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Efficacy of Nephroprotective Pharmacotherapy in an in Vivo Model of Inherited Nephrotic Syndrome

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Mutations in the NPHS2 gene, encoding the podocyte protein podocin, represent the most common cause of hereditary nephrotic syndrome. Recently, we generated an inducible knock-in mouse model carrying the R140Q podocin mutation, the murine analogue of the most common human mutation R138Q. After induction, R140Q-podocin hemizygous mice develop nephrotic-range proteinuria, focal-segmental glomerulosclerosis und progressive renal failure. Nephroprotective effect of antiproteinuric pharmacotherapy in genetic podocytopathies has not been established yet. Thus the aim of our study was to characterize the mouse model with postnatally induced R140Q hemizygosity including quantification of pathohistological changes and, thereafter, to test the antiproteinuric und nephroprotective effects of RAS antagonists in the same mouse model of hereditary podocytopathy.

C57BL/6 mice with $Nphs2^{lox2/R140Q}Cre^{+}$ genotype were injected with Tamoxifen or vehicle for 5 days to induce hemizygosity for R140Q-mutant podocin. Weight, blood pressure and proteinuria were monitored once weekly. Tissue samples were collected at defined intervals after induction by sacrificing animals 1, 2, 4, 6, 8, 12 and 16 weeks after the end of tamoxifen administration. Renal morphology was evaluated by quantitative histology, immunohistochemistry and electron microscopy. In the second part of the study, induced mice (8 per group) were treated prophylactically with the ACE inhibitor ramipril, the AT1 receptor blocker candesartan, the combination of ramipril and candesartan, the non-RAS antihypertensive amlodipine or remained untreated with either tamoxifen induction (sick controls) or vehicle injections (healthy controls). Biochemical and histopathological changes were examined after 4 weeks.

In induced animals proteinuria ocurred within 1 week and reached a maximum 4 weeks after induction (1.091 ± 0.830 mg/g vs. 0.014 ± 0.009; p=0.0000001). After week 4, animals gradually developed renal failure with diminished weight gain (week 6: 15 ± 9% vs. 24 ± 12% of initial body weight, p=0.005) and advanced uremia at 12-16 weeks (mean glomerular

filtration rate 39% of uninduced controls). The number of podocytes per glomerulus started decreasing at week 2 (mean 48 ± 19 vs. 70 ± 20, p=0.14), whereas glomerular sclerosis index increased from week 4 (mean 1.36 ± 0.19 vs. 0.25 ± 0.08, p=0.000001). Interstitial changes included fibrosis (up to 20 % of section area in end stage renal disease), tubular atrophy and dilatation.

Blood pressure was elevated in sick animals and significantly more markedly reduced by RAS antagonist than by amlodipine administration (mean arterial pressure healthy controls, 85; sick controls, 95; ramipril, 70; candesartan: 66; ramipril+candesartan, 50; amlodipine, 83 mm Hg). Proteinuria was markedly attenuated in animals treated with RAS antagonists (by ramipril+candesartan 78%, relative to sick controls), but not in those receiving amlodipine. After 4 weeks, mice receiving the combination of ACEi and ARB were normo-albuminemic (ramipril+candesartan, 31 vs. amlodipine, 24 g/l; sick controls, 19; healthy controls, 32 g/l) and serum creatinine was increased less than in untreated animals. Histopathologically, animals treated with RAS antagonists scored lower glomerular sclerosis index (ramipril+candesartan, 0.91; sick controls, 1.36; healthy controls, 0.25) and longer podocyte survival (ramipril+candesartan, 79; amlodipine, 63; sick controls, 40; healthy controls, 76 podocytes/glomerulus). Whereas podocin mRNA expression was preserved or even increased in all diseased animals, Western blot analysis showed a subtotal loss of podocin protein in all induced animals irrespective of pharmacological treatment.

The reduction of proteinuria by ACE inhibition seems to be mediated by decrease of systemic arterial blood pressure, reduction of intraglomerular pressure and, possibly, other non-hemodynamical intrarenal effects of ACEis and ARBs. ARB-mediated attenuation of TGFβ-signaling seems to be responsible for deceleration of FSGS, whereas longer podocyte survival results from blockage of production and action of angiotensin II that has proapoptotic effect on podocytes.

The inducible R140Q-podocin mouse is a promising model of the most common genetic cause of human nephrotic syndrome in mouse, with a spontaneous disease course strongly reminiscent of the human disorder. This model allows testing the efficacy of available and novel pharmacological approaches to improve podocyte function and viability in attenuating proteinuria and glomerulosclerosis and delaying progression to renal failure. In these mice, the administration of RAS antagonists markedly attenuates proteinuria and podocyte loss and delays glomerulosclerosis despite persistently increased intracellular degradation of mutant podocin protein. These findings suggest that RAS blockade provides an effective pharmacological nephroprotection in this hereditary podocytopathy.