Renin Angiotensin System Induces Renal Inflammation via Renal TLR2 Activation in Experimental Unilateral Ureteral Obstruction

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OBJECTIVES

Although Toll-like receptor 2(TLR2) may play an important role, inhibition of TLR2 has not shown consistent results of amelioration in renal inflammation of obstructed kidney. There have been some reports that renin angiotensin system (RAS) may affect the activation of TLR signaling. However, there was few study for the relationship between RAS and renal TLR2 activation in experimental unilateral ureteral obstruction(UUO). We investigated the effect of RAS on the activation of renal TLR2 in UUO.

METHODS

Male wild type and TLR2 knokout(KO) mice backgrounded C57BL/6 were divided into the 8 groups; 1)Sham, 2)Angiotensin II(Ang II)+ Sham, 3)AngII+TLR2 KO, 4)Aliskiren+Sham, 5)Aliskiren+TLR2 KO, 6)UUO only 7)TLR2 KO UUO, and 8)Aliskiren + TLR2 KO UUO. Ang II and aliskiren were administrated via an osmotic minipump(AngII;1,000ng/kg/min for 12 days, Aliskiren; 25 mg/kg/day for 8days). We performed realtime RT PCR and immunohistochemistry for molecular study and H&E stain and Masson trichrome (MT) stain for histologic examination of kidneys.

RESULTS

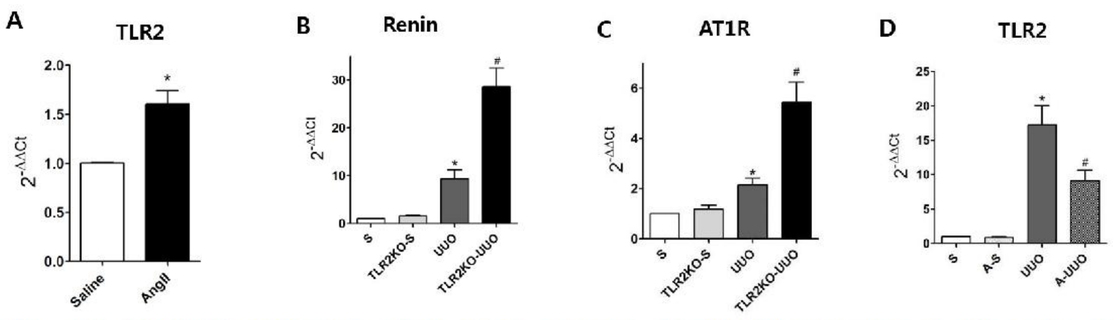

Figure 1. (A) Effects of angiotensin (Ang) II infusion on mRNA expression of *TLR2*. Kidneys from Ang II-infused wild type mice showed higher mRNA expression of *TLR2* than saline-infused wild type mice (*: $p < 0.05$). (B-D) Renin (B), AT1R (C), and TLR2 (D) mRNA expression. *Renin* and *AT1R* mRNA expression was significantly higher in UUO kidneys than in sham kidneys (A, B, *: $p < 0.05$), and in TLR2 KO UUO kidneys compared with wild type UUO kidneys (A, B, #: $p < 0.05$). There were no significant differences in the basal mRNA expression of *renin* and *AT1R* between sham and TLR2 KO kidneys. *TLR2* mRNA expression in UUO kidneys was significantly higher than that in sham-operated kidneys. Aliskiren treatment reduced the mRNA level of *TLR2* in UUO kidneys. S: sham, A: aliskiren, UUO: unilateral ureteral obstruction, AT1R: angiotensin 1 receptor.

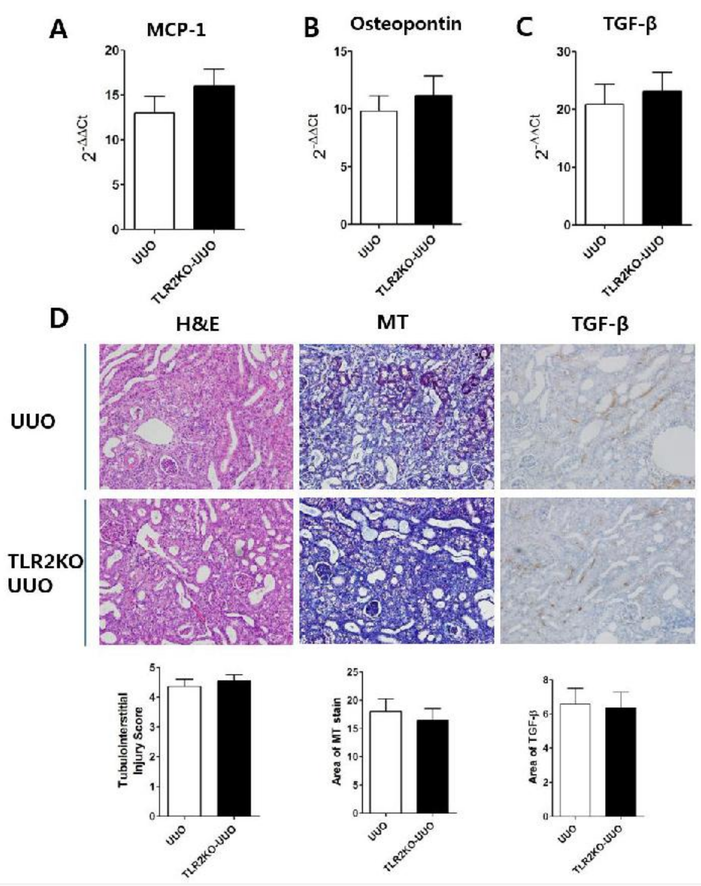

Figure 2 . The effect of TLR2 knockout (KO) on UUO-induced injury. Renal mRNA expression of *OPN* (A), *MCP-1* (B) and *TGF-β* (C) was similar in TLR2 KO and wild type UUO mice. There were no significant differences in tubulointerstitial injury sore, masson trichrome (MT)-stained area, and TGF-positive stained area between wild type and TLR2 KO UUO kidneys (D). UUO: unilateral ureteral obstruction, MCP-1: monocyte chemotactic protein-1, TGF-β: transforming growth factor-β.

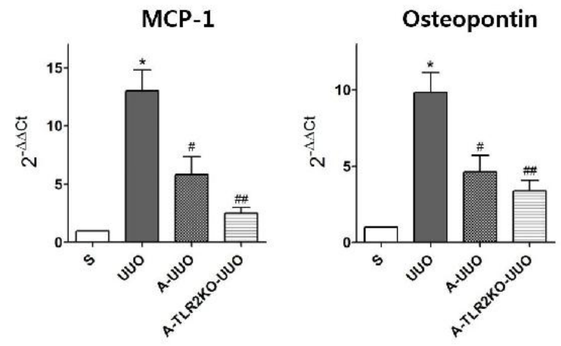

Figure 3. The effect of inhibition of RAS and TLR on MCP-1 and OPN expression 7 days after UUO. Osteopontin (A) and MCP-1 (B) mRNA expression was increased in UUO kidneys compared with sham-operated kidneys ($p < 0.05$). Aliskiren treatment reduced the renal mRNA expression of *OPN* and *MCP-1* in UUO kidneys ($p < 0.05$). Aliskiren-treated TLR2-KO UUO kidneys showed a significant decrease in mRNA expression of *OPN* and *MCP-1* compared with aliskiren-treated wild type UUO kidneys ($p < 0.05$). S: sham, UUO: unilateral ureteral obstruction, A: aliskiren.

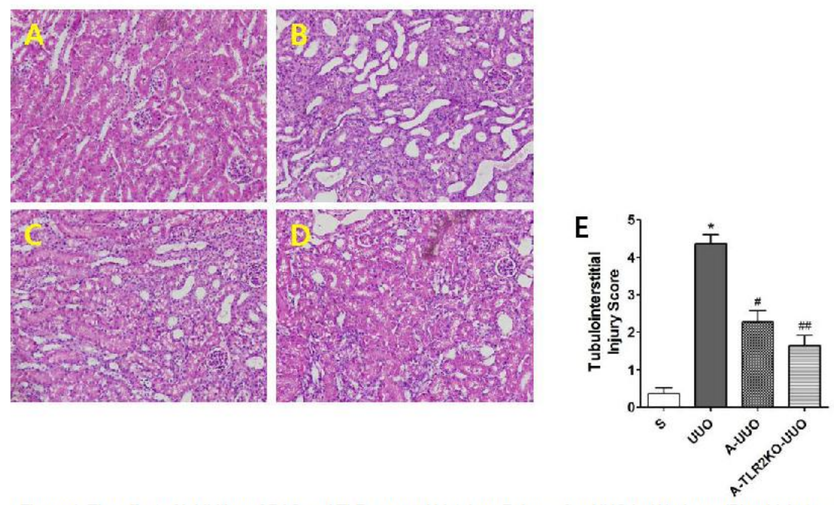

Figure 4. The effect of inhibition of RAS and TLR on renal histology 7 days after UUO in (A) sham, (B) vehicle-treated wild type UUO, (C) aliskiren-treated UUO, and (D) aliskiren-treated TLR2KO UUO mice. Vehicle-treated obstructed kidneys showed marked injury, with mononuclear cells in the interstitium and tubules, and dilatation and desquamation of tubular epithelial cells. In contrast, aliskiren-treated UUO mice showed significantly less tubulointerstitial damage. (E) Aliskiren-treated TLR2-KO UUO kidneys showed a significant decrease in tissue injury score compared with aliskiren-treated UUO kidneys. S: sham, UUO: unilateral ureteral obstruction, A: aliskiren. (*$p < 0.05$ vs sham operation, # $p < 0.05$ vs. UUO, ##$p < 0.05$ vs. A-UUO).

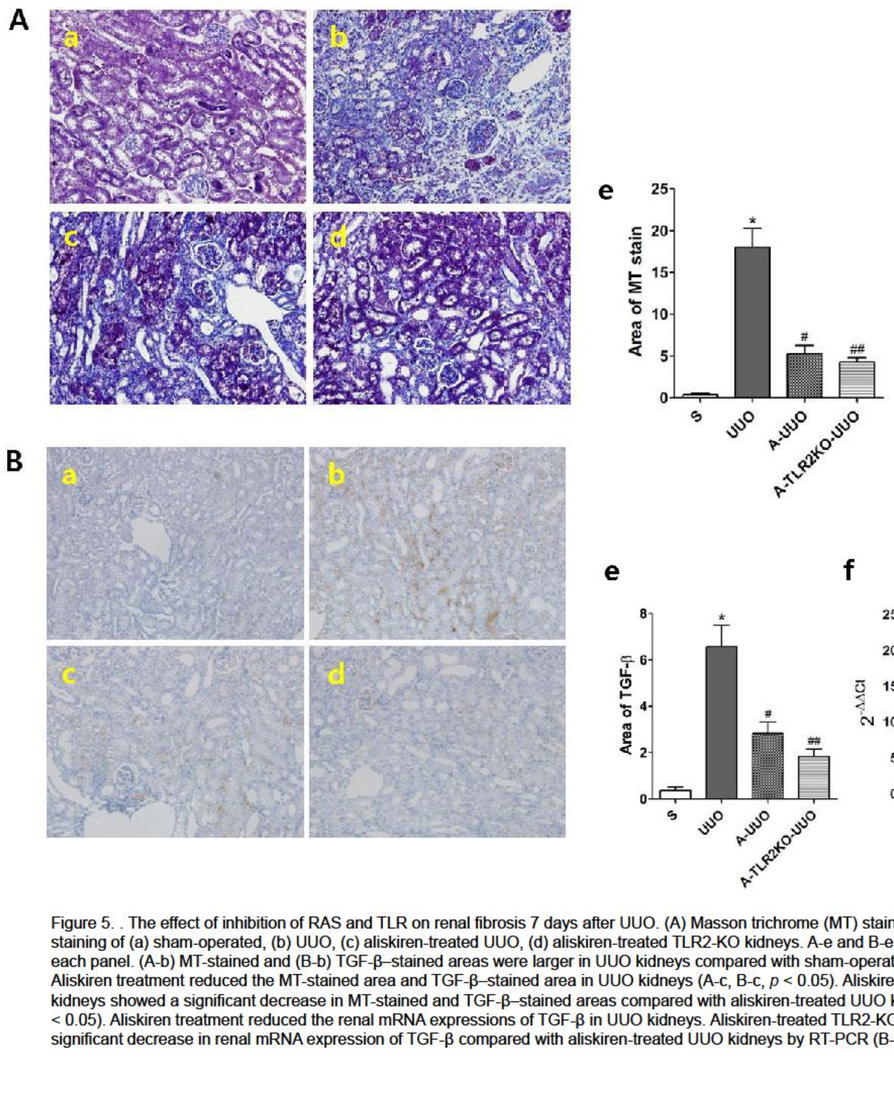

Figure 5. . The effect of inhibition of RAS and TLR on renal fibrosis 7 days after UUO. (A) Masson trichrome (MT) staining and (B) TGF-β staining of (a) sham-operated, (b) UUO, (c) aliskiren-treated UUO, (d) aliskiren-treated TLR2-KO kidneys. A-e and B-e show quantification of each panel. (A-b) MT-stained and (B-b) TGF-β–stained areas were larger in UUO kidneys compared with sham-operated kidneys ($p < 0.05$). Aliskiren treatment reduced the MT-stained area and TGF-β–stained area in UUO kidneys (A-c, B-c, $p < 0.05$). Aliskiren-treated TLR2-KO UUO kidneys showed a significant decrease in MT-stained and TGF-β–stained areas compared with aliskiren-treated UUO kidneys. (A-d, B-d, $p < 0.05$). Aliskiren treatment reduced the renal mRNA expressions of TGF-β in UUO kidneys. Aliskiren-treated TLR2-KO UUO kidneys showed a significant decrease in renal mRNA expression of TGF-β compared with aliskiren-treated UUO kidneys by RT-PCR (B-f, $p < 0.05$).

CONCLUSIONS

Although TLR2 inhibition did not attenuate renal inflammation, inhibition of RAS attenuates renal inflammation in TLR2 KO UUO kidneys. It is speculated that RAS may modulate renal TLR2 activation in experimental unilateral ureteral obstruction.

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G) Acute Renal Failure. G1) Experimental models.
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