Nephroprotective effects of an ALK5 inhibitor in the unilateral ureteral obstruction (UUO) mouse model of kidney fibrosis

Authors
Maria Ougaard, Jacob Nøhr-Meldgaard, Ditte Marie Jensen, Frederikke Emilie Sembach, Ida Rune & Michael Christensen

Gubra, Hørsholm Kongevej 11B, Hørsholm, Denmark

Corresponding author
Michael Christensen MCH@gubra.dk

BACKGROUND & AIM

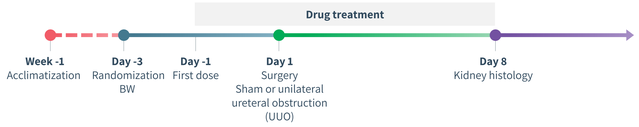
Chronic kidney disease (CKD) often involves development of renal fibrosis, underlying the progressive loss of kidney function and progression to end-stage kidney disease. Preclinical animal models are essential in drug discovery for CKD. The unilateral ureteral obstruction (UUO) mouse is a widely used surgery-induced model of CKD with rapid induction of renal inflammation and fibrosis. Here, we characterized the effect of an anti-fibrotic TGF-β type 1 receptor kinase inhibitor (ALK5 inhibitor, ALK5i) on kidney histopathology in the UUO mouse.

METHODS

Male C57BL/6J mice (8-9 weeks old) were randomised into study groups based on body weight, and were either sham-operated or underwent UUO surgery. Sham and UUO mice received vehicle or ALK5i (30 mg/kg, PO) twice daily for 9 days starting at day -1. At termination both kidneys were weighed, and the obstructed left kidney was processed for quantitative histological assessment of inflammation (F4/80), tubular injury (KIM-1), myofibroblast activation (α-SMA) and fibrosis (Col1a1) .

www.gubra.dk

1 Study Outline



Group	Animal	Gender	Number of animals	Treatment	Administration route	Dosing Frequency	Dosing volume	Dosing concentration
1	Sham Vehicle	Male	8	**Vehicle**	PO	BID	5 ml/kg	-
2	UUO Vehicle	Male	8	**Vehicle**	PO	BID	5 ml/kg	-
3	UUO ALK5i	Male	8	**ALK5i**	PO	BID	5 ml/kg	**30 mg/kg**

2 ALK5i is body weight-neutral, but increases kidney weight

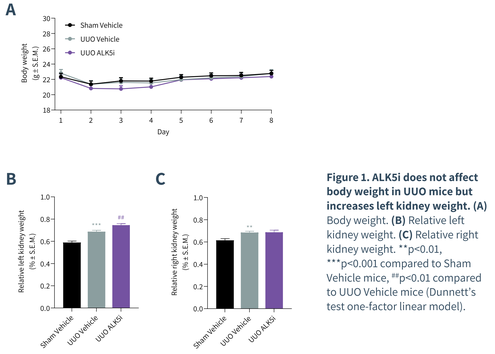


Figure 1. ALK5i does not affect body weight in UUO mice but increases left kidney weight. (A) Body weight. **(B)** Relative left kidney weight. **(C)** Relative right kidney weight. **p<0.01, ***p<0.001 compared to Sham Vehicle mice, ##p<0.01 compared to UUO Vehicle mice (Dunnett's test one-factor linear model).

3 ALK5i reduces kidney inflammation

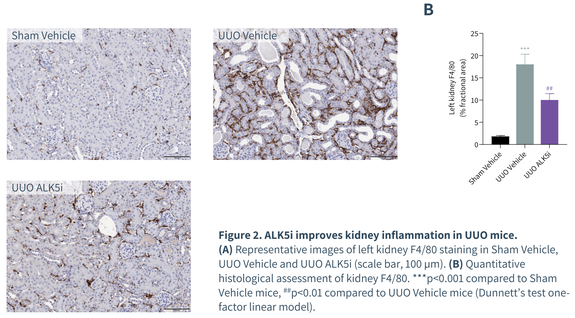


Figure 2. ALK5i improves kidney inflammation in UUO mice. **(A)** Representative images of left kidney F4/80 staining in Sham Vehicle, UUO Vehicle and UUO ALK5i (scale bar, 100 µm). **(B)** Quantitative histological assessment of kidney F4/80. ***p<0.001 compared to Sham Vehicle mice, ##p<0.01 compared to UUO Vehicle mice (Dunnett's test one-factor linear model).

4 ALK5i reduces tubular injury

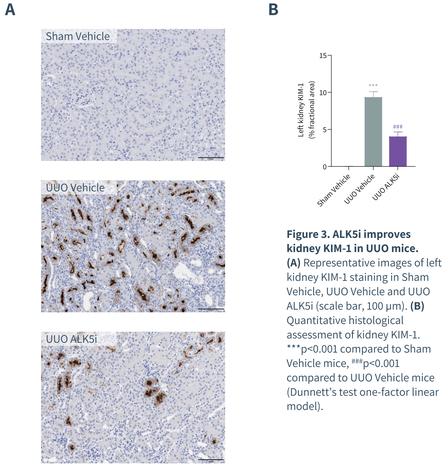


Figure 3. ALK5i improves kidney KIM-1 in UUO mice. **(A)** Representative images of left kidney KIM-1 staining in Sham Vehicle, UUO Vehicle and UUO ALK5i (scale bar, 100 µm). **(B)** Quantitative histological assessment of kidney KIM-1. ***p<0.001 compared to Sham Vehicle mice, ###p<0.001 compared to UUO Vehicle mice (Dunnett's test one-factor linear model).

5 ALK5i reduces myofibroblast activation

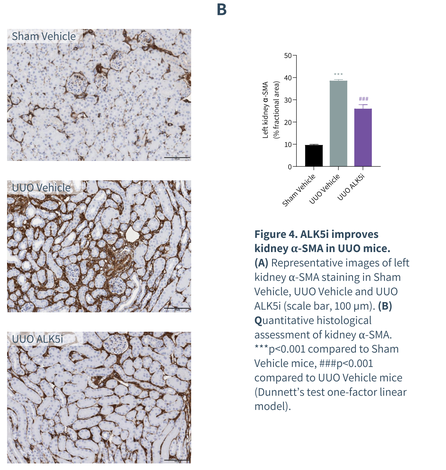


Figure 4. ALK5i improves kidney α-SMA in UUO mice. **(A)** Representative images of left kidney α-SMA staining in Sham Vehicle, UUO Vehicle and UUO ALK5i (scale bar, 100 µm). **(B)** Quantitative histological assessment of kidney α-SMA. ***p<0.001 compared to Sham Vehicle mice, ###p<0.001 compared to UUO Vehicle mice (Dunnett's test one-factor linear model).

6 ALK5i reduces kidney fibrosis

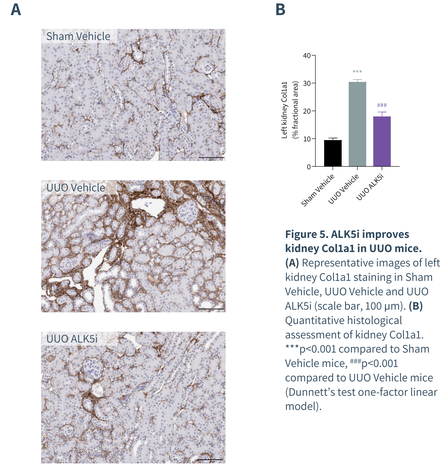


Figure 5. ALK5i improves kidney Col1a1 in UUO mice. **(A)** Representative images of left kidney Col1a1 staining in Sham Vehicle, UUO Vehicle and UUO ALK5i (scale bar, 100 µm). **(B)** Quantitative histological assessment of kidney Col1a1. ***p<0.001 compared to Sham Vehicle mice, ###p<0.001 compared to UUO Vehicle mice (Dunnett's test one-factor linear model).

CONCLUSION

- + UUO surgery does not affect body weight, but increases both right and left kidney weights compared to sham-operated mice.
- + UUO surgery increases levels of F4/80, KIM-1, α-SMA, and Col1a1.
- + Treatment with ALK5i in UUO mice improves these histological markers of inflammation, tubular injury and fibrosis.
- + Rapid induction of kidney fibrosis and inflammation makes the UUO mouse model optimal for screening of test compounds with potential renoprotective effects in CKD.

Scan the QR code to learn more about our services within kidney diseases.