Quantitative Image Analysis Uncovers the Efficacy of the Selective Endothelin A Receptor Antagonist Atrasentan in a Rat Model of IgA Nephropathy

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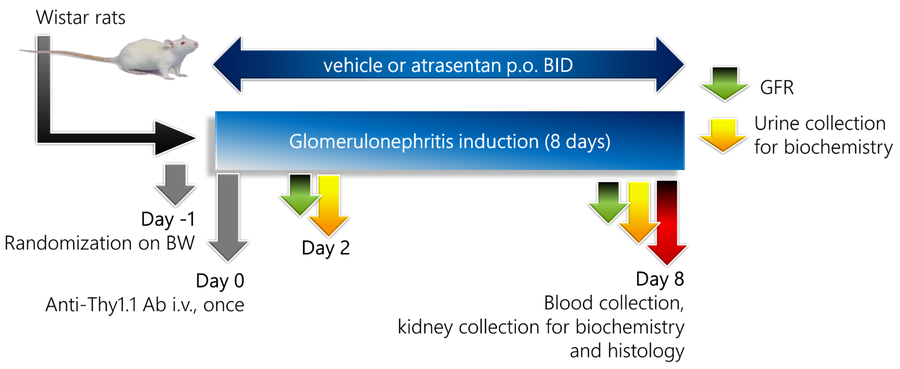
BACKGROUND

Our aim was to validate and optimize a rat model of IgA nephropathy for drug efficacy studies using quantitative image analysis of kidney. To demonstrate the accuracy of our imaging methods, we evaluated atrasentan, a selective Endothelin A receptor Antagonist currently evaluated in phase III clinical trials.

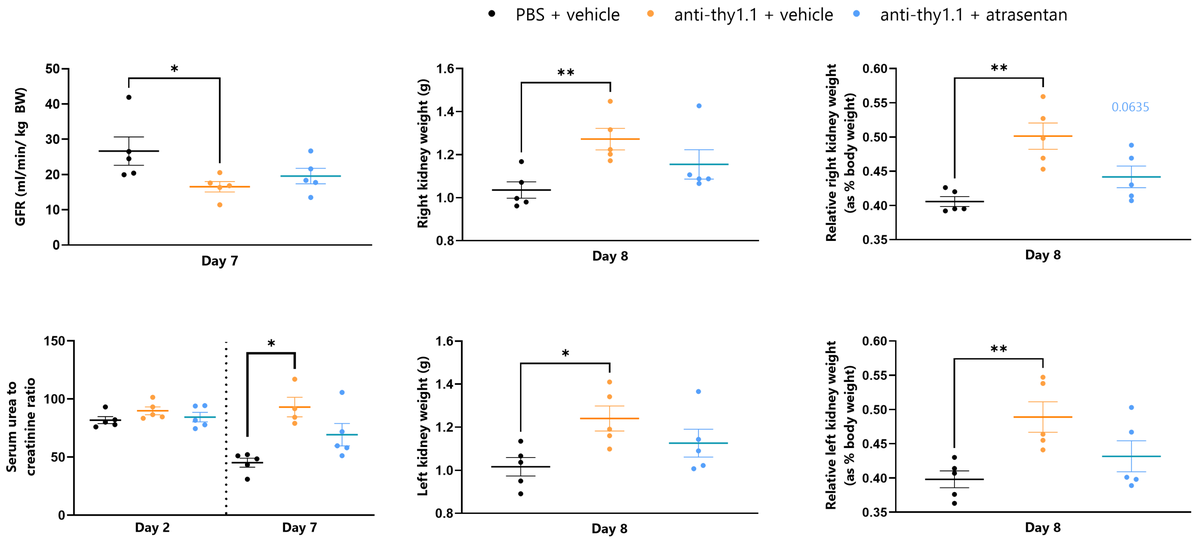
METHODS

On day 0, Wistar male rats received a single i.v. injection of Thy1.1 antibody to induce IgA glomerulonephritis and were then treated orally BID with vehicle or atrasentan 10mg/kg until day 7. A group of rats were injected i.v. with PBS as control. Urine parameters were measured at day 2 and day 7, while Glomerular Filtration Rate (GFR) was measured at day 7. Kidneys were then collected for histology and automated image analysis, including quantitative mesangial expansion and tubular impairments (Kidney AI suite) and Podocyte Exact Morphology Measurement Procedure (PEMP).



RESULTS

1 Anti-Thy1.1 Ab induced IgA nephropathy in Wistar rats leads to lower GFR and greater kidney weight after 8 days

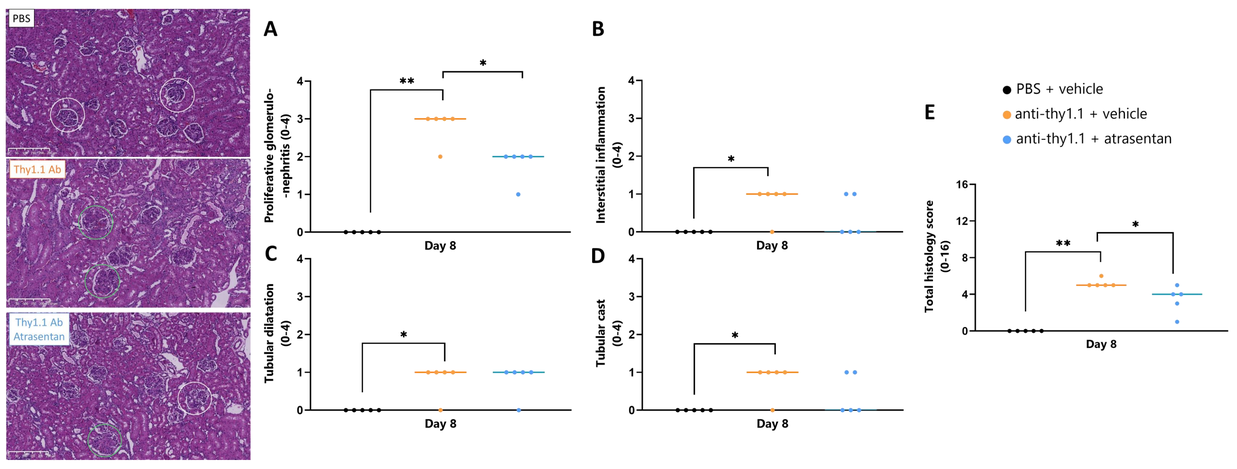


Glomerular filtration rate (A), right kidney weight (B), relative right kidney weight (C), serum urea to creatinine ratio (D) left kidney weight (E) and relative left kidney weight (F) in PBS injected or anti-Thy1.1 Ab injected rats, treated either with vehicle or atrasentan for 8 days.
**p<0.05, **p<0.01 vs. vehicle*

2 Atrasentan improves urine markers of kidney function in rats with IgA nephropathy

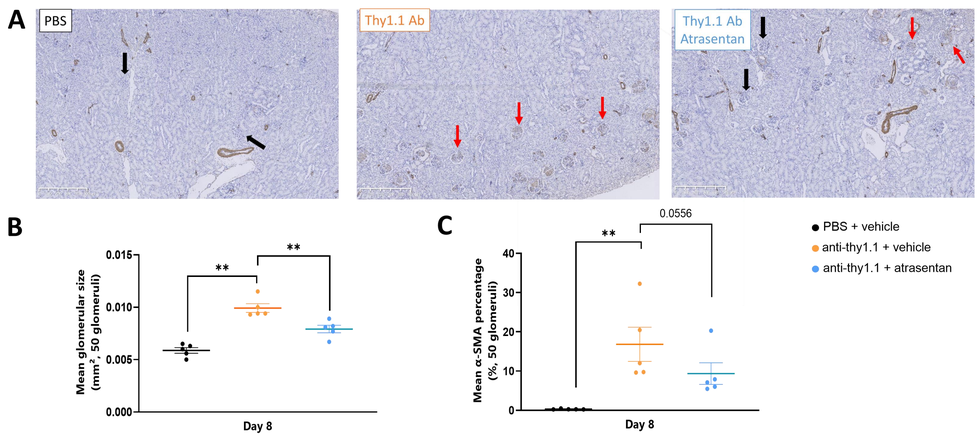
Urinary creatinine excretion (A), proteinuria (B), urinary albumin excretion (C), urine albumin to creatinine ratio (D) urinary Cystatin-C excretion (E) and urinary KIM-1 excretion (F) in rats injected with PBS and treated with vehicule or injected with anti-Thy1.1 Ab and treated with vehicule or atrasentan for 8 days. **p<0.05, **p<0.01 vs. vehicle*

3 Thy1.1 antibody injection leads to a significantly higher kidney histopathological scoring, which is improved by atrasentan treatment



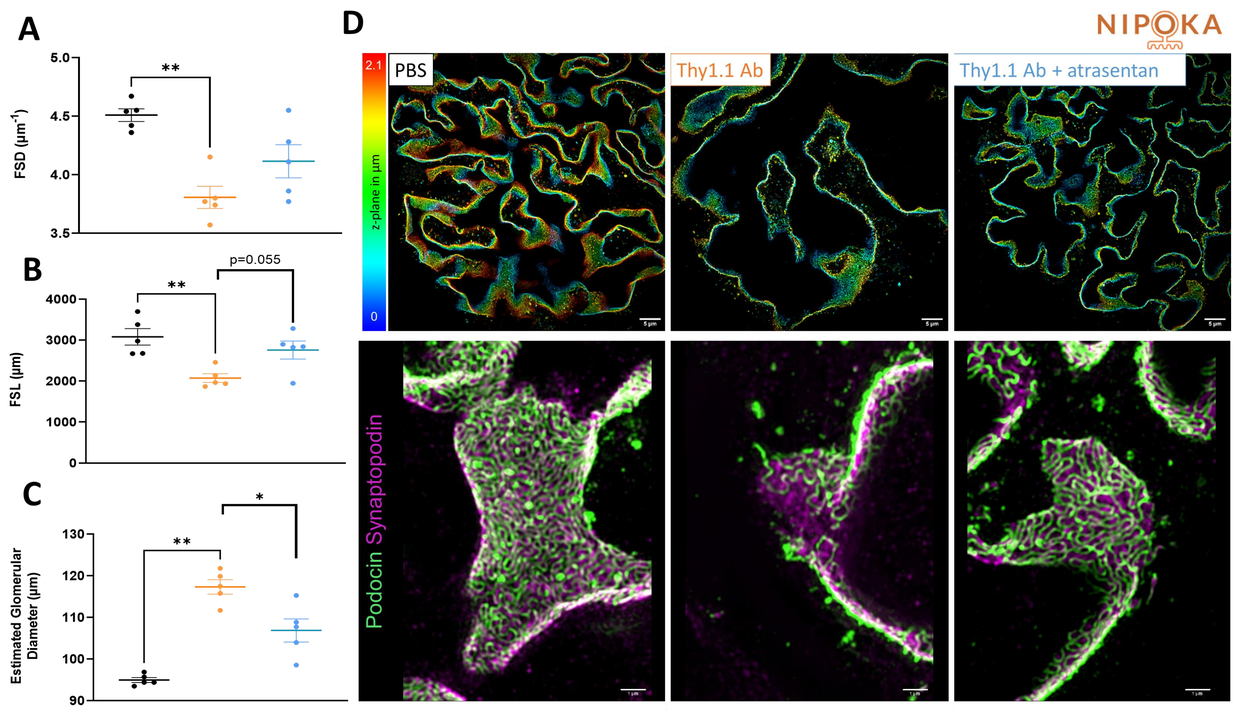
Representative pictures of kidney H&E staining, proliferative glomerulonephritis (A), interstitial inflammation (B), tubular dilatation (C), tubular cast (D) and total histology score (E) in the three groups. *Green circles indicate unhealthy glomeruli and proliferative glomerulo-nephritis. White circles indicate normal glomeruli *p<0.05, **p<0.01 vs. vehicle*

4 Thy1.1 Ab injection induces a significantly higher mean glomerular size and α-SMA labelling; these parameters are improved with atrasentan treatment.



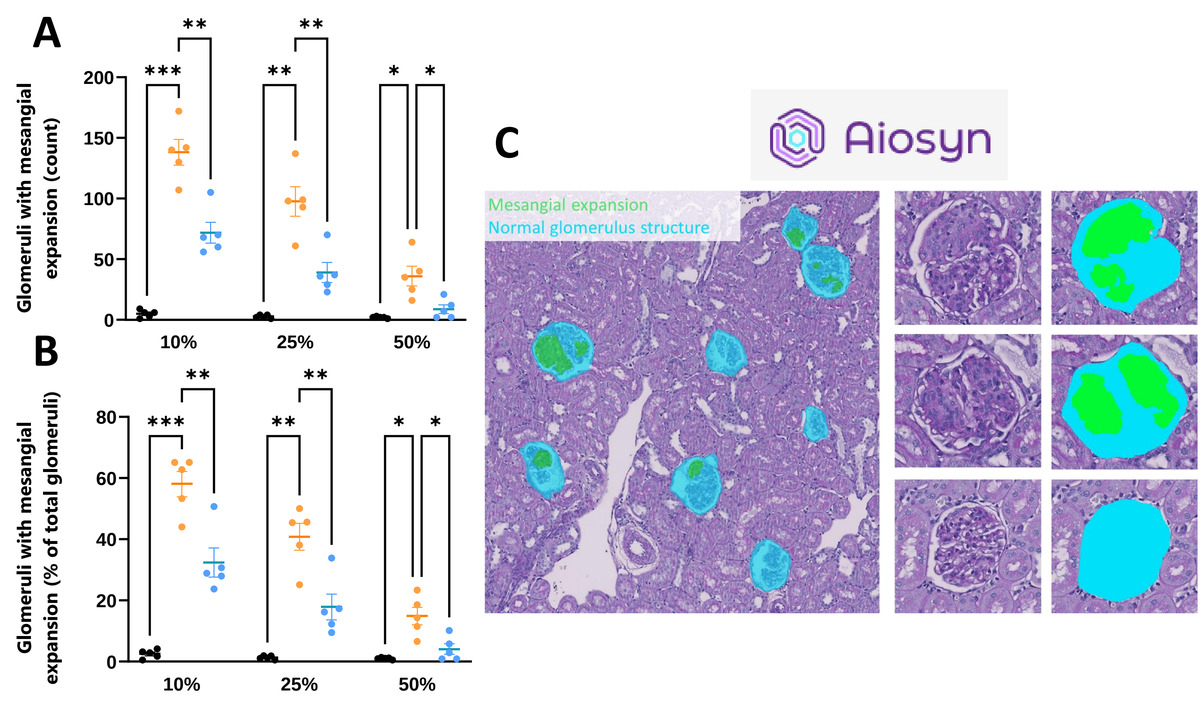
Mean glomerular size (A), mean α-SMA % (B) and representative pictures of α-SMA kidney staining in the three groups. *Red arrows: unheatlhy glomeruli, black arrows: normal glomeruli. *p<0.05, **p<0.01 vs. vehicle*

5 PEMP demonstrates that atrasentan improves podocytes effacement in rats with IgA nephropathy



Assessment of podocyte effacement with PEMP: Filtration Slit Density (FSD) (A), Filtration Slit Length (FSL) (B), estimated glomerular diameter (C), representative images of podocin as maximum intensity projection and podocin and synaptopodin as double staining (D) in the three experimental groups.
**p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 vs. vehicle*

6 Kidney AI suite uncovers how atrasentan reduces the marked mesangial expansion in rats with IgA nephropathy



Assessment of glomeruli with mesengial expension with the Kidney AI suite: count (A), % of total glomeruli (B), representative pictures illustrating both original H&E staining and image analysis layers at different stages of mesenglial expension (C). *Blue zones indicate normal glomerulus structure. Green zones indicate mesangial expansion. *p<0.05, **p<0.01, ***p<0.001 vs. vehicle*

CONCLUSION

- Quantitative image analysis uncovers the efficacy of atrasentan on glomerulonephritis in the present IgA nephropathy rat model.
- This experimental setting will help evaluating the efficacy of drugs targeting IgA nephropathy.

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