Exaggerated Renal Pathology of Partial Ablation-Induced Chronic Renal Failure in eNOS Deficient Mice

Chika YAMASHITA, Naoko TAZAWA, Mamoru OHKITA, and Yasuo MATSUMURA*

Laboratory of Pathological and Molecular Pharmacology, Osaka University of Pharmaceutical Sciences; 4–20–1 Nakanoshima, Takatsuki, Osaka 569–1094, Japan.

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We investigated the role of endothelial nitric oxide synthase (eNOS) in the remnant kidney model of chronic renal failure, by using eNOS-deficient (eNOS−/−) and wild-type mice. There were significant increments of blood urea nitrogen level, plasma creatinine concentration and proteinuria in both wild-type and eNOS−/− mice at 8 weeks after 5/6 nephrectomy, but observed changes were more prominent in eNOS−/− mice. Only 7 out of 30 eNOS−/− mice were alive during 8-week experimental period, whereas survival rate in the wild-type mice was 69%. The glomerular size distribution indicated that the glomeruli of 5/6 nephrectomized eNOS−/− mice tended to be larger compared with cases of wild-type mice. It seems likely that eNOS-derived NO is protective against renal injuries in this disease model.

Key words chronic kidney disease; endothelial nitric oxide synthase; renal function; renal mass reduction

Chronic renal failure is characterized by progressive loss of nephrons caused by increased intraglomerular pressure and hyperfiltration. Vasoactive substances such as angiotensin II, endothelin-1, and nitric oxide (NO) are closely related to the pathogenesis of this progressive renal failure. Angiotensin I converting enzyme inhibitors or angiotensin II type 1 receptor antagonists are known to exhibit a renoprotective effect in patients with chronic renal failure and in subtotally nephrectomized rats, the most frequently employed animal model.1–4 Moreover, chronic treatment with endothelin ETα receptor antagonist to renal mass reduction,10) accumulation of an endogenous NOS inhibitor, asymmetric dimethylarginine, occurs and elevation of its plasma level is closely related to the severity of pathology. In hemodialysis patients with uremia, elevated plasma endothelin-1 levels have been reported, which correlated with the increase in blood pressure.7) On the other hand, there is accumulating evidence indicating that chronic NO inhibition with pharmacological blockade on NO synthase (NOS) leads to progressive hypertension and severe renal injury.8) In patients with chronic kidney disease9) and uremic rats with renal mass reduction,9) accumulation of an endogenous NOS inhibitor, asymmetric dimethylarginine, occurs and elevation of its plasma level is closely related to the severity of pathology. In contrast, oral supplementation of L-arginine, the substrate of NO, is effective in the normalization of renal dysfunction in the partial ablation-induced chronic renal failure rats.11,12) Thus, deficient NO production and/or decrease in its bioavailability play an important role in the pathogenesis of chronic renal failure, but in our knowledge, there is no available evidence evaluating the effect of genetic deficiency of NOS in the partial ablation-induced uremic animals. We now report here that the genetic deficiency of endothelial NOS (eNOS) aggravates the renal dysfunction and survival rate in mice exposed to renal mass reduction.

MATERIALS AND METHODS

Animals and Experimental Design Male C57bl/6J wild-type (n=20) and eNOS-deficient (eNOS−/−, n=36) mice (18—23 g, 8—10 weeks old, Jackson Laboratories, Bar Harbor, U.S.A.) were used. As described originally,13) eNOS−/− mice were indistinguishable in general appearance and histology, except that eNOS−/− mice had lower body weights than wild-type mice. Blood pressure tended to be increased (by approximately 20 mmHg) in eNOS−/− mice compared with wild-type mice (see Results). Animals were housed in a light-controlled room with a 12-h light/dark cycle, and were allowed ad libitum access to food and water. Experimental protocols and animal care methods in the experiments were approved by the Experimental Animal Committee at Osaka University of Pharmaceutical Sciences.

The wild-type and eNOS−/− mice were randomly separated into a sham-operated group (wild-type, n=7; eNOS−/−, n=6) and 5/6 nephrectomy group (wild-type, n=13; eNOS−/−, n=30). The remnant kidney model was induced by surgical renal reduction (5/6 nephrectomy) in two stages. The animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) for all surgical procedures. Initially, a left midflank incision was made and the left kidney was exteriorized. The renal vessel was temporarily occluded with a hemostatic clamp and both poles of the kidney (two-thirds of the functioning kidney mass) were excised with scissors. Bleeding was controlled with thrombin (Mitsubishi Pharma, Tokyo, Japan) administered onto the cut surface. The kidney stump was returned to the abdominal cavity and the incision was closed. Two weeks later for recovery, the remaining right kidney was removed through a right midflank incision after ligation of the right renal artery, vein, and ureter. As the non-ablated control, both stages of the sham operation with manipulation of the renal pedicles involved exteriorizing the kidney and subsequently replacing the intact kidney back into the abdominal cavity (sham-operated control).

After 5/6 nephrectomy, systolic blood pressure was monitored at every two weeks by tail-cuff and a pneumatic pulse transducer (BP-98A, Softron, Tokyo, Japan). Eight weeks after ablation, overnight urine samples were collected from individual survivor (wild-type, n=9; eNOS−/−, n=7) in metabolic cages, and then blood samples were obtained from the tail vein at the end of each urine collection period. These samples were used for measurements of renal functional pa-
observed in eNOS after the 5/6 nephrectomy, but more marked changes were observed in eNOS mice. The survival rate of wild-type mice exposed to renal mass reduction model. By contrast, only 7 out of 30 eNOS mice were alive at the end experimental period (23 mice gradually died during 8 weeks) (Fig. 1). All sham-operated control mice were alive throughout the experimental period.

Renal Histological Findings in eNOS−/− and Wild-Type Mice Using some survivors (n=3) of 5/6 nephrectomized eNOS−/− and wild-type mice, histopathologic examination was done. As shown in Fig. 2, the glomerular size distribution indicated that the glomeruli of 5/6 nephrectomized eNOS−/− mice tended to be larger compared with cases of wild-type mice.

DISCUSSION

The renal mass reduction model has been widely used as the classic model of progressive renal disease. Animals with renal mass reduction develop severe proteinuria and structural changes in the kidney, including glomerulosclerosis, which eventually lead to renal insufficiency. It has been reported that chronic inhibition of NOS activity by pharmacological blockade aggravates the renal damage in the remnant kidney model and that decreased NO production in this model is associated with increased glomerular pressure, which mediates progressive renal disease by initiating injury to the endothelium. On the other hand, administration of

RESULTS

Body Weights, Heart Weights, Systolic Blood Pressure (SBP) and Renal Functional Parameters Body weights, heart weights, SBP and renal functional parameters obtained from survivors were summarized in Table 1. Heart weights were significantly increased in both wild-type and eNOS−/− mice at 8 weeks after the 5/6 nephrectomy. On the other hand, SBP remained baseline level in each group throughout 8-week experimental periods, although the baseline level was higher in eNOS−/− mice. Blood urea nitrogen, plasma creatinine level and urinary excretion of protein were significantly increased in both wild-type and eNOS−/− mice at 8 weeks after the 5/6 nephrectomy, but more marked changes were observed in eNOS−/− mice.

Survival The survival rate of wild-type mice exposed to renal mass reduction at the end of 8-week experimental periods was 69% (4 out of 13 mice died within 4 weeks after 5/6 nephrectomy). By contrast, only 7 out of 30 eNOS−/− mice were alive at the end experimental period (23 mice gradually died during 8 weeks) (Fig. 1). All sham-operated control mice were alive throughout the experimental period.

Statistical Analysis Values were expressed as mean±S.E.M. For statistical analysis, we used one-way ANOVA combined with Bonferroni’s multiple range tests for multiple comparisons. Survival data were analyzed by Kaplan-Meier analysis. For all comparisons, differences were considered significant at p<0.05.

Table 1. Characteristics of Wild-Type and eNOS−/− Mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Wild-type (n=13)</th>
<th>eNOS−/− (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (baseline, g)</td>
<td>21.9±1.2</td>
<td>21.0±0.5</td>
</tr>
<tr>
<td>BW (endpoint, g)</td>
<td>27.9±0.5</td>
<td>24.0±0.8</td>
</tr>
<tr>
<td>HW/BW (mg/g BW)</td>
<td>4.01±0.05</td>
<td>4.30±0.17</td>
</tr>
<tr>
<td>SBP (baseline, mmHg)</td>
<td>106±3</td>
<td>122±2</td>
</tr>
<tr>
<td>SBP (endpoint, mmHg)</td>
<td>110±2</td>
<td>122±2</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>31.3±2.1</td>
<td>35.5±4.9</td>
</tr>
<tr>
<td>Pcr (mg/dl)</td>
<td>0.47±0.04</td>
<td>0.40±0.04</td>
</tr>
<tr>
<td>UproV (mg/24 h/kg BW)</td>
<td>95.24</td>
<td>111.20</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M. Sham, sham-operated control; RMR, renal mass reduction; BW, body weight; SBP, systolic blood pressure; BUN, blood urea nitrogen; Pcr, plasma creatinine level; UproV, urinary excretion of protein. *p<0.05, **p<0.01 vs. wild-type sham. †p<0.01 vs. wild-type RMR.

Fig. 1. The Survival Rate of Wild-Type and eNOS−/− Mice Exposed to Renal Mass Reduction (RMR) and of Sham-Operated Control Mice

The survival rate of eNOS−/− RMR mice was significantly lower than that of wild-type RMR mice (p<0.01, Kaplan-Meier analysis).

Fig. 2. Comparative Data on Glomerular Diameter of Wild-Type and eNOS−/− Mice with Renal Mass Reduction (RMR)

Each column represents mean value of 3 mice. The glomerular size distribution indicated that the glomeruli of eNOS−/− RMR mice tended to be larger compared with cases of wild-type RMR mice.
NO precursor l-arginine to these animals is known to improve the renal dysfunction, reduce proteinuria and preserve renal morphology.\(^1\)\(^2\)\(^3\)\(^4\) Moreover, there is a growing body of evidence to show that asymmetric dimethylarginine, an endogenous NOS inhibitor, is accumulated in patients with chronic kidney disease\(^5\)\(^6\)\(^7\) and uremic rats with renal mass reduction,\(^8\) and its elevation in plasma seems to be a cardiovascular risk factor in patients with end-stage renal disease.\(^9\) It has been indicated that reduced clearance of asymmetric dimethylarginine in the remnant kidney model of rats is associated with attenuated endothelium-dependent vasodilation, which can be reversed by l-arginine administration.\(^9\) Taken together, it seems likely that decreased NO production and its function, in particular in vascular endothelium, are closely related to the pathogenesis of renal mass reduction-induced renal injury. This led us to evaluate the renal mass reduction-induced renal injury in the eNOS\(^{-/-}\) mice. Results clearly indicated that the eNOS\(^{-/-}\) mice revealed higher progression of renal functional insufficiency and severe enlargement of glomeruli. In addition, survival rate after 5/6 nephrectomy was significantly lower in the eNOS\(^{-/-}\) mice compared with that of wild-type mice. Thus, our findings indicate that the deficiency of eNOS-mediated NO production in renal tissues aggravates the development of the ablation-induced progressive renal failure.

Previous studies have suggested several mechanisms underlying pharmacological NOS blockade-induced exacerbation of the ablation-induced progressive renal failure. Consistent findings using rat model are that the blockade of NO synthesis results in higher systemic and glomerular blood pressure, glomerular proteinuria, worse renal function, and more severe glomerulosclerosis.\(^9\) In addition, chronic inhibition of NO production in this rat model is associated with glomerular and peritubular capillary endothelial cell loss which impairs oxygen delivery to the tubules.\(^9\) These alterations seem to lead tubulointerstitial fibrosis as well as severe glomerulosclerosis, and results in microvascular injury in progressive renal disease.\(^2\)\(^0\)\(^2\)\(^1\)

In contrast to rat model, mice model (C57bl/6J) with renal mass reduction is known to be resistant to systemic blood pressure elevation and glomerulosclerosis.\(^2\)\(^2\)\(^2\)\(^3\) Our results also indicated that systemic blood pressure 8 weeks after the 5/6 nephrectomy remained the baseline level, in both wild-type and eNOS\(^{-/-}\) mice. However, baseline of systemic blood pressure was significantly elevated in eNOS\(^{-/-}\) mice, compared with that of wild-type mice, as reported previously.\(^3\) Heart weight to body weight ratio also tended to be increased in eNOS\(^{-/-}\) sham mice. These cardiovascular abnormalities may be at least partly involved in more severe renal injury and the higher mortality in 5/6 nephrectomized eNOS\(^{-/-}\) mice, although influences of blood pressure-independent factors cannot be ruled out.

Augmentation of renal injury and higher mortality observed in 5/6 nephrectomized eNOS\(^{-/-}\) mice may be related to the upregulation of some causal factors of renal mass reduction-induced renal disease. Endothelin-1 is one possible candidate involved in this augmented renal injury. The production of this peptide is enhanced in glomeruli obtained after the renal mass reduction,\(^2\)\(^4\)\(^2\)\(^5\) and is closely related to the pathogenesis of renal mass reduction-induced renal injury.\(^2\)\(^6\) On the other hand, NO is a negative modulator of endothelin-1 production in endothelial and tubular cells.\(^2\)\(^5\)\(^9\) In addition, NO is known to inhibit the mitogenesis and the proliferation of vascular smooth muscle cells.\(^2\)\(^6\) A supplementation of l-arginine, a NO precursor, can antagonize endothelin-1-induced mesangial cell proliferation\(^2\)\(^7\) and ameliorates the progression of renal disease in rats with subtotal nephrectomy.\(^2\) Thus, further studies are still required in order to clarify the downstream event occurring in the remnant kidney of eNOS\(^{-/-}\) mice.

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