Competing Actions of Type 1 Angiotensin Receptors on T Lymphocytes and Kidney Epithelium During Cisplatin-Induced Acute Kidney Injury

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Supplemental Methods

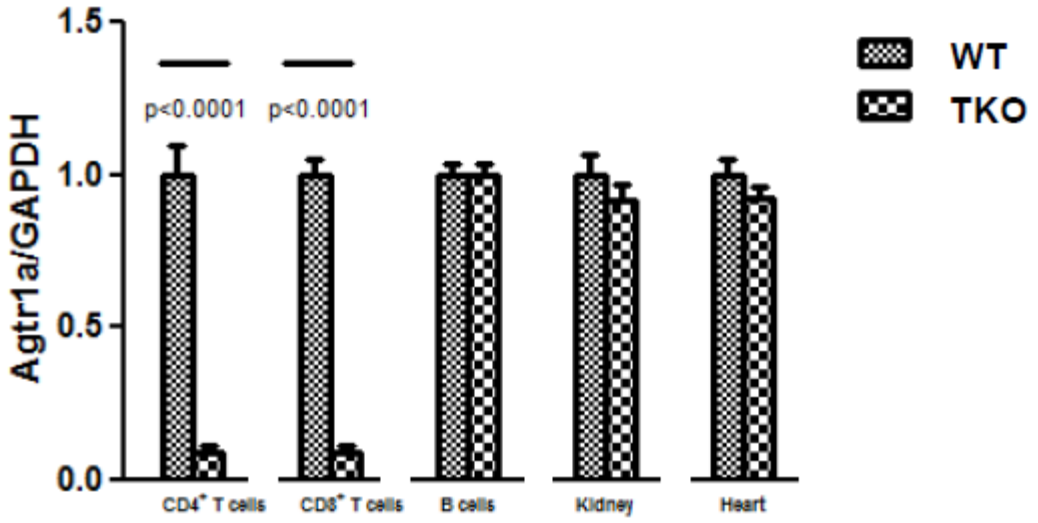
To delete the AT_{1A} receptor selectively from T lymphocytes we bred the *CD4-Cre* mouse line with an *Agtr1a flox* line harboring loxP sites on either side of the coding region for the AT_{1A} receptor. For our experiments, we employed female *CD4* Cre^{+} $Agtr1a^{flox/flox}$ mice (*TKO*) and *CD4* Cre^{-} $Agtr1a^{flox/flox}$ (*WT*) littermates. To confirm T cell-specific deletion of AT_{1A} receptors in our *TKO* animals, we harvested splenocytes and labeled them for the T lymphocyte markers CD4 and CD8 and the B lymphocyte marker CD19. Through fluorescent cell sorting, we isolated cell populations expressing 1 of these 3 markers: $CD4^{+}$ T cells ($CD4^{+}CD8^{-}CD19^{-}$), $CD8^{+}$ T cells ($CD8^{+}CD4^{-}CD19^{-}$), and B lymphocytes ($CD19^{+}CD4^{-}CD8^{-}$). RNA was subsequently harvested from these purified immune cell populations and from kidney and heart. We then performed real-time PCR for the *Agtr1a* gene to confirm the degree and precision of T cell-specific deletion. Compared with their *WT* littermates, *TKO* mice exhibited over 90% deletion of the *Agtr1a* gene from both $CD4^{+}$ and $CD8^{+}$ T cells, but preserved AT_{1A} receptor expression in all other tissues examined (Fig. S1).

Supplemental Figure Legends

Supplemental Figure 1. Verification of specific AT_{1A} receptor deletion in T lymphocytes from female TKO mice. Splenocytes were harvested from female *CD4 Cre*$^{-}$ *Agtr1a*$^{flox/flox}$ mice (WT) and *CD4-Cre*$^{+}$ *Agtr1a*$^{flox/flox}$ littermates (TKO), and sorted into 3 subpopulations (n=6 per group). Figure shows mRNA expression of *Agtr1a* in different tissues and cell subpopulations from WT and TKO groups.

Supplemental Figure 2. AT_1 receptor blockade equalizes AKI levels in cisplatin-treated WT and TNF KKO mice. (A-B) Kidney function in WT and TNF KKO ("Ksp TNF KO") mice treated with cisplatin plus losartan as measured by (A) BUN and (B) serum creatinine (n≥9).

Supplemental Figure 1



Supplemental Figure 2