UniversitätsKlinikum Heidelberg

Nephroprotective Effect of RAS-Antagonists in Mice Carrying R140Q Podocin Mutation

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Background

Mutations in the NPHS2 gene, encoding the podocyte specific protein podocin, cause hereditary nephrotic syndrome progressing to renal failure. Recently, we generated an inducible knock-in mouse model carrying the R140Q mutation, the murine analogue of the most common human mutation R138Q. This model develops a nephropathy strongly reminiscent of the human disorder. The aim of this study was to test the antiproteinuric und nephroprotective efficacy of RAS antagonists in this mouse model.

Methods

Model

Nphs2$^{Flox/R140Q}$, Cre+ podocin KI mice received 33 mg/kg/day of tamoxifen i.p. at age 6 weeks for 5 days to induce hemizygosity for the R140Q mutation.

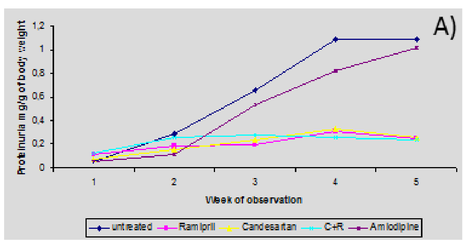
Pharmacological treatment

Prophylactic treatment was started 3 days before initiation of tamoxifen administration. Eight animals per group received an ACE Inhibitor (ramipril), an Angiotensin-II type I receptor blocker (candesartan), the combination of both drugs (ramipril and candesartan) or the Ca channel blocker amplodipine. All drugs were administered at a dose of 10 mg/kg/day admixed to the food.
Proteinuria and blood pressure were monitored weekly.
At week 4 the animals were sacrificed and the glomerular filtration rate, biochemical parameters and histopathological changes were examined (glomerulosclerosis, tubulointerstitial changes, podocyte loss) .

Results

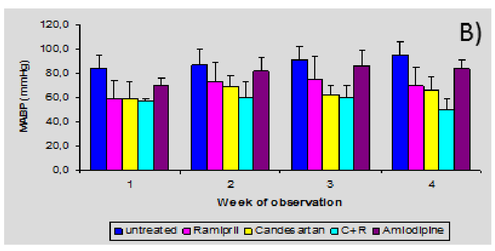
Proteinuria

Attenuated proteinuria in mice treated with ACE-inhibitors and/or angiotensin Receptor blockers.



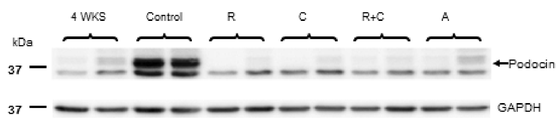
Blood pressure

Treatment with anti-hypertensive drugs displayed lower MABP values comparing to sick untreated animals.



A) Proteinuria was markedly attenuated in animals treated with ramipril and/or candesartan (20% of untreated) and moderately attenuated in those receiving amlodipine (66% of untreated animals).
B) Blood pressure was significantly lower in treated animals. Mean 49.8 mmHg R+C ($p<0.001$) vs. 81.1 mmHg A ($p<0.001$) vs. 95.7 mmHg untreated, week 4.

Podocin protein expression



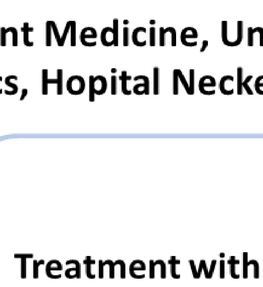
Abolished podocin abundance in untreated as well as in treated animals

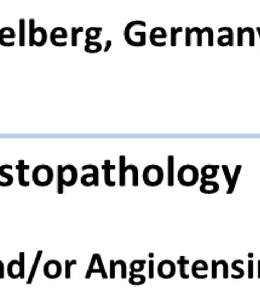
Western blot analysis of homogenized kidney tissue (30 µg) gathered from animals at the beginning of week 5 after induction. Treatment showed no effect on podocin expression.
4 WKS untreated sick animals at week 4; R Ramipril; C Candesartan; R+C Ramipril+Candesartan; A Amlodipine.

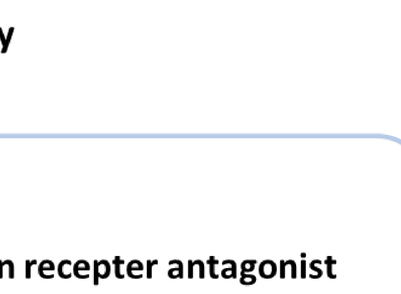
Histopathology

Treatment with ACE-Inhibitor and/or Angiotensin recepter antagonist preserves number of podocytes per glomerulus and reduces glomerular area affected by fibrosis.

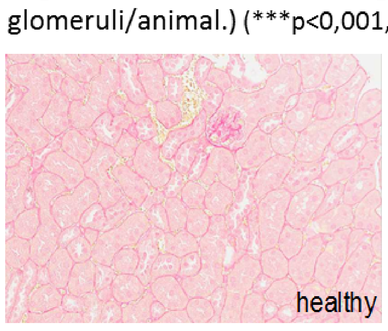
WT1-immunostaining of glomerular cross-sections (4µm & 10 µm) revealed a preserved number of podocytes per glomerulus. Quantification was done with ImageJ according to Animal Models of Diabetic Complications Consortium protocol.
N=5 (R), N=6 (C), N=6 (R+C), N=3 (A)

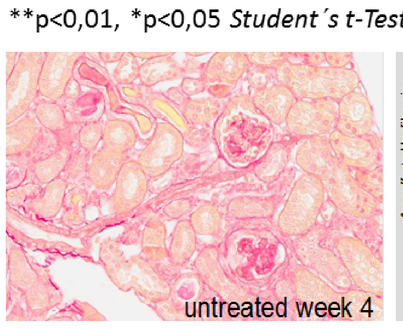


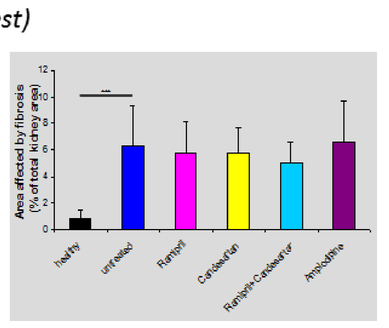




PAS-staining (periodic acid-Schiff) of paraffin sections (3µm) was done to determine the degree of sclerosis within the glomerular tuft (glomerular sclerosis index – GSI, 50 glomeruli/animal.) (***$p<0,001$, **$p<0,01$, *$p<0,05$ *Student´s t-Test)*



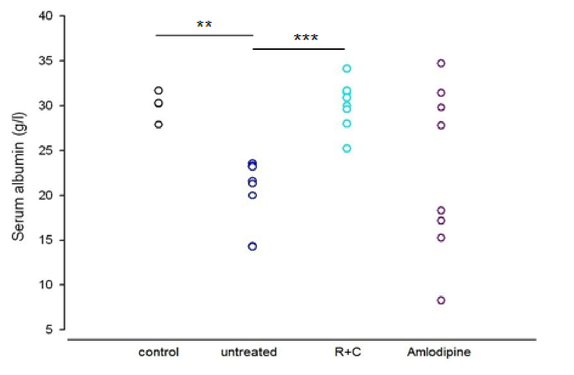




Sirus Red Staining was performed to determine tubulointerstitial fibrosis). Slightly reduction in treated animals was observed (n.s.).
Quantification was done with Image ProPlus (Media Cybernetics, Silver Spring, MD).

Serum Albumin

Treatment with combination of Ramipril+Candesartan increases plasma albumin concentration, whereas treatment with amlodipine showed no significant effect.



Plasma was obtained via cardiac puncture at the time of sacrificing. (mean values 31.2 g/l R+C vs. 24 g/l A vs.19.1 g/l untreated (***$p<0.001$, **$p<0.01$)

Conclusion

In mice carrying the most common human podocin mutation, administration of ACE inhibitors and ARBs, attenuates proteinuria and glomerulosclerosis despite persistently increased intracellular degradation of mutant podocin protein. Our findings suggest that RAS blockade provides effective pharmacological nephroprotection in children with this hereditary podocytopathy.

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SEVENTH FRAMEWORK PROGRAMME

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