Temporal characterization of kidney injury, inflammation and fibrosis after unilateral ureteral obstruction in C57BL/6J mice

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Background & Aim

Unilateral ureteral obstruction (UUO) is a widely used preclinical model of surgery-induced chronic kidney disease characterized by robust inflammation and tubulointerstitial fibrosis. However, the temporal relationship between tubular injury, inflammatory responses, fibrotic remodeling, and associated transcriptional changes remains elusive. This study aimed to characterize histological and molecular markers of disease progression in the UUO mouse model.

Methods

Male C57BL/6J mice (8–9 weeks old) were randomized by body weight and subjected to UUO or sham surgery. UUO animals were terminated on day 3, 5, or 8 post-surgery. Sham-operated animals were terminated on day 8. Vehicle was administered orally once daily from study day -1 until termination. Affected kidney weight and hydroxyproline (HP) content (collagen deposition) were measured. Kidney tissue was analyzed by immunohistochemistry for macrophage infiltration (F4/80), tubular injury (KIM-1), myofibroblast activation (α-SMA), and fibrosis (Col1a1). Bulk RNA sequencing was performed on kidney samples for differential gene expression and pathway enrichment analysis.

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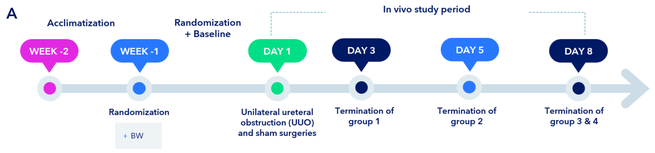
1 Model study design, protocol and body weight profile



Group	Group name	Gender	Number of animals	Treatment	Dosing frequency
1	UUO – day 3	Male	8	Vehicle	BID
2	UUO – day 5	Male	8	Vehicle	BID
3	UUO – day 8	Male	10	Vehicle	BID
4	Sham – day 8	Male	8	Vehicle	BID

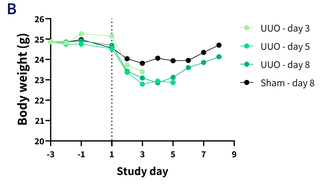


Figure 1. **(A)** Study outline and **(B)** body weight profile.

2 Kidney hydroxyproline content

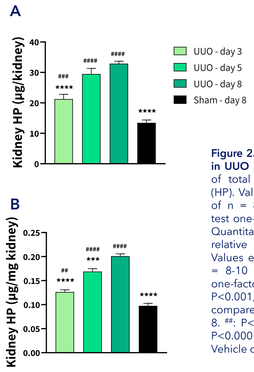


Figure 2. Kidney hydroxyproline in UUO mice. **(A)** Quantification of total kidney hydroxyproline (HP). Values expressed as mean of n = 8-10 + SEM. Dunnett's test one-factor linear model. **(B)** Quantitative assessment of relative kidney HP content. Values expressed as mean of n = 8-10 + SEM. Dunnett's test one-factor linear model. ***: P<0.001, ****: P<0.0001 compared to UUO - Vehicle day 8. ##: P<0.01; ###: P<0.001, ####: P<0.0001 compared to Sham - Vehicle day 8.

3 Progressive kidney inflammation and tubular injury

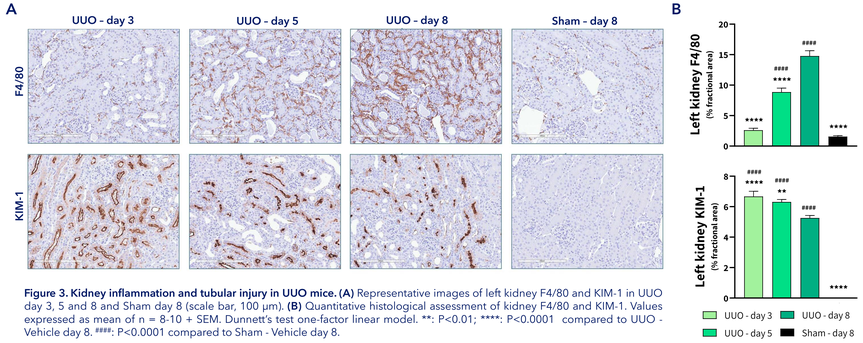


Figure 3. Kidney inflammation and tubular injury in UUO mice. **(A)** Representative images of left kidney F4/80 and KIM-1 in UUO day 3, 5 and 8 and Sham day 8 (scale bar, 100 µm). **(B)** Quantitative histological assessment of kidney F4/80 and KIM-1. Values expressed as mean of n = 8-10 + SEM. Dunnett's test one-factor linear model. **: P<0.01; ****: P<0.0001 compared to UUO - Vehicle day 8. ####: P<0.0001 compared to Sham - Vehicle day 8.

4 Progressive kidney myofibroblast activation and fibrosis

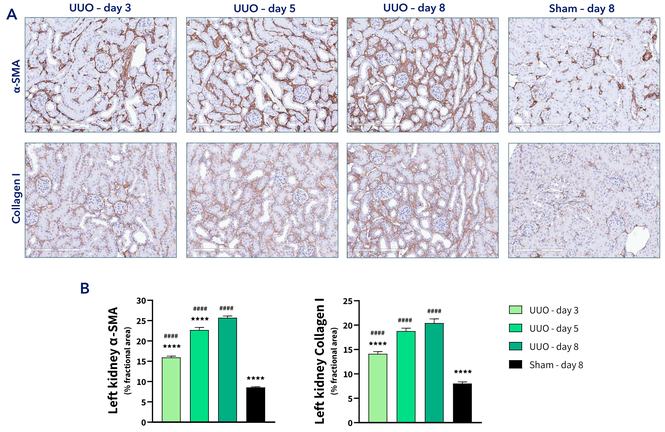


Figure 4. Kidney myofibroblast activation and fibrosis in UUO mice. **(A)** Representative images of left kidney α-SMA and Col1a1 in UUO on day 3, 5 and 8 and Sham day 8 (scale bar, 100 µm). **(B)** Quantitative histological assessment of kidney α-SMA and Col1a1 . Values expressed as mean of n = 8-10 + SEM. Dunnett's test one-factor linear model. ****: P<0.0001 compared to UUO - Vehicle day 8. ####: P<0.0001 compared to Sham - Vehicle day 8.

5 Time-dependent transcriptomic remodeling following UUO

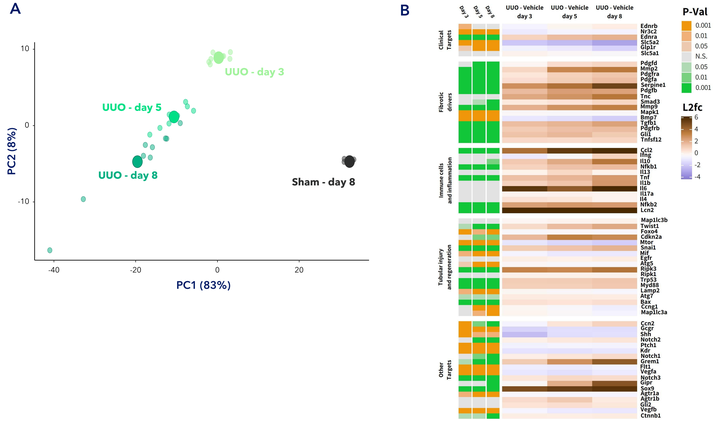


Figure 5. Differential gene expression analysis and pathway enrichment analysis. **(A)** Principal component analysis based on the top 500 most varying genes across the dataset. The axes describe the percentage of variability explained by the principal component 1 (PC1) and principal component 2 (PC2). Each small point represents a sample, and each big point represents the group average. **(B)** Heatmap of gene expression of CKD drug targets. Log2-fold change in gene expression of CKD drug targets from controls to UUO animals over time. Rows are unscaled. Drug targets are separated into several groups, based on function.

Conclusion

- UUO surgery caused a progressive increase in kidney hydroxyproline content
- Histological markers of inflammation (F4/80), myofibroblast activation (α-SMA), and fibrosis (Col1a1) increased progressively from day 3 to day 8 following UUO surgery
- Tubular injury (KIM-1) was elevated in vehicle-treated UUO animals at days 3 and 5, with lower levels observed by day 8 post-UUO
- RNA-seq revealed time-dependent transcriptional changes following UUO, characterized by progressive regulation of genes involved in extracellular matrix remodeling, fibrosis, and inflammation

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