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 nucleases) to knock out the MMP-2 gene in the SS rat genetic background (MMP-2 ZN KD 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 KD 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, SS mg/kg (i.p.) to induce diabetes. A third group consisted MMP-2 ZN KD rats treated with STZ. Rats were fed a low salt diet to minimize the development of hypertension. At 18 weeks of age, proteinuria (indication of renal injury) increased to 27±6 mg/dl/day in STZ treated SS rats versus 12±1.3 mg/dl/day in vehicle treated rats. Proteinuria was significantly reduced in the MMP-2 ZN KD strain (13±1.10 mg/dl/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 is related to an increase in MMP-2 activity and suggests that MMP inhibition holds 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 of the lack of an appropriate rodent model. The Dahl salt-sensitive (SS) rat is a genetic model of salt-sensitive that rapidly develops 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 knock out the MMP-2 gene in the SS rat genetic background (MMP-2 ZN KD strain).

RESULTS

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

Figure 1. Tail cuff pressure in SS and MMP-2 ZN KD rats fed either a HS or HS diet for 28 days. MMP-2 was significantly reduced in both SS and MMP-2 ZN KD 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 LS rats fed a HS diet.

Figure 2. Time course of the development of proteinuria in SS and MMP-2 ZN KD fed either a HS or HS diet for 28 days. Proteinuria was 4-fold higher in SS fed a LS diet compared to the LS group. However, the development of proteinuria was markedly reduced in the MMP-2 ZN KD 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 by western blot analysis of MMP-2 in the SS rats fed either a HS or HS diet for 28 days. MMP-2 protein levels (measured by ELISA) were significantly increased in rats fed a LS diet versus SS rats maintained on a LS diet. There was a 10-fold reduction in MMP-2 protein levels in the MMP-2 ZN KD strain compared to the LS group fed a HS diet for 28 days. * P<0.05 vs. the corresponding values in LS rats fed a HS diet. # P<0.05 vs. the corresponding values in LS rats fed an LS diet.

Figure 4. The measurement of MMP-2 activity (z-scores) in the renal cortex by a fluorescent gelatin degradation assay. MMP-2 activity was 2-fold higher in SS fed a LS diet compared to their LS counterparts. Knocking down the MMP-2 gene in SS rats (the MMP-2 ZN KD strain) reduced MMP-2 activity by 50-0%. # P<0.05 vs. the corresponding values in SS rats fed a LS diet and 7 P<0.05 vs. the corresponding values in SS rats fed a HS diet.

Figure 5. Comparison of the measurement of MMP in control and STZ treated SS rats maintained on a LS diet. There were no differences in MMP 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 SS SB 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.

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

Figure 7. Comparison of the development of proteinuria in control and STZ treated SS rats maintained on an HS diet. Proteinuria was 4-fold higher in SS treated with a HS diet versus control SS rats. However, the development of proteinuria was markedly reduced in the MMP-2 ZN KD strain. ** 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 progression of 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 inhibition holds the potential to prevent the progression of diabetes-induced renal disease in the millions of patients suffering from chronic kidney disease.

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