Murine Recombinant ACE2 Reduces Renal Fibrosis in Experimental Alport Syndrome

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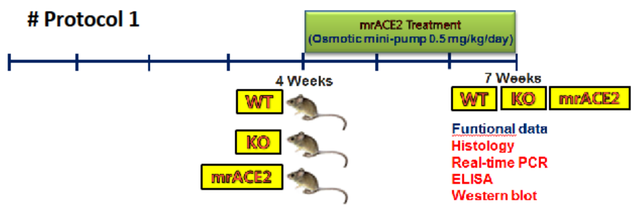
ABSTRACT

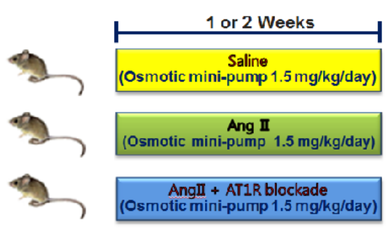
ACE2 is a monocarboxypeptidase in the renin angiotensin system that catalyzes the breakdown of Angiotensin II (AngII) to Ang1-7. We have reported that ACE2 expression and activity in the kidney are reduced in experimental AS but the impact of this finding on kidney disease progression has not been studied. Accordingly, we evaluated the effects of treatment with murine recombinant ACE2 (mrACE2) in*Col4A3*$^{-/-}$ mice, a model of AS characterized by proteinuria and progressive renal injury. The mrACE2 (0.5 mg/kg/day) was administered from 4 -7 weeks of age via osmotic mini-pump. We also evaluated TNF-α converting enzyme (TACE) expression and activity in AS and Ang type 1 receptor (AT1R) blocker-treated mice. Treatment with mrACE2 led to an increase in both kidney renal ACE2 expression and the urinary ACE2 excretion rate in 7-week-old *Col4A3*$^{-/-}$ mice compared to untreated group. Kidney AngII levels declined and kidney Ang1-7 levels increased. These effects were associated with a significant decrease in proteinuria in the treated 7-week-old *Col4A3*$^{-/-}$ mice compared to the untreated group. ACE2 expression and activity were decreased and TACE expression and activity were increased in 7-week-old *Col4A3*$^{-/-}$ or Ang II-treated mice which were attenuated by mrACE2 or AT1R blocker treatment. The inflammatory cytokine IL-6 and F4/80, a macrophage marker, were also reduced by treatment with mrACE2. Transforming growth factor-β1 (TGF-β1), col1α1, and alpha smooth muscle actin levels were increased in the kidneys of 7-week-old *Col4A3*$^{-/-}$ mice and all were reduced by mrACE2. In summary, treatment with mrACE2 alters angiotensin peptide metabolism in the kidneys of *Col4A3*$^{-/-}$ mice and attenuates the progressionof AS nephropathy.

METHODS

 # Protocol 1



 # Protocol 2



RESULTS

Animal data and functional parameters

	WT (n=8)	KO (n=8)	mrACE2(n=8)
Body weight (g)	20.70±0.62	18.10±0.70*	19.02±0.49*,#
Kidney weight(g)	0.157±0.006	0.170±0.004	0.171±0.008
KW(g)/BW (Kg)	7.58±0.11	9.39±0.20*	8.90±0.22*
Urine output (mL)	1.56±0.22	3.38±0.27*	2.45±0.25*,#
UalbV(μg/24hr)	17.8±2.26	247.6±18.18*	150.9±16.72*,#

Abbreviations: WT, wild type; KO, Col4A3$^{-/-}$; *n*, number of mice; BW, body weight; KW, kidney weight, UalbV, urinealbuminover 24 hours. BW and kidney weight were recorded at time of sacrifice. Urine output was measured for 24 hours the day before sacrifice. Creatinine levels were measured in frozen plasma samples. *p< 0.05, compared to WT. *p< 0.05, compared to KO. Values are means + SE.

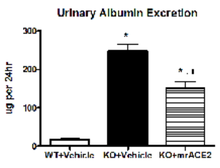


Figure 1. Urinay albumin excretion
Each column represents mean ± SE. *p< 0.05 compared with the WT. # p<0.05 compared with KO.

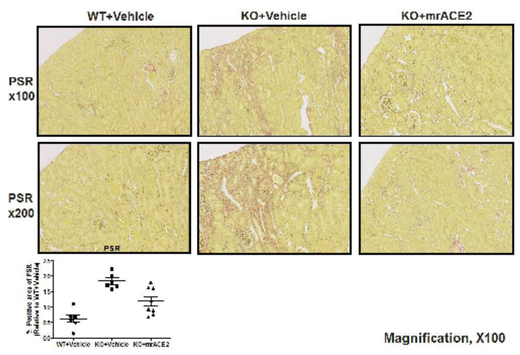


Figure 2. Picrosirius red (PSR) shows increased collagen deposit in KO mice, which is decreased by mrACE2 treatment. Each column represents mean ± SE. *p< 0.05 compared with the WT. # p<0.05 compared with KO.

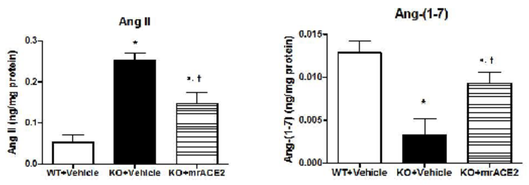


Figure 3. Angiotensin (Ang) II and Ang-(1-7) peptides in kidneys. Peptide levels were determined by enzyme immunoassay and normalized to total protein. Ang II peptide level was increased and Ang-(1-7) level was decreased in KO mice which was counter-regulated by mrACE2 treatment. Each column represents mean ± SE. *p< 0.05 compared with the WT. # p<0.05 compared with KO.

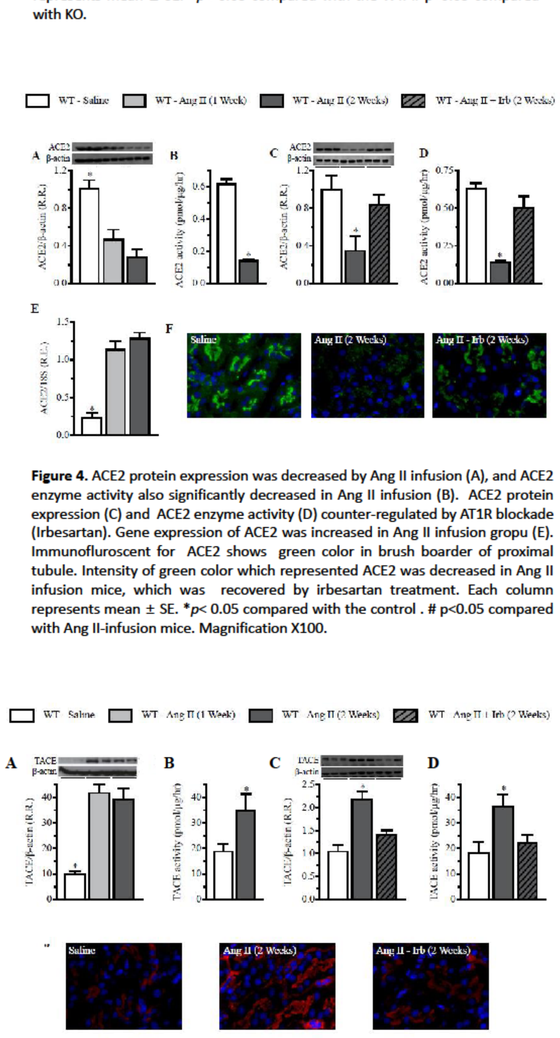


Figure 4. ACE2 protein expression was decreased by Ang II infusion (A), and ACE2 enzyme activity also significantly decreased in Ang II infusion (B). ACE2 protein expression (C) and ACE2 enzyme activity (D) counter-regulated by AT1R blockade (Irbesartan). Gene expression of ACE2 was increased in Ang II infusion gropu (E). Immunofluroscent for ACE2 shows green color in brush boarder of proximal tubule. Intensity of green color which represented ACE2 was decreased in Ang II infusion mice, which was recovered by irbesartan treatment. Each column represents mean ± SE. *p< 0.05 compared with the control . # p<0.05 compared with Ang II-infusion mice. Magnification X100.

Figure 5. TACE protein expression was increased by Ang II infusion (A), and TACE enzyme activity also significantly decreased in Ang II infusion (B). TACE protein expression (C) and ACE2 enzyme activity (D) counter-regulated by AT1R blockade (Irbesartan). Immunofluroscent for ACE2 shows green color in brush boarder of proximal tubule. Intensity of red color which represented TACE was decreased in Ang II infusion mice, which was recovered by irbesartan treatment. Each column represents mean ± SE. *p< 0.05 compared with the control . # p<0.05 compared with Ang II-infusion mice. Magnification X100

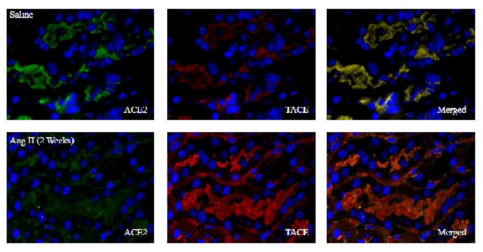


Figure 6. Immunofluoresecent for ACE2 and TACE expression in kidney proximal tuule. Merged image shows increased TACE expression after Ang II infusion. Magnification X100.

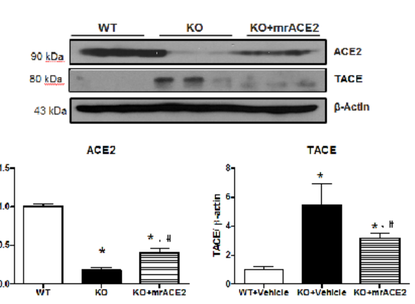


Figure 7. Westernblot for ACE2 and TACE expression in kidneys. ACE2 expression was decreased and TACE expression was increased in KO mice, which were counter-regulated by mrACE2 treatment. Each column represents mean ± SE.*p< 0.05 compared with the WT. # p<0.05 compared with KO.

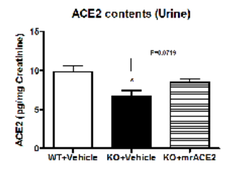


Figure 8. Urinay ACE2 excretion
Each column represents mean ± SE.
*p< 0.05 compared with the WT.

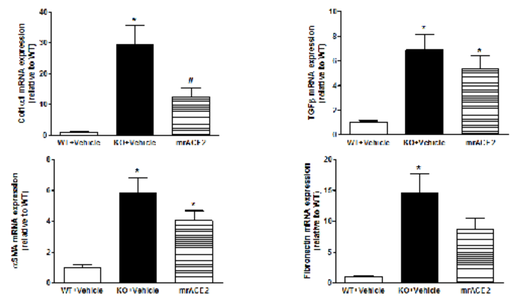


Figure 9. Real-time PCR data for fibrosis markers such as Clo1a1, aSMA, TGFß and fibronectin. mRNA expresion of Col1a1 was significantly increased in KO mice which was attenuated by mrACE2 treatment. Each column represents mean ± SE.*p< 0.05 compared with the WT. # p<0.05 compared with KO.

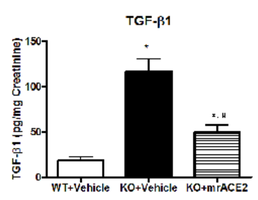


Figure 10. TGFß1 Urinary excretion. Each column represents mean ± SE. *p< 0.05 compared with the WT. # p<0.05 compared with KO.

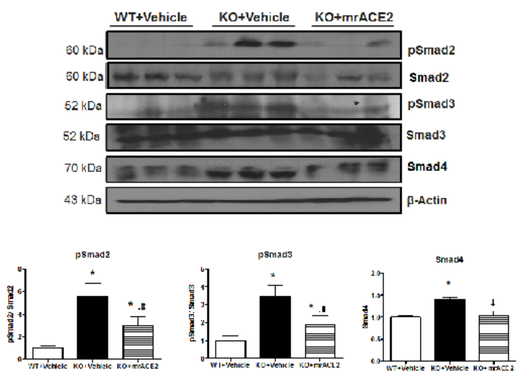


Figure 11. Westernblot image for Smad signaling. The protein expression of phosphoSmad 2 and 3, and Smad 4 was increased in KO mice, which was attenuated by mrACE2 treatment. Each column represents mean ± SE.*p< 0.05 compared with the WT. # p<0.05 compared with KO.

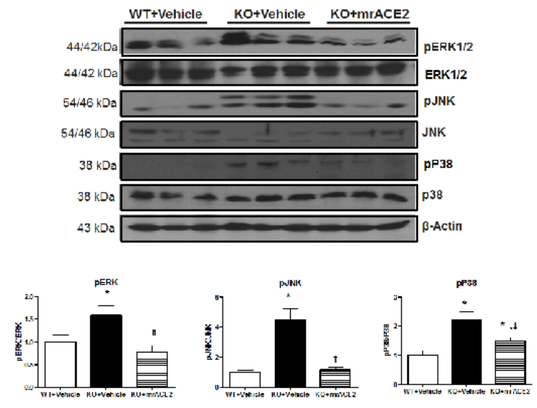


Figure 12. Westernblot image for MAPK kinase pathway. The protein expression of phosphoSmad 2, 3 and Smad 4 was increased in KO mice, which was attenuated by mrACE2 treatment. Each column represents mean ± SE.*p< 0.05 compared with the WT. # p<0.05 compared with KO.

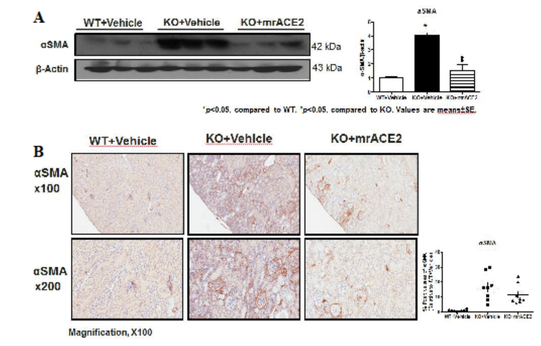


Figure 13. Westernblot image for aSMA. The protein expression of aSMA was increased in KO mice, which was attenuated by mrACE2 treatment. Immunohistochemistry for aSMA shows increased expression in KO mice, which was attenuated by mrACE2 treatment (B). p< 0.05 compared with the WT. # p<0.05 compared with KO.

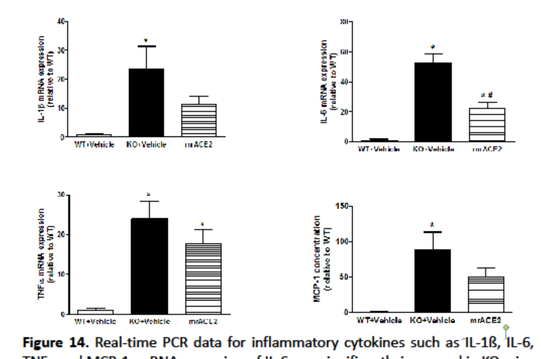


Figure 14. Real-time PCR data for inflammatory cytokines such as IL-1ß, IL-6, TNFa and MCP-1. mRNA expresion of IL-6 was significantly increased in KO mice which was attenuated by mrACE2 treatment. Each column represents mean ± SE.*p< 0.05 compared with the WT. # p<0.05 compared with KO.

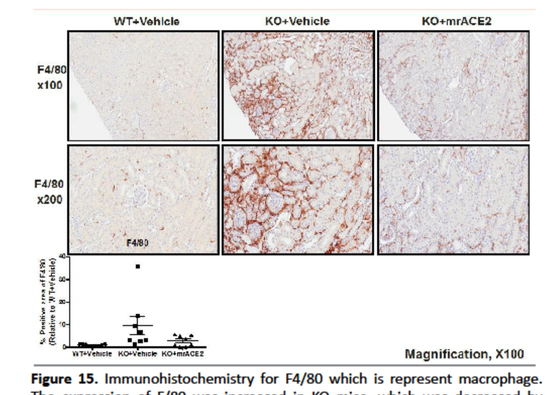
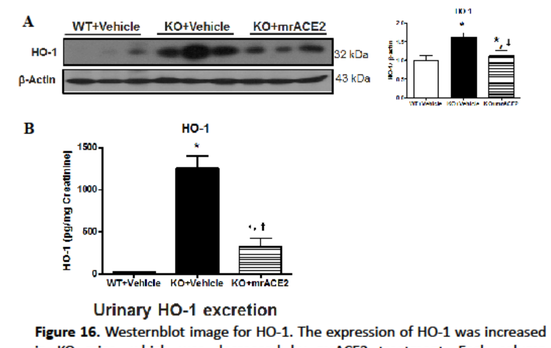


Figure 15. Immunohistochemistry for F4/80 which is represent macrophage. The expression of F/80 was increased in KO mice, which was decreased by mrACE2 treatment. Each column represents mean ± SE.*p< 0.05 compared with the WT. # p<0.05 compared with KO.



Urinary HO-1 excretion

Figure 16. Westernblot image for HO-1. The expression of HO-1 was increased in KO mice, which was decreased by mrACE2 treatment. Each column represents mean ± SE.*p< 0.05 compared with the WT. # p<0.05 compared with KO.

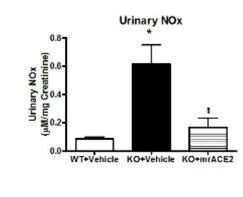


Figure 17. Urinay nitric oxide metabolite excretion
Each column represents mean ± SE. *p< 0.05 compared with the WT. # p<0.05 compared with KO.

SUMMARY

- Treatment with mrACE2 alters angiotensin peptide metabolism in the kidneys of *Col4A3*$^{-/-}$ mice.
- Treatment with mrACE2 reduce proteinuria and attenuates progression of AS nephropathy.
- Decreased ACE2 in AS kidney is associated with increased TACE activation which is attenuated by mrACE2 treatment.
- Renal protective effects of mrACE2 in AS kidney is associated with counter-regulation of TGFβ-Smad signaling and MAPK pathway, anti-inflammation, anti-oxidative pathway.

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DOI: 10.3252/pso.eu.52era.2015
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