INTRODUCTION

TGF-β is recognized as a critical mediator of tissue remodeling and fibrosis. Smad3 has been indentified as an important intermediate of the TGF-β signaling pathway. We have recently found that genetic deficiency of Smad3 protects against kidney injury in an acute ischemia-reperfusion model and in a renal artery stenosis model. Several studies have shown that Smad3 knockout (KO) mice are protected from myocardial damage in a reperfused myocardial infarction model. In an angiotensin-II-induced hypertension model, hypertensive cardiac inflammation and fibrosis were developed in Smad3 wild-type (WT) but not in Smad3 KO mice. The aim of this study was to determine whether cardiac fibrosis could be prevented in Smad3 KO mice subjected to the 2-kidney, 1-clipping (2K1C) model of renovascular hypertension.

METHODS

- The 2K1C RAS model was established by anesthetizing WT and Smad3 KO mice with ketamine and xylazine, exposing the right kidney through a small flank incision, isolating the renal artery and placing a 0.2 mm polytetrafluoroethylene cuff on it. Sham surgeries involved a flank incision, isolation of the renal artery without placing a cuff.
- Blood pressure was measured by the tail cuff method using the XBP1000 Non-invasive Blood Pressure System (Kent Scientific).
- Renin activity was assessed via angiotensin I production using the RIA Kit provided by DiaSorin.
- Mice were sacrificed six week after surgery.
- Extent of cardiac fibrosis was assessed by quantitative image analysis of trichrome and collagen III stained sections of myocardium.
- Statistical analysis was done using median ± SE, and the Mann-Whitney test. P value < 0.05 were considered significant.

RESULTS

- 40/42 (95%) of WT animals and 32/51 (63%) of KO animals became hypertensive after surgery as defined by >20% change in blood pressure. Median peak systolic blood pressure was higher in WT mice than KO mice (148 vs 120 mmHg, P < 0.0001). (Figure 1)
- In WT mice, renin activity was increased at 2 weeks (P < 0.05), but was not increased 6 weeks after surgery (P = 0.52). In KO mice, renin activity was increased at both 2 and 6 weeks after surgery (P < 0.05). Renin activity was greater in KO than WT mice (P < 0.01, Figure 2).
- In both genotypes, the cuffed mice had higher cardiac fibrosis than the control/sham mice, as determined by quantitative histologic analysis of trichrome (WT sham 0.4%, KO sham 0.9%, WT RAS 5.3%, KO RAS 6.5%; P < 0.05) (Figure 3) and collagen III stained sections (WT sham 0.6%, KO sham 0.9%, WT RAS 4.3%, KO RAS 4.9%; P < 0.05, Figure 4).
- Cardiac fibrosis did not differ between cuffed mice of both genotypes, as shown by quantitative histologic analysis of trichrome and collagen III stained sections (Figure 3, 4).

CONCLUSIONS

We conclude that an intact Smad3 signaling pathway is not necessary for the development of cardiac fibrosis in the 2K1C model of renovascular hypertension.

REFERENCES