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A central role of Endothelin A (ETA) Receptor Activation in Mesangial Cell – Podocyte Crosstalk In IgA Nephropathy and Other Mesangio-Proliferative Glomerulopathies

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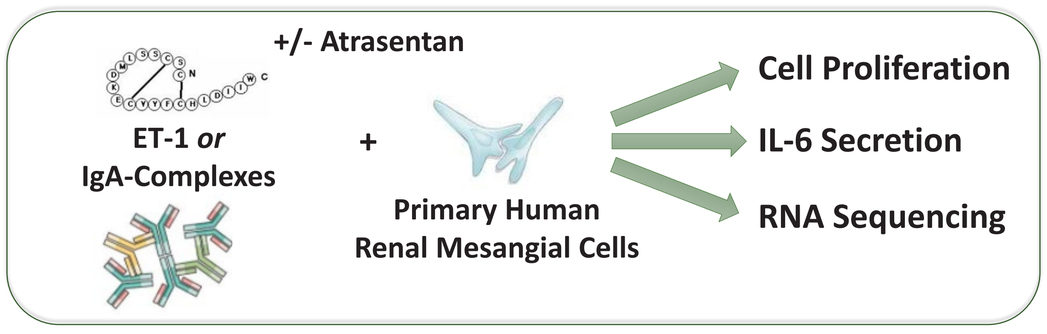
Background & Objectives

- Mesangial cell (MC) activation by IgA-immune complexes is the initiating intra-renal event in the pathogenesis of IgAN.[1]
- Subsequent MC-podocyte crosstalk results in proteinuria, the strongest predictor of progression.[1]
- However, the molecular mechanisms responsible have not been well defined.
- The objective of these studies was to determine the role of the ETA receptor in MC activation and subsequent proteinuria in IgAN and other mesangio-proliferative glomerulopathies, using the potent and selective ETA antagonist atrasentan.

Methods and Materials

Primary human renal mesangial cells (HRMC) in culture (≤4 passages), were stimulated with ET-1 (4 nM) for up to 72 hours ± atrasentan and proliferation (BrdU) and cytokine production (ELISA) were measured. Global transcriptional responses were assessed at 24 h by RNASeq.

IgA-complexes were isolated from the serum of either IgAN patients or age and sex matched healthy controls using jacalin-agarose affinity chromatography[2] and applied to HRMCs in culture for 72 hours ± atrasentan; proliferation was analyzed by BrdU incorporation.

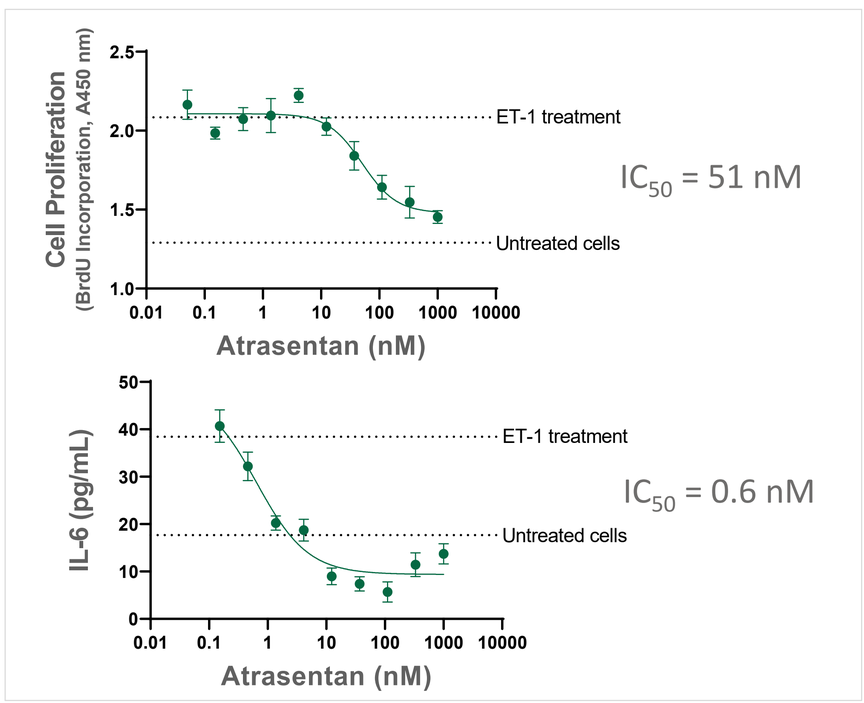


Mesangio-proliferative GN, to model MC injury and recovery, was induced in 5-6 w male Wistar rats (n=6/group) by a single IV injection of anti-Thy1.1 antibody (0.5 mg/kg, Day 0). Rats were randomized to receive atrasentan (10 mg/kg, po bid) or vehicle control beginning on Day -1 through Day 7. Non-diseased control rats (n=4) received PBS IV.

Spot UPCR was measured on Days 1, 3, 5 and 7. On Day 7, kidneys were weighed, fixed (10% neutral buffered formalin), paraffin embedded, sectioned at 4-6 microns, stained (H&E and PSR) and evaluated by light microscopy using a semi-quantitative grading scale by a board-certified veterinary pathologist, blinded to treatment allocations.

Results

A ET-1 induced HRMC proliferation and IL-6 secretion which was blocked by atrasentan in a concentration-dependent manner



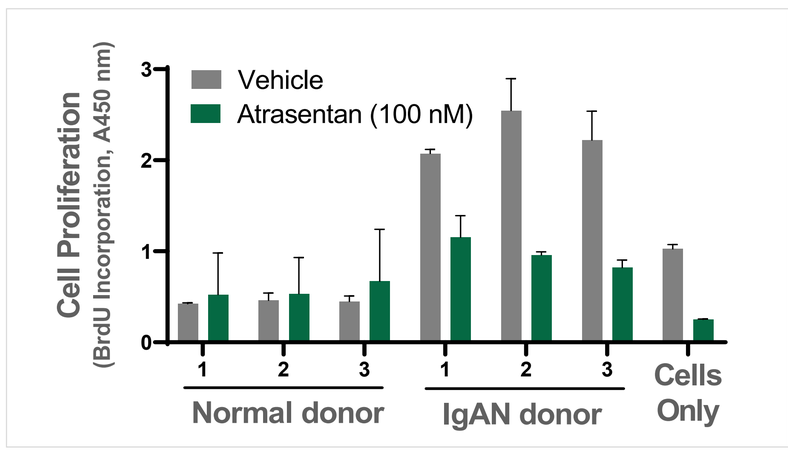
HRMCs were incubated for 72 hours with 4 nM ET-1; atrasentan blocked the increase in cell proliferation and IL-6 secretion with IC_{50} values of 51 nM and 0.6 nM, respectively

B Gene set enrichment analysis identified upregulation of cell proliferation, pro-fibrotic and pro-inflammatory pathways with ET-1 stimulation in HRMCs, which were blocked by atrasentan

HRMCs were incubated with 4 nM ET-1 for 24 hours ± 1 or 25 nM atrasentan and differential gene expression was determined following RNA sequencing

Results (continued)

C Atrasentan prevented hyperproliferation of HRMCs in response to IgA-complexes purified from IgAN patients

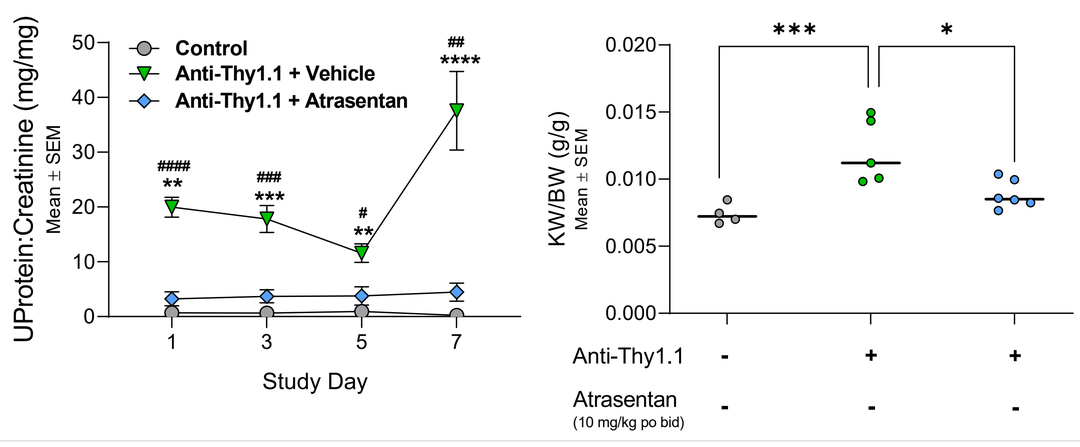


Serum IgA and Gd-IgA1 were increased in IgAN patients (913 ± 248 & 4.4 ± 0.6 µg/mL) compared to matched healthy controls (586 ± 261 & 1.9 ± 0.4 µg/mL)

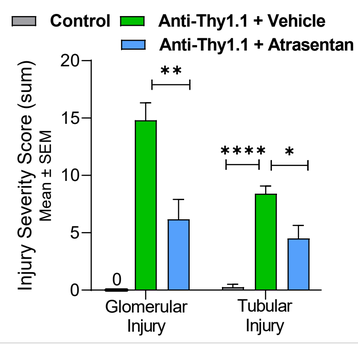
IgAN patient derived immune complexes caused 5.1-fold increase in HRMC proliferation compared to normal donors following 72 hours treatment

Atrasentan significantly ($p<0.01$) attenuated proliferation induced by IgA-containing immune complexes purified from IgAN patients (57 ± 6% reduction)

D Atrasentan reduced proteinuria and kidney weight in anti-Thy1.1 induced mesangio-proliferative GN

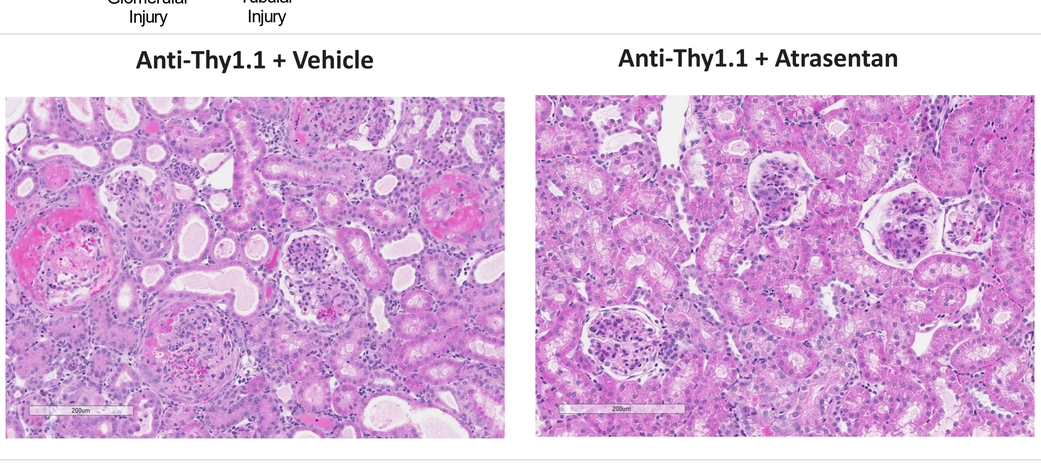


E Atrasentan attenuated glomerular and tubulointerstitial injury histologically in anti-Thy1.1 induced mesangio-proliferative GN



Glomerular injury score includes mesangial hypercellularity and matrix expansion, synechia, segmental mesangiolysis and glomerulosclerosis

Tubulointerstitial injury score includes protein casts, tubular degeneration, tubular dilation and interstitial fibrosis



F Atrasentan was recently reported to rapidly reduce albuminuria and downregulate intra-renal pro-inflammatory and pro-fibrotic transcriptional networks in the gddY mouse model of spontaneous IgAN - WCN21-0358.[3]

Conclusion

- These studies suggest an important role of the ETA receptor in mesangial cell activation and subsequent proteinuria in IgAN and other models of immune mediated mesangio-proliferative GNs.

These results support the therapeutic potential of atrasentan in IgAN patients, not only via its well characterized effect to reduce proteinuria, but also by potentially reducing mesangial cell activation, a hallmark of IgAN and other mesangio-proliferative glomerulopathies

- The Phase 3 ALIGN trial is assessing the efficacy, safety and tolerability of atrasentan in IgAN patients at risk of progressive kidney function loss, despite optimized RAS blockade. (ClinicalTrials.gov Identifier: NCT04573478)

References

1. Lai et al., *Nature Reviews.* 2:1601, 2016
2. Novak et al., *Kidney Int*. 67(2): 504-13, 2005
3. King et al., *Kidney Int*. Reports 6(4): S164, POS-378 2021