Combination of Chronic Exercise and Antihypertensive Therapy Enhances Renoprotective Effects in Rats With Renal Ablation

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BACKGROUND
We assessed the renal protective effects of treatment with moderate exercise (EX), with EX plus olmesartan (OLS), with EX plus azelnidipine (AZN), and with the three together in a rat model of chronic renal failure (CRF).

METHODS
Male 5/6-nephrectomized Wistar Kyoto (WKY) rats were divided into six groups according to the following treatments for: (i) no EX (C); (ii) moderate EX with treadmill running (20 m/min for 60 min/day, 5 days/week) (EX); (iii) EX+OLS (10 mg/kg/day); (iv) EX+AZN (3 mg/kg/day); (v) EX+OLS (5 mg/kg/day)+AZN (1.5 mg/kg/day); and (vi) sham operation (S). The rats were then treated for 12 weeks.

RESULTS
EX, EX+OLS, EX+AZN, and EX+OLS+AZN showed decreases in the serum creatinine (Scr), an index of glomerular sclerosis (IGS), the relative interstitial volume of the renal cortex (RIV), the number of ED-1 (monoclonal antibody) positive cells (ED1 +) and the glomerular expression score of α-smooth muscle actin (α-SMA +). EX+OLS, EX+AZN, and EX+OLS+AZN blocked the development of hypertension, increased the number of Wilm’s tumor-1 (WT-1) positive cells (WT1 +); EX+OLS and EX+OLS+AZN blunted the increases in proteinuria. In particular, blood urea nitrogen (BUN), ED1 +, α-SMA +, WT1 +, IGS, and RIV in the EX+OLS+AZN were the lowest among all the nephrectomized groups.

CONCLUSIONS
In the results, simultaneous treatment of EX, OLS, and AZN showed renal protective effects in this rat model suggesting that the treatment may affect the macrophage infiltration to the glomerulus, the fibroblast accumulation in the glomerulus, the mesangial activation, and the podocyte differentiation.


Patients with chronic renal failure (CRF) have low exercise (EX) tolerance and experience progressively worsening disability.¹ Although advances in dialysis treatment have extended the lifespan of patients with CRF, this treatment alone does not ensure preservation of the quality of life. It has been suggested that appropriate EX may produce an improvement in the physical strength in patients with CRF.²⁻⁴ In addition, it has been reported that chronic EX improved the renal function and morphology in rats with renal ablation.⁵⁻⁶ However, immunohistochemical studies on the effect of EX in CRF have not been reported. An immunohistochemical study may clarify whether the kidneys are improved by EX.

Recent evidence suggests that control of the blood pressure and suppression of the renin–angiotensin system are important in CRF treatment. Antihypertensive therapy reduces the rate of decline of renal function in established progressive renal disease.⁷ Previously, we reported that treatment of EX combination enalapril (angiotensin-converting enzyme inhibitor) provided greater renoprotective effects than enalapril alone in Wistar Kyoto (WKY) rats with 5/6-nephrectomy (NX).⁶ The results of clinical trials and experimental studies suggest that angiotensin II (ANG II) type 1 receptor blockers (ARB) and calcium antagonists have comparable renal protective effects.⁸⁻⁹ Accordingly, such antihypertensive drugs in combination with EX may be beneficial for patients with CRF.

Therefore, in the present study, we assessed the renal protective effects of moderate EX in a rat model of CRF. We also assessed the effect of treatment with EX plus olmesartan (OLS), with EX plus azelnidipine (AZN), and with the three together, on glomerular lesions by immunohistochemistry.

METHODS
Animals. Six-week-old male WKY rats (Charles River Japan, Yokohama, Japan) were subjected to 5/6-NX⁶ by removal of the left kidney and two-third infarction of the right kidney under ether anesthesia. The rats were housed in a metabolic cage (model ST; Sugiyamagen, Tokyo, Japan) designed to prevent feces-urine contact and kept in a humidity and temperature-controlled room (55 ± 10% and 22 ± 2°C) with a 12-h light/dark cycle. The rats...
were fed a regular diet (0.18 wt% sodium, 18.3 wt% protein; Nusan Corp., Yokohama, Japan) and had free access to tap water.

When the rats were 9-weeks old, baseline measurements of the body weight, systolic blood pressure (SBP), urine volume and 24-h urinary excretion of protein (UP) were made. A treadmill test was performed for the measurements of EX intensity, and the oxygen consumption (VO2) when rats were running at a speed of 20 m/min, 0 grade incline, and the peak VO2 were measured using an O2/CO2 metabolism measuring system (model MK-5000, MK-680AT/02R, Murumachikikai, Kyoto, Japan). The rats were then randomly assigned to six groups according to the following treatment regimens: (i) NX without EX (C, n = 7); (ii) NX with treadmill running (KN-73, Natsume Industries, Tokyo, Japan) at a speed of 20 m/min for 60 min/day, 5 days/week (EX, n = 6); (iii) NX with EX and receiving OLS (10 mg/kg/day) (EX+OLS, n = 5); (iv) NX with EX and receiving AZN (3 mg/kg/day) (EX+AZN, n = 9); (v) NX with EX and receiving both OLS (5 mg/kg/day) and AZN (1.5 mg/kg/day) (EX+OLS+AZN, n = 8); (vi) sham operation (S, n = 9). The rats were then treated for 12 weeks. OLS and AZN were mixed with 0.5% carboxymethyl cellulose before administration and were administered once daily (9 AM) by gavage.

The SBP was monitored in conscious rats by the tail-cuff method (UR5000, Elquest, Ciba, Japan).11 The urine volume was measured gravimetrically, and urine was collected and stored at −80°C.

Later 12 weeks the initiation of treatment, the rats were killed by decapitation and trunk blood was collected in polyethylene tubes for the determination of serum creatinine (Scr) and blood urea nitrogen (BUN). The UP, Scr, and BUN were measured by a standard autoanalysis technique (Synchron-CX-3, Beckman Coulter, Fullerton, CA).

Renal histology. Portions of the remnant kidneys (removed when the rats were killed) were fixed in 10% neutral buffered formalin, and the index of glomerular sclerosis (IGS) and the relative interstitial volume of the renal cortex (RIV) were assessed by methods previously described.6

Immunohistochemistry of the kidney. Other portions of the remnant kidneys were fixed in 95% cold ethanol and then embedded in paraffin blocks. The primary antibodies used were mouse antimonoclonal antibody ED-1 (Serotec, Oxford, UK), mouse anti α-smooth muscle actin (α-SMA) (1A4; Dako, Glostrup, Denmark), and rabbit anti-Wilms’ tumor-1 (WT-1) (C19; Santa Cruz Biotechnology, Santa Cruz, CA) antibodies. The technique for immunohistochemistry was previously described.12 The number of ED-1-positive cells (ED1+), the number of WT-1-positive cells (WT1+) and the glomerular expression score of α-SMA (α-SMA+) were assessed by methods previously described.12

Statistical analysis. Values are expressed as the means ± s.e.m. With respect to peak VO2, comparison between the NX group and S group was made using the unpaired t-test. For Scr, BUN, RIV, ED1+, and WT1+ were compared between the different groups of rats using one-way analysis of variance (ANOVA) and Bonferroni/Dunn test. For SBP, UP, and body weight comparisons between the different groups of rats were made by ANOVA with repeated measures over the duration of the study. Statistically significant differences on each day were assessed between groups by Bonferroni/Dunn test. For IGS and α-SMA+ comparisons between the different groups of rats were made using Kruskal–Wallis test and Bonferroni/Dunn test. Values of <0.05 were considered statistically significant. The statistical analysis in the present study was performed using the statistical software package Statview version 5.0 (Abacus Concepts, Berkeley, CA).

This study conformed to the principles for the use of live animals outlined in the Declaration of Helsinki and those of the ethics committee of Tohoku University Graduate School of Medicine.

RESULTS
Peak VO2 in the NX group was significantly decreased (P < 0.001) compared with that in the S group. The VO2 when NX rats were running at a speed of 20 m/min corresponded to about 80% of the peak VO2.

The SBP in the C group progressively increased to 190 ± 6 mm Hg during the 12-week experimental period and was significantly higher (P < 0.0001, repeated measures ANOVA) than that in the S group (Figure 1). The SBP in the EX+OLS, EX+AZN, and EX+OLS+AZN groups was significantly decreased (P < 0.0001, P < 0.01, and P < 0.0001, respectively, repeated measures ANOVA) compared with that in the C group. The SBP in the EX+OLS+AZN group was significantly decreased compared with that in the EX, EX+OLS, EX+AZN groups (P < 0.0001, P < 0.05, P < 0.01, respectively, repeated measures ANOVA).

**Figure 1** | Sequential systolic blood pressure (SBP) values in the following groups (classified according to treatment): no exercise (C); sham operation (S); moderate exercise with treadmill running (20 m/min for 60 min/day, 5 days/week) (EX); EX+OLS (10 mg/kg/day); EX+AZN (3 mg/kg/day); EX+OLS+AZN (5 mg/kg/day)+AZN (1.5 mg/kg/day) groups, during the 12-week experimental period. Values are expressed as the means ± s.e.m. filled triangles, C (n = 7); open circles, S (n = 9); open squares, EX (n = 6); open triangles, EX+OLS (n = 5); filled circles, EX+AZN (n = 9); filled squares, EX+OLS+AZN (n = 8). **P < 0.05,** **P < 0.01,** **P < 0.0001** vs. C group; **P < 0.05,** **P < 0.01,** **P < 0.001** vs. EX group; **P < 0.05** vs. EX+OLS group; **P < 0.05** vs. EX+AZN group.
The UP in the C group progressively increased and was significantly higher ($P < 0.01$, repeated measures ANOVA) than that in the S group (Figure 2). The UP in the EX+OLS and EX+OLS+AZN groups was significantly decreased ($P < 0.05$, $P < 0.01$, respectively, repeated measures ANOVA) compared with that in the C group. Furthermore, UP in the EX+OLS+AZN group was significantly lower ($P < 0.05$, repeated measures ANOVA) than that in the EX+AZN group.

The values of Scr and BUN in the C group were significantly higher ($P < 0.0001$) than those in the S group (Table 1). The Scr in the EX, EX+OLS, EX+AZN, and EX+OLS+AZN groups were significantly lower compared with that in the C group ($P < 0.05$, $P < 0.05$, $P < 0.01$, and $P < 0.0001$, respectively). Furthermore, Scr in the EX+OLS+AZN group was significantly lower than that in the EX+AZN group ($P < 0.05$). The BUN in the EX+OLS+AZN group was significantly lower ($P < 0.01$) than that in the C group.

The body weight in the C group was significantly lower ($P < 0.0001$, repeated measures ANOVA) than that in the S group (Table 1). Body weight in the EX+OLS+AZN group was significantly heavier ($P < 0.05$, $P < 0.01$, respectively, repeated measures ANOVA) compared with that in the C and EX groups, and was significantly lower ($P < 0.01$) than that in the S group.

Renal histology
The C group demonstrated focal and segmental glomerular structural lesions and increased cortical interstitial volume. Such lesions were milder in the EX, EX+OLS, EX+AZN, and EX+OLS+AZN groups than those in the C group (Figure 3).

The IGS and RIV in the C group were significantly higher ($P < 0.0001$) than those in the S group (Table 1). The IGS and RIV in the EX, EX+OLS, EX+AZN, and EX+OLS+AZN groups were significantly lower than those in the C group (IGS: $P < 0.0001$; RIV: $P < 0.01$), and were significantly higher than those in the S group (IGS: $P < 0.0001$; RIV: $P < 0.001$, $P < 0.01$, $P < 0.01$, and $P < 0.05$, respectively). The IGS in the EX+OLS+AZN group was significantly lower than that in the EX ($P < 0.01$) and EX+AZN ($P < 0.05$) groups; the RIV in the EX+OLS+AZN group was significantly lower than that in the EX+AZN group ($P < 0.01$).

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Table 1 | Values of serum creatinine (Scr), blood urea nitrogen (BUN), body weight (BW), index of glomerular sclerosis (IGS), relative interstitial volume of the renal cortex (RIV), the number of ED-1-positive cells (ED1+), the glomerular expression score of α-SMA (α-SMA+) and the number of WT-1-positive cells (WT1+) at 12 weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>S</th>
<th>C</th>
<th>EX</th>
<th>EX+OLS</th>
<th>EX+AZN</th>
<th>EX+OLS+AZN</th>
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<tbody>
<tr>
<td>Scr (mg/dl)</td>
<td>0.34 ± 0.02†</td>
<td>1.39 ± 0.15D</td>
<td>1.00 ± 0.06c</td>
<td>1.00 ± 0.16c</td>
<td>1.00 ± 0.04**D</td>
<td>0.65 ± 0.05f§</td>
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<tr>
<td>BUN (mg/dl)</td>
<td>16.9 ± 0.4†</td>
<td>57.3 ± 8.4D</td>
<td>52.8 ± 1.9c</td>
<td>53.0 ± 8.2B</td>
<td>54.0 ± 3.6c</td>
<td>34.8 ± 3.4**s</td>
</tr>
<tr>
<td>BW (g)</td>
<td>376 ± 5†</td>
<td>312 ± 8D</td>
<td>312 ± 12D</td>
<td>327 ± 12c</td>
<td>319 ± 9D</td>
<td>340 ± 8**Ba</td>
</tr>
<tr>
<td>IGS</td>
<td>0.13 ± 0.2†</td>
<td>2.62 ± 0.07D</td>
<td>1.51 ± 0.04**D</td>
<td>1.30 ± 0.08**D</td>
<td>1.40 ± 0.07**D</td>
<td>1.11 ± 0.06**D§</td>
</tr>
<tr>
<td>RIV (%)</td>
<td>2.70 ± 0.2†</td>
<td>21.0 ± 0.8D</td>
<td>13.6 ± 0.5**sc</td>
<td>13.7 ± 1.9**sc</td>
<td>15.4 ± 0.7**B§</td>
<td>8.6 ± 0.1**AI</td>
</tr>
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<td>α-SMA+</td>
<td>0.87 ± 0.3†</td>
<td>2.38 ± 0.07D</td>
<td>1.86 ± 0.11**D</td>
<td>1.85 ± 0.07**D</td>
<td>1.61 ± 0.03**D†</td>
<td>1.47 ± 0.05**D§</td>
</tr>
<tr>
<td>ED1+</td>
<td>1.59 ± 0.2†</td>
<td>8.84 ± 0.67D</td>
<td>5.71 ± 0.44**D</td>
<td>7.00 ± 0.62**D</td>
<td>5.67 ± 0.48**D</td>
<td>4.60 ± 0.43**D§</td>
</tr>
<tr>
<td>WT1+</td>
<td>12.5 ± 0.44†</td>
<td>7.1 ± 0.35D</td>
<td>8.40 ± 0.37D</td>
<td>9.5 ± 0.47**D</td>
<td>10.6 ± 0.46Ba</td>
<td>10.3 ± 0.51**Ca</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, †P < 0.0001 vs. C group, ‡P < 0.01, §P < 0.001 vs. EX group, ¶P < 0.01, §P < 0.001 vs. EX+OLS group, †P < 0.05, ‡P < 0.01 vs. EX+AZN group, §P < 0.05, ¶P < 0.01, †P < 0.001, ‡P < 0.001 vs. S group. C (n = 7), S (n = 9), EX (n = 6), EX+OLS (n = 5), EX+AZN (n = 9), EX+OLS+AZN (n = 8).
Immunohistochemistry of the kidney

α-SMA+ and ED1+ in the C group were significantly higher ($P < 0.0001$) than those in the S group (Table 1 and Figure 4). α-SMA+ and ED1+ in the EX, EX+OLS, EX+AZN, and EX+OLS+AZN groups were significantly lower than those in the C group (α-SMA+: $P < 0.0001$; ED1+: $P < 0.0001$, $P < 0.05$, $P < 0.0001$, $P < 0.0001$, respectively) and were significantly higher than those in the S group ($P < 0.0001$). α-SMA+ in the EX+AZN and EX+OLS+AZN groups was significantly lower than that in the EX and EX+OLS groups ($P < 0.01$ EX+AZN vs. EX; $P < 0.01$ EX+OLS+AZN vs. EX; $P < 0.0001$ EX+OLS+AZN vs. EX+OLS). ED1+ in the EX+OLS+AZN group was significantly lower than that in the EX+OLS ($P < 0.01$).

WT1+ in the C group was significantly lower ($P < 0.0001$) than that in the S group (Table 1 and Figure 4). WT1+ in the EX+OLS, EX+AZN, and EX+OLS+AZN groups was significantly higher than that in the C group ($P < 0.01$, $P < 0.0001$, and $P < 0.0001$, respectively) and significantly lower than that in the S group ($P < 0.0001$, $P < 0.01$, and $P < 0.001$, respectively). Furthermore, WT1+ in the EX+AZN and EX+OLS+AZN groups was significantly higher ($P < 0.01$) than that in the EX group.

**DISCUSSION**

In the present study, we observed macrophage accumulation, podocyte damage, myofibroblast infiltration, focal and segmental glomerular sclerosis and an increased cortical interstitial volume associated with a progressive increase in proteinuria in nephrectomized WKY rats; these findings are consistent with the development of hypertension. EX, EX+OLS, EX+AZN, and EX+OLS+AZN showed decreased Scr, IGS, RIV, ED1+, and α-SMA+. EX+OLS, EX+AZN, and EX+OLS+AZN blocked the development of hypertension and increased WT1+; EX+OLS and EX+OLS+AZN blunted the increases in proteinuria. In particular, BUN, ED1+, α-SMA+, WT1+, IGS, and RIV in the EX+OLS+AZN group were the lowest among all nephrectomized groups. Moreover, SBP, UP, Scr, and BUN in the EX+OLS+AZN group were not significantly different compared with the S group. These results suggest that EX, EX+OLS, EX+AZN, and EX+OLS+AZN all have renal protective effects, and EX+OLS+AZN has greater renal protective effects compared with the other treatments.

The biochemical events associated with glomerular and tubular cell activation in response to protein stress include upregulation of the genes encoding vasoactive and inflammatory substances and synthesis of the corresponding protein products, such as endothelin-1, monocyte chemoattractant protein-1, and regulation upon activation of normal T-cell expression and secretion. Such events cause the accumulation of macrophages in the glomerulus giving rise to the inflammatory reaction. Renal function then progressively deteriorates. ED1+ reflects the number of macrophages that have accumulated in the glomerulus by the accelerated inflammatory reaction induced by glomerular injury. A decrease of ED1+ would suggest there was a protective effect against the infiltration of macrophages. In the present study, EX, EX+OLS, EX+AZN, and EX+OLS+AZN all reduced ED1+ suggesting that these treatments may have blunted the accumulation of macrophages, thereby reducing the inflammatory reaction. The ED1+ in the EX+OLS+AZN group was the lowest among these treatment groups suggesting that EX+OLS+AZN was the most effective treatment.

The loss of podocytes could be pathogenically important in leading to proteinuria and glomerular scarring. Podocyte injury is closely associated with glomerulosclerosis in many human and animal kidney diseases. Our method of counting podocytes relied on accurately identifying podocyte nuclei (WT-1). A decrease of WT1+ suggests that podocytes have been damaged and are progressively being lost. In the present study, EX+OLS, EX+AZN, and EX+OLS+AZN all increased WT1+, but EX monotherapy did not. Moreover,
the WT1+ in the EX+OLS+AZN group was the highest among these treatments groups. This suggests that EX+OLS, EX+AZN and EX+OLS+AZN may prevent podocyte loss more than EX alone.

In addition, with increasing proteinuria, mesangial cells and parietal epithelial cells and, later, tubular cells became damaged. Then, glomerular cells show the proliferation of such cells and myofibroblast infiltration. This occurs early in glomerular sclerosis. A relationship between an early differentiation process of α-SMA and the late development of chronic dysfunction has been recently suggested. In the present study, treatment groups all showed reduced α-SMA+. Moreover, α-SMA+ in the EX+AZN, EX+OLS+AZN groups was significantly lower than in the EX and EX+OLS groups suggesting that both EX+AZN and EX+OLA+AZN may cause a greater reduction in myofibroblast infiltration to the glomerulus than the other treatments.

It has not yet been elucidated whether EX causes the development and progression of nephropathy. In the present study, EX reduced Scr, ED1+, α-SMA+, and improved RIV and IGS. On the other hand, there have been few reports regarding the influence of EX on the progression of renal disease, and the results have been controversial. Osato et al. reported that a decrease in Scr, an increase in glomerular filtration rate, and an alleviation of glomerulosclerosis resulted from 2 h/day swimming for 20 weeks in Lewis rats. Heifets et al. reported an increase in glomerular filtration rate, a decrease in UP, and an alleviation of glomerulosclerosis compared with the values in sedentary rats brought about by swimming for 2 h/day for 2 months in rats with 3/4-NX. In contrast, Cornacoff et al. reported that, in an acute serum nephritis rabbit model, EX by 45 to 60 min treadmill running for 4 weeks increased BUN and UP. Bergamaschi et al. reported that treadmill EX for 30 min for 60 days in Munich-Wistar rats with 5/6-NX did not cause any significant change in glomerular filtration rate, UP, and the IGS. The reason for the discrepant effects of EX in the present study may be differences in the animal models used as well as the variety, intensity, and duration of EX.

The mechanism by which EX protected the remnant kidney in the present study has not been fully elucidated. Although we did not assess the intraglomerular hypertension and hyperfiltration in the present experiments, if the glomerular capillary pressure is reduced by EX, renal protective effects may appear. It has been reported that the levels of kinin and nitric oxide (NO) in blood were increased after EX. NO has been proposed to play a significant role in the regulation of oxygen consumption by both skeletal and cardiac muscle, but there are no reports on whether NO also has such a role in the kidney. It has been postulated that the increases of kinin and NO may dilate renal efferent arterioles to improve the glomerular hypertension by EX. Moreover, Bergamaschi et al. reported the dilation of renal efferent arterioles and an improvement of glomerular hypertension by EX in Munich-Wistar rats with CRF. Provided that these findings are also true for WKY rats with CRF, it is conceivable that EX exerts a protective effect on kidneys by improving the glomerular hypertension.

Until the present study, there have been no reports about the effect of treatment with EX plus ARB or with EX plus a calcium antagonist in CRF. In the present study, EX+OLS and EX+AZN blocked the development of hypertension and reduced WT1+ compared with EX alone. EX+OLS blunted the increases in proteinuria. These results indicate that EX in combination with either OLS or AZN has greater renal protective effects compared with EX monotherapy.

ARB dilates the efferent arterioles, leading to a reduction in the intraglomerular pressure. ARB reduces hyperfiltration damage in remnant kidney nephrons in CRF, and has beneficial effects in reducing UP and preserving the renal function. ARB prevents the binding of ANG II to ANG II type 1 receptor and increases the levels of plasma renin and ANG II. Increased ANG II may potentially induce signaling through the ANG II type 2 receptor. Recent studies have suggested that ANG II type 2 receptor–mediated signaling induces the inhibition of cell growth or apoptosis by counteracting the ANG II type 1 receptor signal. Platelet-derived growth factor-BB chain and transforming growth factor-β1 are known to play critical roles in promoting mesangial cell proliferation and extracellular matrix accumulation, respectively. Blockade of these growth factors prevents glomerular injury and this occurs through the ANG II type 2 receptor–NO pathway. EX and ARB may have comparable renal protective effects, with additional benefits from combination therapy. The mechanisms underlying the renoprotective effects of EX+OLS may be the reduction in the intraglomerular capillary pressure resulting from inhibition of the compensatory glomerular enlargement and the mesangial activation.

The mechanism underlying the renoprotective effects of EX+AZN might in part be the AZN induced reduction of the intraglomerular capillary pressure due to the efficient reduction in the systemic blood pressure. In addition to preglomerular vasodilatation, calcium antagonists have many other actions in the kidney, including effects on ANGII-induced glomerular contraction, ANGII-mediated stimulation of mesangial cell growth, ANGII-induced anti-innatiuresis, and renal tubule handling of sodium and water. However, the precise mechanisms of the renoprotective effects of calcium antagonists remain to be elucidated.

In this study, we did not examine the effect of OLS and AZN monotherapy on the progression of renal impairment. The renoprotective effects of OLS and AZN have already been reported. The treatment with OLS (10 mg/kg/day) for 8 weeks decreased SBP and glomerular sclerosis in WKY and spontaneous hypertensive rats with renal ablation. Kanazawa et al. reported that treatment with AZN (3 mg/kg/day) for 12 weeks in spontaneous hypertensive rats with 5/6-NX, decreased SBP and the values of Scr, BUN, IGS, RIV, and UP. Moreover, the treatmen with AZN protected kidneys in experimental studies and clinical trials. Acute EX may cause proteinuria and may decrease the renal blood flow and glomerular filtration rate. However, EX showed renal protective effects in the present study. Moreover, OLS or AZN in combination with EX did not aggravate the
renal function, and deceased SBP and the values of BUN, IGS, RIV and UP. Immunohistochemical study clarified that these combination treatments decreased macrophage accumulation, podocyte damage and myofibroblast infiltration in the glomerulus. These results suggest that antihypertensive drugs in combination with EX may be beneficial for CRF.

Even when the dosages of OLS and AZN were reduced to half, EX+OLS+AZN had greater renal protective effects compared with either EX+OLS or EX+AZN. The mechanism underlying the renoprotective effects of EX+OLS+AZN may be that OLS dilated the efferent arterioles and AZN dilated the afferent arterioles, resulting in a more effective reduction of the glomerular capillary pressure than EX+OLS or EX+AZN.

Acknowledgment: We are grateful to Sankyo, Tokyo, Japan for supplying olmesartan and azelnidipine.

Disclosure: The authors declared no conflict of interest.