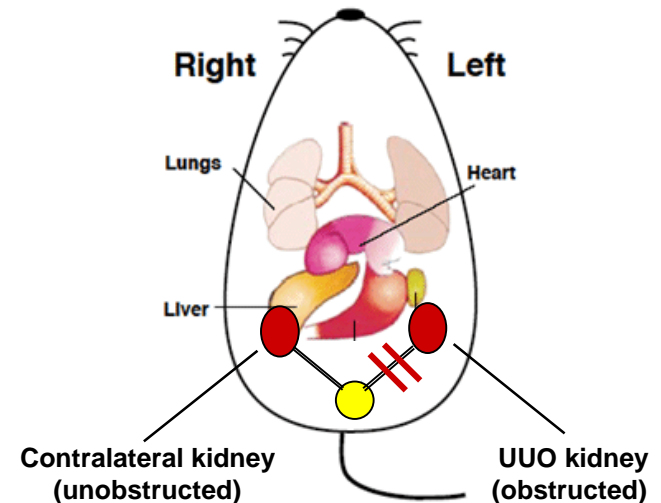
Blood → Urea nitrogen (*a marker of renal function*)

Kidney → Hydroxyproline assay (*estimation of renal collagen content*)

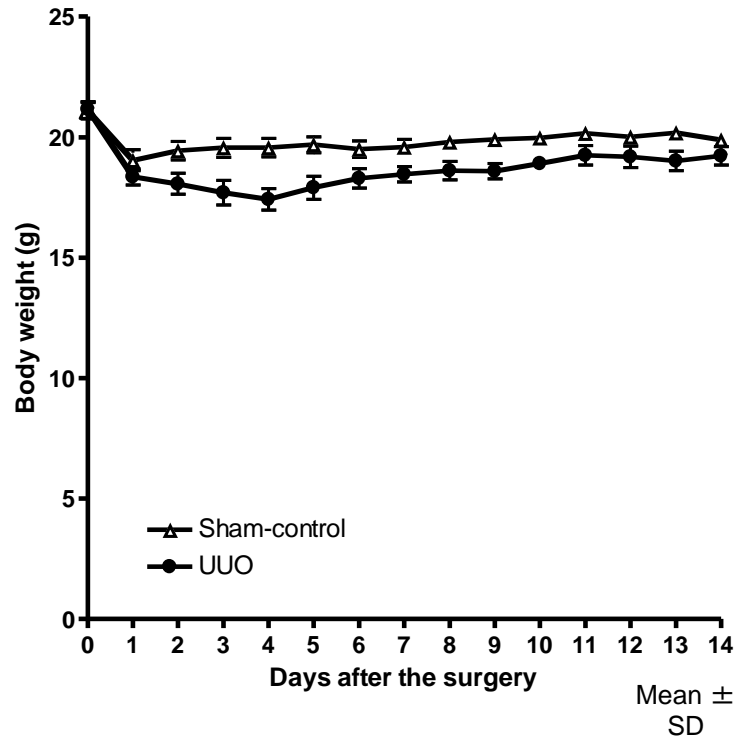
→ Histological analyses (*evaluation of renal injury, collagen deposition*)

→ Gene expression analysis (*for marker genes of fibrosis, inflammation, etc.*)

Mouse UUU



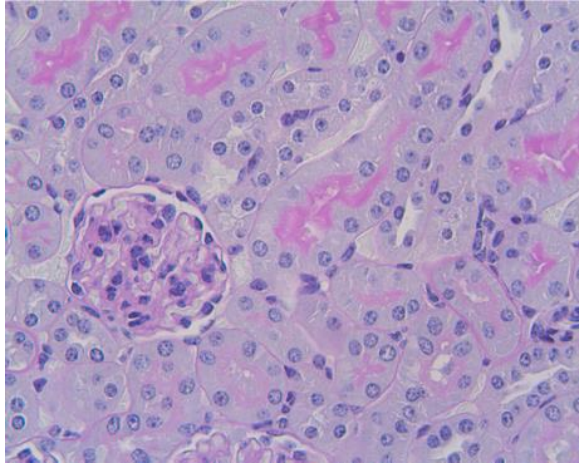
Body weight changes



- Body weight decreases by 2.5-15% after surgery (both in Sham-control and UUO mice)
- No statistical difference after Day 6 between sham-control and UUO mice

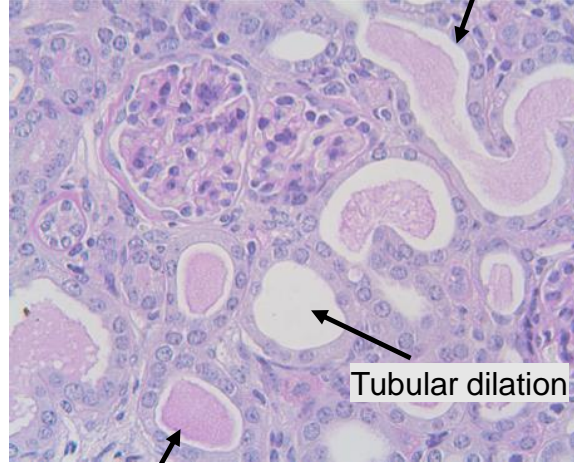
PAS staining

Sham-control



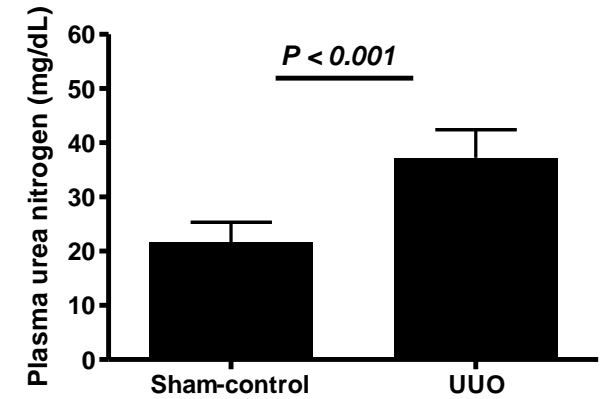
X400, cortical region, Day 14

UUO



Tubular cast

Blood urea nitrogen



Unpaired t-test
Mean \pm SD
Day 14

The UUO model exhibits;

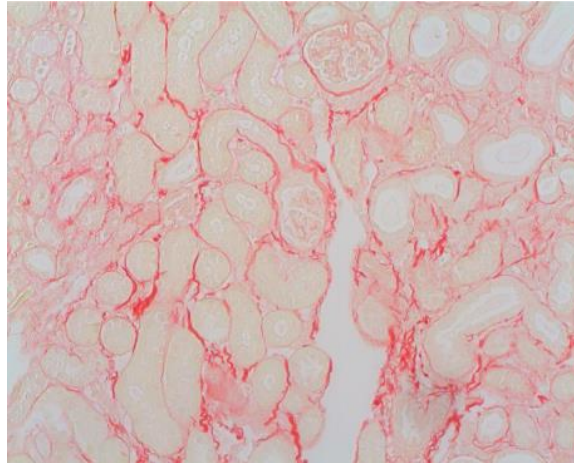
- renal injury – tubular dilation, atrophy, cast formation and necrosis
- increases in blood urea nitrogen levels

Sirius red staining

Sham-control



UUO



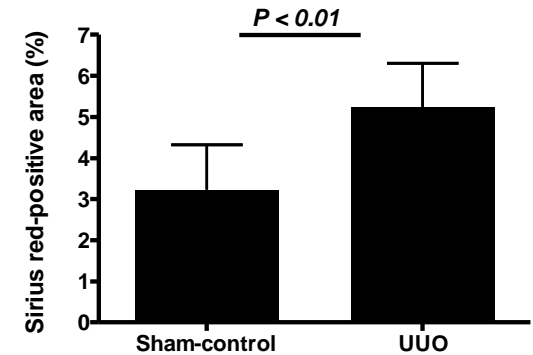
X200, corticomedullar region, Day 14

Positive area: red

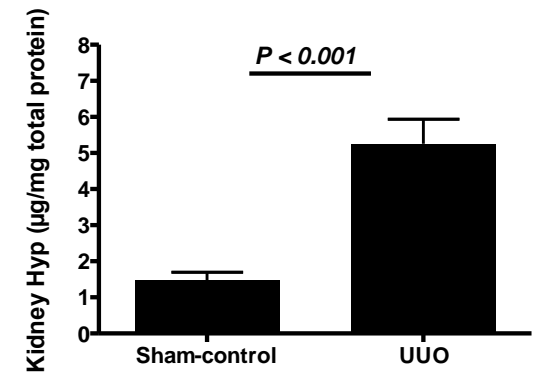
The UUO model exhibits;

- severe interstitial fibrosis with a statistically significant increase in the Sirius red-positive area
- a 4-5 times increase in kidney hydroxyproline content

Sirius red positive area

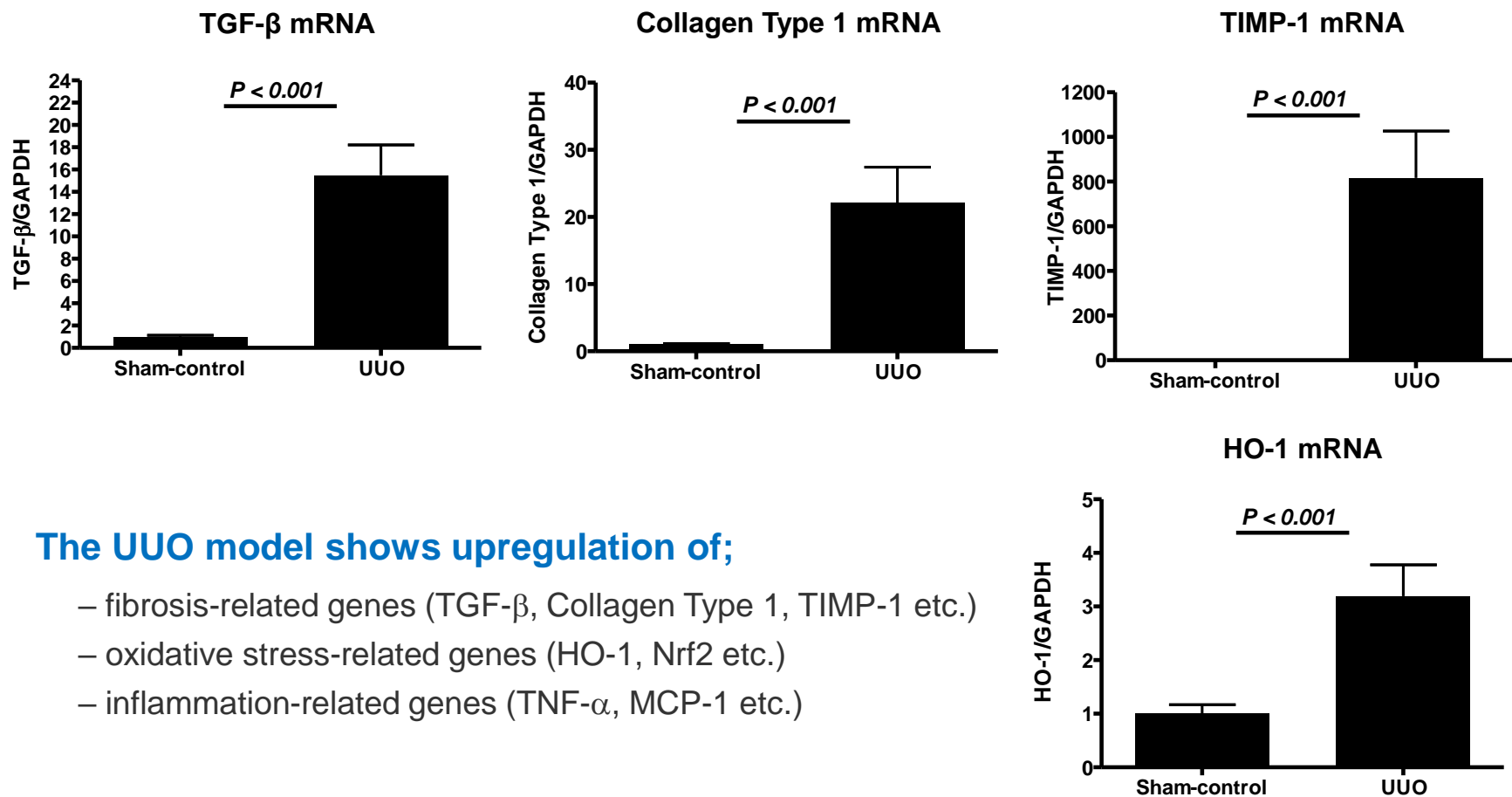


Kidney hydroxyproline



Unpaired t-test
Mean \pm SD
Day 14

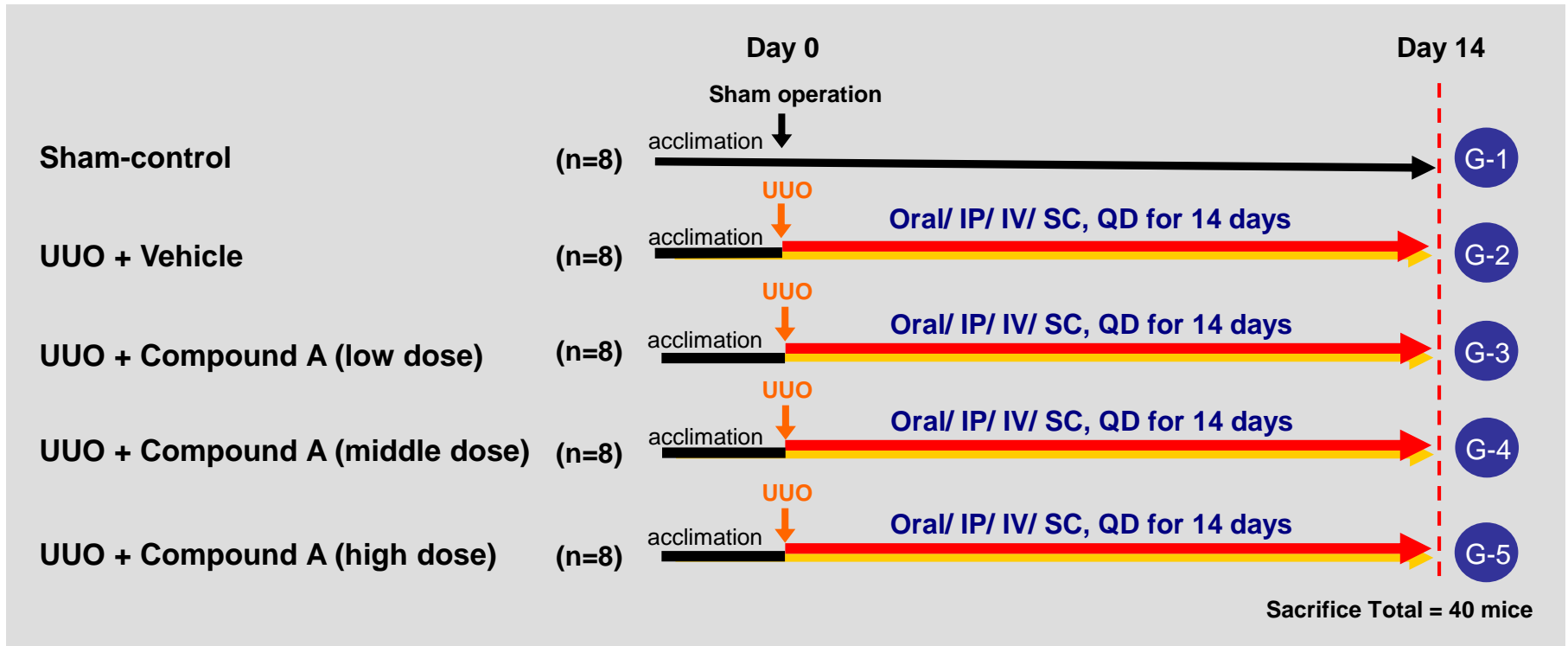
Quantitative RT-PCR



The UUO model shows upregulation of;

- fibrosis-related genes (TGF-β, Collagen Type 1, TIMP-1 etc.)
- oxidative stress-related genes (HO-1, Nrf2 etc.)
- inflammation-related genes (TNF-α, MCP-1 etc.)

Unpaired t-test
Mean ± SD (n=8)
Day 14



Analyses

1. General condition

- Body and organ weight

2. Biochemistry

- Plasma urea nitrogen
- Kidney hydroxyproline content

3. Histopathological analyses

- PAS staining
- Sirius red staining (fibrosis area)

4. Gene expression analysis

- α -SMA
- TGF- β
- TIMP-1
- Collagen Type 1