ROLE OF MMP-2 DURING THE PROGRESSION OF RENAL INJURY IN DAHL SALT-SENSITIVE RATS

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ABSTRACT

Recently, our laboratory demonstrated that SS rats develop a form of diabetic nephropathy (DN) following induction of diabetes with streptozotocin (STZ) that is similar to patients with DN. The progression of renal injury in this model is associated with increased levels of matrix metalloproteinase-2 (MMP-2) in the renal cortex. In the current study, we used new *in vivo* technology *(Zinc finger nuclease)* to knockout the MMP-2 gene in the SS rat genetic background (MMP-2 ZN KO strain). Results from preliminary experiments indicate that the renal cortical protein expression and activity of MMP-2 and the progression of renal injury was significantly reduced in the MMP-2 ZN KO strain compared to SS rats when fed a HS diet without differences in BP. The aim of the present study was to determine the specific role of MMP-2 during the progression of diabetes-induced renal injury in the SS rat. Nine week-old SS rats were treated with either (1) vehicle or (2) STZ, 50 mg/kg (i.p.) to induce diabetes. A third group contained MMP-2 ZN KO rats treated with STZ. Rats were fed a low salt diet to minimize the development of hypertension. At 18 weeks of age, proteinuria (indicator of renal injury) increased to 276±39 mg/day in STZ-treated SS rats versus 103±13 mg/day in vehicle treated rats. Proteinuria was significantly reduced in the MMP-2 ZN KO strain (125±10 mg/day) compared to the values observed in STZ-treated SS rats. These data indicate that the progression of both hypertension and diabetes-induced renal injury in SS rats are related to an increase in MMP-2 activity and suggests that MMP inhibitors hold the potential to prevent the progression of diabetes-induced renal disease in the millions of patients suffering from chronic kidney disease.

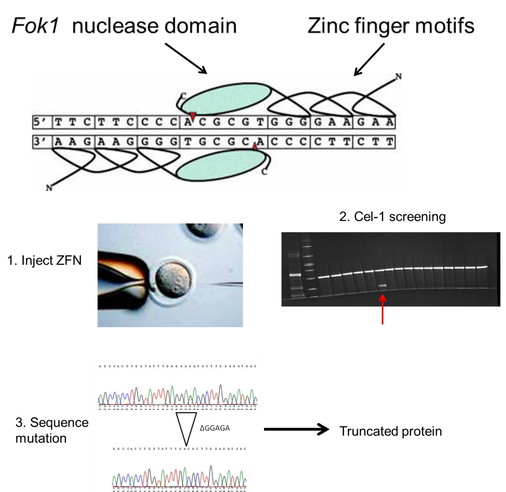
INTRODUCTION

- Hypertension and diabetes are the most common causes of chronic kidney failure and end-stage renal disease (ESRD) in the United States.
- Despite the magnitude of the problem, little is known about the pathogenesis of hypertension and/or diabetes-induced renal injury because the lack of an appropriate rodent model.
- The Dahl salt-sensitive (SS) rat is a genetic model of salt-sensitive of that rapidly develops progressive proteinuria and focal glomerulosclerosis leading to ESRD when challenged with a high salt (HS) diet.
- Preliminary studies from our laboratory have demonstrated that SS rats treated with STZ exhibit progressive proteinuria with renal abnormalities similar to patients with diabetic nephropathy.
- Interestingly, the expression of MMP-2 protein was significantly elevated in the renal cortex of SS rats fed a HS diet or treated with STZ compared to their control counterparts.

OBJECTIVE

The present study examined the specific role of MMP-2 during the progression of, both, hypertension and diabetes-induced renal injury in SS rats by using new *in vivo* technology *(Zinc finger nucleases)* to knockout the MMP-2 gene in the SS rat genetic background (MMP-2 ZN KO strain).

ZINC FINGER NUCLEASE TECHNOLOGY



RESULTS

KNOCK OUT OF MMP2 REDUCES HYPERTENSION-INDUCED RENAL INJURY IN SS RATS

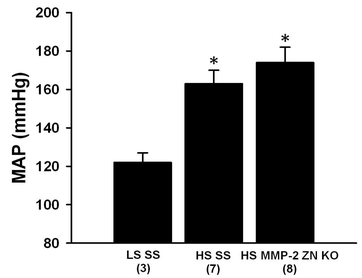


Figure 1. Tailcuff pressure in SS and MMP-2 ZN KO rats fed either a LS or HS diet for 28 days. MAP was significantly elevated in both SS and MMP-2 ZN KO rats fed a HS diet compared to SS rats maintained on a LS diet. Numbers in parentheses indicate the number of animals studied. * P<0.05 vs. the corresponding values in SS rats fed a LS diet.

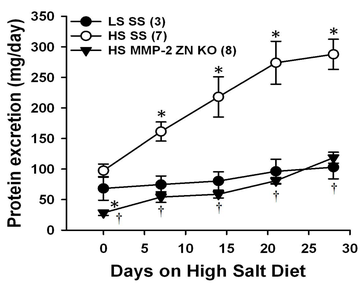


Figure 2. Time course of the development of proteinuria in SS and MMP-2 ZN KO rats fed either a LS or HS diet for 28 days. Protein excretion was 3-fold higher in SS rats fed a HS diet compared to the LS SS group. However, the development of proteinuria was markedly reduced in the MMP-2 ZN KO strain. * P<0.05 vs. the corresponding values in SS rats fed a LS diet. † P<0.05 vs. the corresponding values in SS rats fed a HS diet.

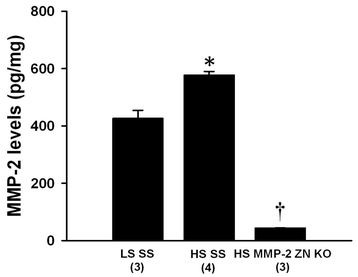


Figure 3. The measurement of MMP-2 protein levels in the renal cortex of SS and MMP-2 ZN KO rats fed either a LS or HS diet for 28 days. MMP-2 protein levels (measured by ELISA) were significantly increased in SS rats fed a HS salt diet versus SS rats maintained on a LS diet. There was a 13-fold reduction in MMP-2 protein levels in the MMP-2 ZN KO strain compared to SS placed on a HS diet for 28 days. * P<0.05 vs. the corresponding values in SS rats fed a LS diet. † P<0.05 vs. the corresponding values in SS rats fed a HS diet.

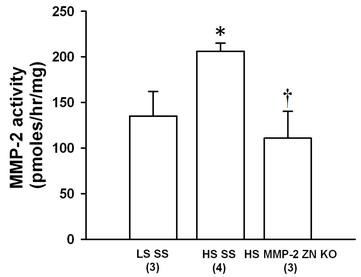


Figure 4. The measurement of MMP-2 activity (ELISA based fluorescent substrate) in the renal cortex of SS and MMP-2 ZN KO rats fed either a LS or HS diet for 28 days. There was a 40% increase in renal cortical MMP-2 activity in SS rats fed a HS diet compared to their LS counterparts. Knocking down the MMP-2 gene in SS rats (the MMP-2 ZN KO strain) reduced MMP-2 activity by 50%. * P<0.05 vs. the corresponding values in SS rats fed a LS diet. † P<0.05 vs. the corresponding values in SS rats fed a HS diet.

INDUCTION OF DIABETS PRODUCED RENAL INJURY INDEPENDENT OF HYPERTENSION IN SS RATS

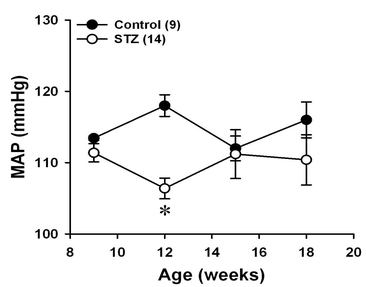


Figure 5. Comparison of the measurement of MAP in control and STZ-treated SS rats maintained on a LS diet. There were no differences in MAP between control and STZ-treated SS rats. * P<0.05 vs. the corresponding values in control SS rats.

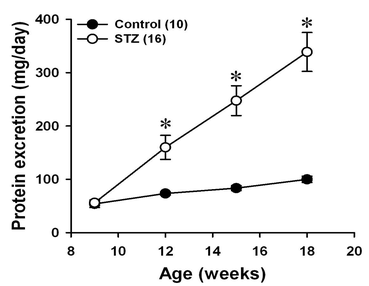


Figure 6. Time course of the development of proteinuria in control and STZ-treated SS rats maintained on a LS diet. The STZ-SS group developed progressive proteinuria when compared to the values observed in the control SS group. * P<0.05 vs. the corresponding values in control SS rats.

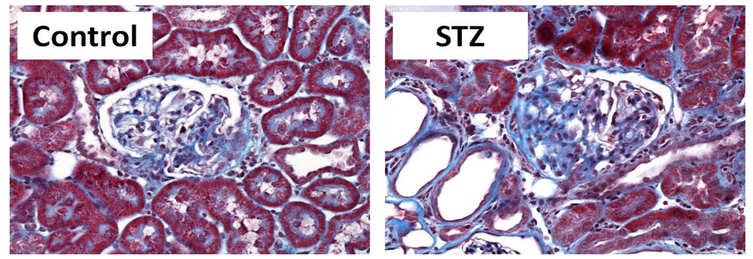


Figure 7. Comparison of renal histopathology in control and STZ-treated SS rats. STZ treated rats exhibited renal histological abnormalities typical of diabetes including mesangial expansion, glomeruloslcerosis, and interstitial fibrosis.

RENAL INJURY IN STZ-TREATED SS RATS IS ASSOCIATED WITH ELEVATED MMP2 PROTEIN LEVELS

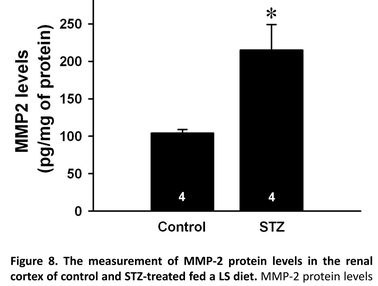


Figure 8. The measurement of MMP-2 protein levels in the renal cortex of control and STZ-treated fed a LS diet. MMP-2 protein levels (measured by ELISA) were greater than 50% increased in STZ-treated SS rats. * P<0.05 vs. the corresponding values in SS rats fed a LS diet.

KNOCK OUT OF MMP2 REDUCES THE DEVELOPMENT OF SEVERE PROTEINURIA IN STZ-TREATED SS RATS

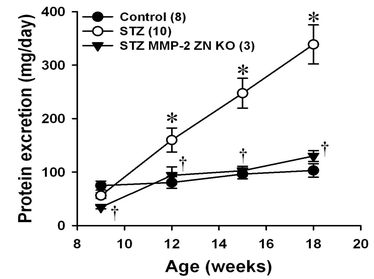


Figure 9. Comparison of the development of proteinuria in STZ-treated SS and MMP-2 ZN KO rats maintained on a LS diet. Protein excretion was 3-fold higher in STZ-treated SS rats versus control SS rats. However, the development of proteinuria was markedly reduced in the MMP-2 ZN KO strain. * P<0.05 vs. the corresponding values in control SS rats. † P<0.05 vs. the corresponding values in SS rats treated with STZ.

SUMMARY

- SS rats treated with either a HS diet or STZ developed severe proteinuria with renal histological abnormalities typical of patients with hypertension and diabetes-induced renal disease including mesangial expansion, glomerulosclerosis, and interstitial fibrosis.
- We observed that the progressive renal injury in SS rats treated with either a HS diet or STZ was associated with increases in the protein levels and activity of MMP-2 in the renal cortex compared to the values observed in their control counterparts.
- Knocking down the MMP-2 gene within the SS genetic background significantly reduced the severe development of, both, hypertension and diabetes-induced renal injury observed in SS rats.

CONCLUSION

These data indicate that the progression of both hypertension and diabetes-induced renal injury in SS rats are related to an increase in MMP-2 activity and suggests that MMP inhibitors hold the potential to prevent the progression of diabetes-induced renal disease in the millions of patients suffering from chronic kidney disease.

ACKNOWLEDGEMENTS

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