Resistance to Tubulointerstitial Injury in a Mouse Remnant Kidney Model

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Abstract

Numerous animal models of CKD have been developed, but mice are relatively resistant to kidney injury. The remnant kidney model mimics progressive renal failure, and widely used in CKD research. The present study was performed to evaluate the effects of combined high-protein diet (HPD) loading and 5/6 nephrectomy (Nx) in a susceptible strain of mice (129/Sv). Mice underwent 5/6 Nx or sham surgery, then 2 weeks later were switched to an HPD, and cardiovascular parameters, kidney function, and renal histology were assessed after 4, 8, or 12 weeks. The 5/6 Nx group showed blood pressure elevation, cardiac hypertrophy, renal function decline, severe albuminuria, and glomerular hypertrophy. However, the glomerulosclerosis by 5/6 Nx was very mild and there was only modest tubulointerstitial inflammation and fibrosis in the 5/6 Nx group, even after 12 weeks of HPD loading. Furthermore, the sham group showed no histological changes. Thus, an HPD alone is insufficient to cause renal pathology, and a combination of 5/6 Nx and HPD loading induces mild renal pathology. Therefore, future studies of CKD using 5/6 Nx should be performed over a relatively long period of time in mice, or should be performed in other animals that are susceptible to kidney injury.

Introduction

The number of patients with chronic kidney disease (CKD) continues to increase worldwide. The progression of CKD culminates with end-stage kidney disease, which eventually necessitates renal replacement therapy[1–3]. Patients with CKD are at a high risk of mortality because of a high incidence of cardiovascular disease[4,5]. In 2017, there were 697.5 million cases of CKD worldwide and 1.2 million people died from CKD[6]. Therefore, the development of interventions that would prevent the progression of CKD represents a global challenge.

CKD is diagnosed based on a low glomerular filtration rate (GFR; < 60 mL/min/1.73m^2) and/or findings indicative of kidney injury, such as proteinuria, that has persisted for more than 3 months[7]. Common renal histopathological findings in CKD are glomerular sclerosis, tubulointerstitial inflammatory cell infiltration, tubulointerstitial fibrosis, and tubular atrophy[8,9]. Since patients with CKD are typically also hypertensive[10–12], hypertensive renal arteriolar damage is also often identified[13,14]. To elucidate the mechanisms involved in the pathogenesis of CKD and the effects of interventions, numerous animal models of CKD have been developed over the years[15].

The remnant kidney model, which is created by performing a 5/6 nephrectomy (Nx) to reduce the renal mass of rodents, mimics progressive renal failure. This model has been widely used in research into CKD because it is simple and recapitulates several features of human CKD[16]. Although mice are often used to create such experimental models, including the 5/6 Nx model, they are resistant to kidney injury[17–19]. Therefore, it is necessary to carefully evaluate the pathology of renal injury in murine experimental models[16]. Additionally, their susceptibility to renal injury is strain-dependent; for example, the 129/Sv strain is relatively susceptible to renal damage when compared with the C57BL/6 strain[20].

High-protein diet (HPD) loading results in the dilation of afferent arterioles and the constriction of efferent arteries, which increases intraglomerular pressure[21]. The glomerular hyperfiltration that is induced by HPD loading is similar to that induced by 5/6 Nx[22], which implies that HPD loading may accelerate the renal damage induced by 5/6 Nx. Long-term glomerular hyperfiltration leads to glomerulosclerosis and hypoxia in the tubulointerstitium, and consequent tubulointerstitial inflammation and fibrosis[23].

In the present study, we aimed to evaluate the renal histological injury caused to 129/Sv mice, which are relatively susceptible to kidney injury, by 5/6 Nx followed by HPD loading for 3 months. The results of the present study would provide important basic insights for researchers.

Results

Remnant kidney/HPD-fed mice demonstrate significantly lower body mass gain, higher blood pressure, and cardiac hypertrophy

The baseline body weight (BW) of the sham and 5/6 Nx 129/Sv mice were identical. After surgery, followed by HPD loading, the BW gain of the 5/6 Nx group was significantly less than that of the sham group (2-way repeated measures ANOVA, $F = 11.70$, $P < 0.01$) (Fig. 1a). Although the systolic blood pressure (BP) of the sham group was unaffected by 12 weeks of HPD loading consumption (baseline, 125.9 ± 2.8 mmHg; 12 weeks, 119.1 ± 6.5 mmHg; paired *t*-test, $P = 0.3655$), that of the 5/6 Nx group significantly increased (sham group *vs*. 5/6 Nx group, 2-way repeated measures ANOVA, $F = 49.21$, $P < 0.001$) (Fig. 1b). On the other hand, the heart rates of the two groups were identical during the experimental period (Fig. 1c). Because cardiac hypertrophy is closely associated with high BP, heart weight/body weight ratio was also examined. The heart weight/body weight ratio was significantly higher in the 5/6 Nx group than in the sham group after 4, 8, and 12 weeks of HPD loading (Fig. 1d). These findings indicate that HPD alone did not increase BP and that 5/6 Nx induced the development of hypertension in the 129/Sv mice.

Remnant kidney mice show significant impairments in renal function and higher urinary albumin excretion than sham-operated mice after HPD loading

We next assessed the renal function of the mice by measuring the plasma creatinine and blood urea nitrogen (BUN) concentrations. The plasma creatinine concentration of the 5/6 Nx group was significantly higher than that of the sham group after 4, 8, and 12 weeks of HPD loading (4 weeks: 5/6 Nx group 0.24 ± 0.01 mg/dL vs. sham group 0.13 ± 0.02 mg/dL, $P < 0.01$; 8 weeks: 5/6 Nx group 0.24 ± 0.02 mg/dL vs. sham group 0.15 ± 0.01 mg/dL, $P < 0.01$; 12 weeks: 5/6 Nx group 0.18 ± 0.01 mg/dL vs. sham group 0.11 ± 0.02 mg/dL, $P < 0.01$) (Fig. 2a). Similarly, the BUN of the 5/6 Nx group was significantly

higher than that of the sham group after 4, 8, and 12 weeks of HPD loading (4 weeks: 5/6 Nx group 63.7 ± 2.3 mg/dL vs. sham group 29.9 ± 0.8 mg/dL, $P < 0.001$; 8 weeks: 5/6 Nx group 55.7 ± 1.6 mg/dL vs. sham group 34.1 ± 4.9 mg/dL, $P < 0.01$; 12 weeks: 5/6 Nx group 46.0 ± 3.7 mg/dL vs. sham group 29.5 ± 2.3 mg/dL, $P < 0.05$) (Fig. 2b). Furthermore, urinary albumin excretion tended to be higher in the 5/6 Nx group than that in the sham group after 4 weeks of HPD loading, but the difference did not achieve statistical significance (5/6 Nx group 1,862 ± 767 μg/day vs. sham group 84 ± 16 μg/day, $P = 0.060$) (Fig. 2c). However, it was significantly higher in the 5/6 Nx group than in the sham group after 8 and 12 weeks of HPD-feeding (8 weeks: 5/6 Nx group 4,825 ± 1,715 μg/day vs. sham group 132 ± 18 μg/day, $P < 0.05$; 12 weeks: 5/6 Nx group 3,353 ± 795 μg/day vs. sham group 143 ± 29 μg/day, $P < 0.05$) (Fig. 2c). These results indicate that 5/6 Nx causes renal function decline and albuminuria in 129/Sv mice, alongside the development of hypertension.

Remnant kidney mice show modest glomerular sclerosis and glomerular hypertrophy after HPD loading

We next analyzed the histology of periodic acid-Schiff (PAS)-stained kidney sections (Fig. 3a–i) (Table 1). After 4 and 8 weeks of HPD loading, the 5/6 Nx group hardly exhibited glomerular sclerosis, but after 12 weeks, it showed modest glomerular sclerosis and mesangial expansion. In the tubulointerstitium, there was minimal inflammatory cellular infiltration in the 5/6 Nx group, even after 12 weeks of HPD loading, although urinary casts were observed. However, the sham group did not show glomerular sclerosis and tubulointerstitial inflammation, even after 12 weeks of HPD loading. These results indicate that HPD loading alone did not cause renal histopathology and that 5/6 Nx followed by 12 weeks of HPD loading provoked only minor glomerular injury and minimal tubulointerstitial injury, even in 129/Sv mice, which are relatively susceptible to renal damage.

Table 1
Results of the histological analysis of kidneys from the sham-operated and 5/6 nephrectomized mice after 4, 8, and 12 weeks of high-protein diet feeding

Histopathological findings						
Mouse Number	HPD loading (weeks)	Surgery	Mesangial expansion	Glomerulosclerosis	Urinary casts	Inflammatory cell infiltration in interstitium
22	4	Sham	-	-	-	-
23		Sham	-	-	-	-
27		Sham	-	-	-	-
28		Sham	-	-	-	-
18		5/6 Nx	-	-	-	-
19		5/6 Nx	-	-	-	-
20		5/6 Nx	-	-	-	-
21		5/6 Nx	-	-	±	-
15	8	Sham	-	-	-	-
16		Sham	-	-	-	-
17		Sham	-	-	-	-
25		Sham	-	-	-	-
26		Sham	-	-	-	-
11		5/6 Nx	-	-	-	-
12		5/6 Nx	±	-	±	-
13		5/6 Nx	+	±	++	+
14		5/6 Nx	-	-	-	-
2	12	Sham	-	-	-	-
4		Sham	-	-	-	-
7		Sham	-	-	-	-
9		Sham	-	-	-	-
1		5/6 Nx	±	-	+	-

5/6 Nx: 5/6 nephrectomy; HPD: high-protein diet; -: no abnormality, ±: minor abnormality, +: mild abnormality, ++: moderate abnormality, and +++: severe abnormality.

Histopathological findings					
3	5/6 Nx	±	±	+	-
5	5/6 Nx	±	±	+	-
6	5/6 Nx	-	-	±	-
8	5/6 Nx	+	+	+	±
10	5/6 Nx	±	±	+	-

5/6 Nx: 5/6 nephrectomy; HPD: high-protein diet; -: no abnormality, ±: minor abnormality, +: mild abnormality, ++: moderate abnormality, and +++: severe abnormality.

With respect to glomerular hypertrophy, after 4 weeks of HPD loading, the 5/6 Nx group exhibited a significantly larger glomerular area than the sham group (2,458 ± 93 μm^2 *vs.* 2,123 ± 38 μm^2, $P < 0.05$) (Fig. 3j), and the difference in size increased further with longer periods of consumption (8 weeks: 5/6 Nx group 2,577 ± 106 μm^2 *vs.* sham group 2,162 ± 64 μm^2, $P < 0.01$; 12 weeks: 5/6 Nx group 2,818 ± 126 μm^2 *vs.* sham group 2,305 ± 36 μm^2, $P < 0.05$) (Fig. 3a).

Remnant kidney/HPD mice show little tubulointerstitial fibrosis

We next assessed the extent of tubulointerstitial fibrosis by examining Masson's trichrome-stained kidney sections (Fig. 4a–i). There was little tubulointerstitial fibrosis in the 5/6 Nx group after either 4 or 8 weeks of HPD loading. After 12 weeks of HPD loading, there was modest tubulointerstitial fibrosis in the 5/6 Nx group, but the sham group showed almost no histological evidence of fibrosis.

Discussion

Mice are the most commonly used mammalian species for *in vivo* experiments[24]. Almost all murine genes share their functions with those of humans[25], but mice have a short generation time, in the order of 10 weeks from birth to parenthood[26]. Additionally, mice are small and require little space for breeding. Finally, they are genetically tractable and well characterized; therefore, they are frequently used for genetic modification studies. However, compared with other species such as rats, dogs, and cats, mice are relatively resistant to kidney damage. For example, the C57BL/6 mouse strain, which is often used in experiments, does not develop glomerular or tubulointerstitial injury and is resistant to hypertension when used to create a remnant kidney model[16].

Long-term dietary HPD loading causes renal glomerular hyperfiltration and sclerosis in rats[21]. The loading of an HPD to rats increases sodium-dependent amino acid reabsorption in their proximal tubules, resulting in tubuloglomerular feedback, which causes dilation of the afferent arterioles and constriction

of the efferent arterioles. This leads to higher intraglomerular pressure and excessive filtration, resulting in glomerulosclerosis[27]. Independently of this tubuloglomerular feedback mechanism, vascular endothelial growth factor (VEGF) is also involved in the pathogenesis of HPD-induced glomerular hyperfiltration[28,29]. Additionally, HPD also increases renal sterol-regulatory element binding protein 1 (SREBP-1) expression, which promotes renal lipogenesis and triglyceride accumulation in obese Zucker$^{fa/fa}$ rats[30]. Such renal triglyceride accumulation promotes renal interstitial fibrosis *via* the transforming growth factor-β (TGF-β) signaling pathway[31]. However, in the present study, 129/Sv mice fed an HPD alone for 12 weeks did not develop any renal histological lesions, such as glomerulosclerosis or tubulointerstitial fibrosis. Furthermore, the systolic BP of the 129/Sv mice was not affected by the consumption of HPD alone. Thus, even in the 129/Sv strain, which is susceptible to renal damage, HPD loading does not cause hypertension or renal histopathology to develop, and so the feeding of mice with an HPD is probably insufficient to cause kidney disease on its own.

Remnant kidney models are characterized by low GFR, and therefore they have been widely used in research into CKD. The mechanism of renal parenchymal injury in the remnant kidney model is thought to involve an increase in single-nephron GFR in the remaining glomeruli to compensate for nephron loss[32]; this increase in intraglomerular pressure leads to intraglomerular hypertension, and the resulting barotrauma induces glomerulosclerosis, proteinuria, and lower filtration. In Sprague-Dawley rats, 5/6 Nx results in albuminuria, glomerulomegaly, glomerulosclerosis, mild interstitial fibrosis, and tubular atrophy within 8 weeks, and more extensive glomerulosclerosis and interstitial fibrosis within 12 weeks[15]. In the present study, the 129/Sv mice underwent 5/6 Nx, followed by HPD loading for 12 weeks. The combination of HPD loading and 5/6 Nx significantly increased BP and albumin excretion, and caused cardiac hypertrophy. However, histological analysis showed that the glomerulosclerosis induced was very mild and there was only modest tubulointerstitial inflammation and fibrosis. A previous study showed that angiotensin II-infused C57BL/6 mice are resistant to kidney injury, possibly because of higher expression of renal cortical angiotensin type II receptor and anti-oxidative factors such as heme oxygenase-1[33]. In the present study, the factor responsible for the renal protection in the remnant kidney model remains to be identified. Therefore, further studies should aim to elucidate the molecular mechanisms of the renal protection in mice.

The present study had some limitations. We did not include a control group that was fed a normal diet and relatively few animals were studied. Nevertheless, the findings of the present study provide important information that mice are resistant to renal parenchymal damage induced by glomerular hyperfiltration via a combination of 5/6 Nx and HPD loading. Therefore, to determine the protective effects of drugs against the development of CKD *in vivo*, long-term studies of mouse 5/6 Nx models are required. Alternatively, a rat 5/6 Nx model may be more suitable because renal pathology develops more rapidly in this species. For the same reason, the genetic modification of rats, combined with 5/6 Nx, may facilitate the investigation of candidate genetic mediators of CKD, although the genetic modification of rats is more difficult than that of mice[34]. Therefore, researchers should consider the advantages and

disadvantages of using both mice and rats when designing studies of the mechanisms of, and potential therapies for, CKD.

Materials And Methods

Animals

All animal experiments were reviewed and approved by the Animal Studies Committee of Yokohama City University, which is in compliance with the ARRIVE guidelines. Efforts were made to minimize the number of animals used and ensure minimal sufferings. Male 129/Sv mice were purchased from Japan SLC (Shizuoka, Japan). They were housed in a controlled environment on a 12-hour light/dark cycle at a temperature of 25°C, and were allowed free access to a standard diet (0.3% NaCl, 3.6 kcal/g and 13.3% energy as fat; Oriental MF, Oriental Yeast Co, Ltd, Tokyo, Japan) and water.

5/6 nephrectomy and high-protein diet loading

Male 8–11-week-old mice were assigned to remnant kidney or sham-operated groups. For the remnant kidney group, a right subcapsular Nx was performed, followed by surgical resection of the upper and lower one-third of the left kidney under isoflurane anesthesia, as described previously[35]. Two weeks after the surgery, the mice were switched to an HPD (45% protein, 38% carbohydrate, 17% fat; Clea, Osaka, Japan) for up to 12 weeks.

BP measurement

Systolic BP was measured using the tail-cuff method (BP-Monitor MK-2000; Muromachi Kikai Co., Tokyo, Japan), as described previously[36,37] between 09:00 and 14:00. At least 10 measurements were performed in each mouse and the mean values were analyzed.

Biochemical analysis

Mice were anesthetized in the fed state, and blood samples were collected by cardiac puncture and centrifuged at 3,000 rpm (MR-150, Tomy Seiko Co., Ltd., Tokyo, Japan) at 4°C for 10 minutes to separate the plasma, which was stored at − 80°C until use. Plasma creatinine, BUN, urinary creatinine, and urinary albumin concentrations were measured using a Hitachi 7180 autoanalyzer (Hitachi, Tokyo, Japan).

Metabolic cage analysis

Urine samples were collected by housing mice in metabolic cages, as described previously[38,39]. The mice had free access to tap water and fed an HPD.

Histological analysis

Kidneys were fixed in 4% paraformaldehyde and embedded in paraffin. Four-μm-thick sections were stained with PAS or Masson's trichrome, as described previously[40,41]. Images of the sections were acquired using a BZ-X800 microscope (Keyence, Osaka, Japan).

Statistical analysis

Statistical analysis was performed using GraphPad Prism software (GraphPad Software, La Jolla, CA, USA). All quantitative data are expressed as means ± SEMs. Differences were analyzed using the unpaired Student's *t*-test. $P < 0.05$ was considered to represent statistical significance.

Declarations

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Author contributions:

S.T. and H.W. designed and conducted the study. S.T., H.W., and K.A. wrote the manuscript. S.T., H.W., K.A., S.T., T.Y., S.U., T.S., E.A., S.T., T.Y., T.Y., R.K., T.K., S.K., A.Y., and T.I. performed the experiments. S.T. and H.W. analyzed the data. K.T. supervised the study. All authors approved the final manuscript.

Institutional Review Board Statement:

This study was performed in accordance with the National Institutes of Health guidelines for the use of experimental animals. All the animal experiments were reviewed and approved by the Animal Studies Committee of Yokohama City University.

Informed Consent Statement:

Not applicable.

Data Availability Statement:

The data used to support the findings of this study are included within the article.

Conflicts of Interest:

The authors declare no conflict of interest.

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