Apoptosis and Glomerular Injury After Prolonged Nitric Oxide Synthase Inhibition in Spontaneously Hypertensive Rats

Hidehiko Ono, Yuko Ono, Atsuko Takanohashi, Hiroaki Matsuoka, Edward D. Frohlich

Abstract—This study was designed to investigate the relationship between apoptosis and glomerular injury in spontaneously hypertensive rats (SHR) with hypertensive disease that was exacerbated by inhibition of NO synthesis. Development of glomerular cell apoptosis was evaluated by assessment of renal hemodynamics, glomerular morphometric changes, and participation of the renin-angiotensin system. Three groups of 20-week-old SHR were investigated: control male SHR and 2 similar groups given 2 doses of $N^\text{G}$-nitro-$L$-arginine methyl ester (L-NAME, 50 or 80 mg/L, respectively) for 3 weeks. Mean arterial pressure and renal vascular resistance increased, whereas effective renal plasma flow and glomerular filtration rate were diminished by L-NAME. The small artery wall:lumen ratio increased as the glomerular-tuft area diminished. Renal cortical tissue levels of angiotensin II increased in response to the L-NAME, thereby inducing afferent arteriolar injury. Apoptosis and proliferative index (PCNA) of nonsclerotic glomeruli were induced by the low-dose L-NAME as the glomerular cell number decreased. In contrast, the PCNA index was downregulated with the high-dose L-NAME. These results indicate that angiotensin II activation, induced by L-NAME, was related to glomerular cell deletion and apoptosis together with the pathophysiological changes of severe nephrosclerosis and impaired renal dynamics. (Hypertension. 2001;38:1300-1306.)

Key Words: apoptosis ■ nitric oxide ■ L-NAME ■ glomerular structure ■ nephrosclerosis ■ arterial wall thickness ■ renal hemodynamics ■ rats, spontaneously hypertensive

Apoptosis, or programmed cell death, was first reported by Kerr as a distinctive morphological entity of hepatocytes with “shrinkage necrosis.” Kerr et al thereupon provided evidence that apoptosis is a basic biologic phenomenon having wide-ranging implications for tissue kinetics. This phenomenon of apoptosis was held to be different pathologically from necrosis or mitosis in terms of its ultrastructural and biochemical features demonstrating cytoplasmic and nuclear condensation, subsequent formation of membrane-bound bodies (nuclear segmentation), and extensive chromosomal DNA degeneration, and unlike necrosis, it is unassociated with inflammatory cell infiltration.\(^3\)–\(^6\) Glomerular cell apoptosis has been induced in the rat remnant kidney,\(^7,8\) in experimental and clinical glomerulonephritis,\(^8–11\) and by reactive oxygen species.\(^12,13\) However, the relation between apoptosis and glomerular injury in hypertensive renal disease remains unresolved.

We have previously reported that prolonged NO synthase (NOS) inhibition induced apoptosis in coronary arterial smooth muscular and left ventricular endothelial cells.\(^14\) Furthermore, it produced marked proteinuria and severe hypertensive nephrosclerosis in the spontaneously hypertensive rat (SHR) manifested by intense afferent and efferent arteriolar constriction, glomerular hypertension, and fibrinoid necrosis.\(^15\) Moreover, this severe hypertensive nephrosclerosis was prevented and reversed by ACE inhibition or certain calcium antagonists, and it was exacerbated by a diuretic.\(^16–18\) The present study was designed to investigate the regulation of glomerular cell apoptosis during the progressive hypertensive glomerular injury and severely impaired glomerular hemodynamics induced by prolonged NOS inhibition in that model.

Materials and Methods

Twenty-nine male 17-week-old SHR (Charles River Laboratories, Wilmington, Mass), with body weights of 290 to 330 g (mean ±1SEM; 306±5g), were used in this study, which had been approved in advance by our institutional Animal Care Committee, using methods described in greater detail earlier.\(^14–18\) In brief, 10 SHR were administered only tap water for 3 weeks, thereby serving as untreated controls. Another 19 SHR were given L-NAME (Sigma Chemical Co, St Louis, Mo) in their drinking water for 3 weeks (10 rats: 50 mg/L, 9.0±0.4 mg/kg per day; 9 rats: 80 mg/L, 14.3±0.7 mg/kg per day). Furthermore, 11 SHR were given L-NAME (80 mg/L in drinking water), and 10 SHR were given only tap water as controls for the measurements of renal cortical tissue levels of angiotensin (Ang) II. The drinking water with L-NAME was changed daily, and the volume consumed measured in order to determine the precise amount of L-NAME received.

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Hypertension is available at http://www.hypertensionaha.org
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All SHR were deprived of food on the night prior to study but were allowed free access to water. They were anesthetized with Inactin (100 mg/kg IP Byk-Gulden, Constance, Germany) and placed on a heating pad to maintain body temperature; after a tracheostomy, the abdominal aorta was cannulated by polyethylene catheter via the right femoral artery for blood sampling and measurement of mean arterial pressure (MAP) and heart rate (HR). Polyethylene catheters were inserted into the right and left femoral veins. The former catheter was used for [1H]methoxyaminulin (850 μCi/mL) infusion (0.1 mL/100 g body weight per hour), and the latter was used for infusion of a saline solution containing 5.6% p-aminohippurate (PAH; Merck Sharp & Dohme; 0.2 mL/100 g body weight per hour) and 12.5% albumin (bovine albumin, Sigma Chemical Co, St Louis, Mo; 0.5 mL/100 g body weight per hour for the initial 45 to 60 minutes and 0.1 mL/100 g body weight per hour thereafter). Urine was collected over two 30-minute periods, with blood samples withdrawn at their midpoint. Urine and plasma samples were counted for [1H]inulin radioactivity by placing them in 10-mL scintillation vials (Bio-Safe II, Mount Prospect, Ill) in a β-scintillation counter; glomerular filtration rate (GFR) was calculated by the standard clearance formula. Hematocrit was determined for all arterial samples by the Beardwell method22 and then centrifuged (12 000 g for 2 minutes). The extract was dried under vacuum and reconstituted in Tris buffer with the distilled water, 0.5 N HC1-acetone, and petroleum ether. The supernatant was added to Florisil (Sigma Chemical Co, St Louis, Mo) by the Beardwell method23 and then centrifuged (1000 g for 2 minutes). The supernatant was discarded, and the pellet was washed with an AutoCal Model 643 flame photometer (Instrumentation Laboratory Inc). Serum creatinine and uric acid concentrations were measured with the creatinine autoanalyses (Creatinine Analyzer, Beckman Instruments Inc, Fullerton, Calif).

Measurement of Renal Tissue Ang II Level

Ang II from the renal cortical tissue was measured using radioimmunoassay.20,22 In brief, renal cortical tissue was homogenized with 9 vol of ice-cold saline containing 0.1 N HC1 and 5% aprotonin and then centrifuged (12 000 g at 4°C for 2 minutes). The collected supernatant was added to Florisil (Sigma Chemical Co, St Louis, Mo) by the Beardwell method23 and then centrifuged (1000 g for 2 minutes). The supernatant was discarded, and the pellet was washed with the distilled water, 0.5 N HCl-acetone, and petroleum ether. The extract was dried under vacuum and reconstituted in Tris buffer (pH 8.5) for radioimmunoassay. The incubation mixture consisted of a sample or standard and the anti-Ang II rabbit antiserum (SRL). This Ang II antibody has been reported to have very low cross-reactivities (0.3%) with Ang I (30.8%), with Ang-(2-8) (46%), and with Ang-(3-8). In addition, these other metabolites might also be measured since the assay was carried out without HPLC extraction.24 The incubation was carried out (at 4°C for 24 hours) and then followed by the late addition of 125I-Ang II and further incubation (at 4°C for 8 hours.). To separate bound radioactivity from free ligands, anti-rabbit Ig goat antiserum and polyethylene-glycol were added, incubated (at 4°C for 24 hours), and centrifuged (2000 g for 20 minutes). The radioactivity in the precipitate was counted in a γ-spectrometer.

Histological Studies

The left kidney of each rat was fixed in fresh 10% paraformaldehyde and then removed and weighed. Sections (3 μm thick) were stained with hematoxylin-eosin, periodic acid–Schiff, Elastica-Masson, and periodic acid–methenamine-silver reactions for assessment of glomerular and intrarenal vascular injuries, and tubulo-interstitial changes, extracellular matrix, fibrinogen, and collagen. Afferent arteriolar and glomerular lesions were determined for arteriolar (AIS; n = 870 in total) and glomerular injury (GIS; n = 2900 in total) scores as described previously.14–18

Grading of GIS and afferent AIS was performed as previously described.14–18 GIS was graded from 0 to 3+ on glomerular injuries and sclerosis; 0 was no injury, 1+ on glomerular injuries and sclerosis; 0 was no injury, 1+ was injury of up to one third (≤1/3), 2+ was one third to two thirds injury, and 3+ was injury of more than two thirds (>2/3) of glomerular involvement. The AIS was also graded 0 to 3+ on afferent arteriolar hyalinosis and sclerosis (arteriolosclerosis); 0 was no injury at all, 1+ demonstrated arteriolar lesions up to 50% of the mural circumference, 2+ demonstrated lesions between 50% and 100% of the wall circumference but without luminal narrowing, and 3+ was complete mural hyalinosis with luminal encroachment. These scores were obtained by independent study by 2 renal pathologists, and the scoring of all tissue was conducted in a blinded manner.

The frequency of glomerular pathological profiles was determined at 2 renal depths, subcapsular and juxtamedullary cortex, each obtained by serial section. Whole-kidney GIS was expressed as the total scores of the 2 (subcapsular plus juxtamedullary GIS) scores.

Immunohistochemistry Studies, TUNEL, PCNA Index

The right kidneys were placed immediately for 2 hours in fresh 10% paraformaldehyde and then transferred to a 30% sucrose-PBS solution before being embedded in paraffin. For each specimen, several 5-μm sections were obtained for classic histological analysis, in situ determination of apoptosis, and immunohistochemical cell proliferation. For the morphometric studies, the glomerular area was determined by computer-assisted image analysis (ImageQuest, Hamamatsu Photonics K.K., Hamamatsu, Japan) as described previously.15,20 In situ detection of apoptotic cells (terminal deoxyribonucleotidyl transferase-mediated biotin-dUTP nick-end labeling of fragmented DNA, TUNEL) was performed as reported by Gavrieli et al.24 Tissue sections were stained using the In Situ Apoptosis Detection Kit (Oncor, Gathersburg, Md) and analyzed by the dioxigenin-nucleotide method, by which the detected cells show internucleosomal DNA fragmentation. To detect nonquiescent cells, an immunohistochemical staining using anti-PCNA antigen (DAKO) was employed. For quantification of the TUNEL method and PCNA immunohistochemistry, at least 30 nonsclerotic glomerular sections were examined from each rat, and the number of positively stained nuclei per intraglomerular cell number (Np/G) was determined (n = 1785 in total glomeruli). The average of each of the rats from the 3 groups were considered as TUNEL and PCNA labeling indices, respectively.

Electron Microscopy

Electron microscopic studies were performed to evaluate the structure of apoptotic cells and bodies in glomerulus. For this purpose, tissue samples were fixed at room temperature in 2.5% glutaraldehyde (TAAB) in 0.1 mol/L sodium cacodylate. They were then stained en bloc in uranyl maleate for 1 hour, postfixed in 1% osmium tetroxide, dehydrated, and then embedded in Epok 812 (Shell Chemical). Ultrathin sections were stained with manylacetate and lead citrate and examined with a JEM-1200EX electron microscope (JEOL Ltd, Tokyo, Japan).

Morphometric Studies

For morphometric analyses, the glomerular (A0) and glomerular capillary tuft (A1) areas were studied on the same kidney sections, which were examined for morphological evidence of glomerular injury.25 All microscopic slides were examined using the computer analyzer system (ImageQuest), and calculations were made using stereologic principles. To determine the A0, the mean cross-sectional glomerular area (not Bowman’s capsule) was obtained by tracing the outlines of those glomerular capillaries having a vascular pole. A0 from 20 glomeruli with minor glomerular abnormalities was studied (n = 645 in total glomeruli), each from the juxtamedullary and
subcapsular cortex areas of 1 kidney. We had demonstrated previously, using scanning electron microscopy, that the glomerular volume of the juxtamedullary glomerulus was greater than the subcapsular glomerulus.26 On the other hand, the AIS was determined (n=3110 in total capillary) by tracing the luminal area of all capillaries in a glomerulus falling within 1 high power field (×400). On average, 200 loops per specimen were measured, each representing 5 glomeruli demonstrating minor glomerular abnormalities of the juxtamedullary and subcapsular layers.

**Wall Thickening Ratio**

The wall thickening ratio (WTR) was assessed as the ratio of media thickness to outer radius of afferent (n=113) and interlobular arteries (n=73) as previously described.20 Inner and outer circumferences were measured using a computer analyzer system. Values were conducted for between-group significance. All data are expressed as mean±1 SEM. A value of P<0.05 was considered to be of statistical significance.

**Statistical Analysis**

One-way ANOVA, followed by Duncan’s multiple range test,27 was conducted for between-group significance. All data are expressed as mean±1 SEM. A value of P<0.05 was considered to be of statistical significance.

**Results**

**Body and Organ Weights, Systemic Hemodynamics**

Ratios of left kidney weight to body weight (4.39±0.23, 4.32±0.09, and 4.31±0.16 mg/g) for each of the 3 rat groups (control, L-NAME 50 mg/L, and L-NAME 80 mg/L, respectively) were similar despite the further increases of MAP in the 2 L-NAME-treated groups (185±4, 230±7, and 221±11 mm Hg, respectively; P<0.01). The respective body weights (333±10, 338±10, and 282±16 g) and absolute left kidney weights (1465±84, 1461±53, and 1226±103 mg) were reduced in the group receiving the higher L-NAME dose, the 80 mg/L group (P<0.01; control and L-NAME 50 mg/L versus L-NAME 80 mg/L). The MAP was elevated in the rats of both L-NAME groups (P<0.01), and the HR was reduced in the high-dose L-NAME (80 mg/L) group (352±10, 368±18, and 302±21 bpm, respectively; P<0.01).

**Renal Hemodynamics**

L-NAME significantly reduced ERPF (P<0.01) and increased renal vascular resistance (RVR), serum creatinine, uric acid, and urinary sodium excretion in a dose-dependent manner (Table 1). GFR and urinary protein excretion were also increased by L-NAME, although dose independently. Notably, these changes occurred in association with increased cortical tissue levels of Ang II in the L-NAME (80 mg/L) rats (P<0.0005). However, the 24-hour urinary NO excretion was markedly reduced in both L-NAME groups of rats (P<0.01) (Table 1).

**Renal Morphology**

The histological appearances of the control SHR arterioles, glomeruli, and tubulo-interstitium were only slightly altered after the 3-week follow-up. In contrast, the low dose of L-NAME produced severe nephrosclerosis, which appeared similar to human malignant nephrosclerosis as we reported previously.28 Thus, the GIS and AIS were markedly increased and associated with an increased arteriolar wall/lumen ratio in both L-NAME–treated groups (Table 2). The nephrosclerosis

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<th>TABLE 1. Renal Hemodynamic Measurements and Parameters in SHR After 3-Week Treatment With L-NAME</th>
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<td>Glomerular filtration rate, mL/1 min/g</td>
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<td>Cortical tissue levels of Ang II, pg/mg weight</td>
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<td>Urinary NO2/NO3 excretion, μmol/d</td>
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</table>

Values are mean±1 SEM. *P<0.01; †P<0.05 vs control; ‡P<0.01; §P<0.05 vs L-NAME 50 mg/L.

<table>
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<th>TABLE 2. GIS, AIS, and Wall Lumen Ratio of Interlobular Arteries in SHR After 3-Week Treatment With L-NAME</th>
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Values are mean±1 SEM. *P<0.01; †P<0.05 vs control; ‡P<0.01; §P vs L-NAME 50 mg/L.

GIS indicates the glomerular injury score in control SHR treated with L-NAME 50 mg/L or L-NAME 80 mg/L. These glomerular lesions were observed and scored as glomerular sclerosis with an increased mesangial matrix, hyalinosis, and thrombosis. Afferent arteriolar lesions also were scored on arteriosclerosis with sclerosis, hyalinosis, and luminal narrowing, using serial sections for comparison between afferent and efferent arterioles.
of the 80 mg/L L-NAME treatment was associated with intense glomerulosclerosis, interstitial fibrosis, and perivascular mononuclear cell infiltration that was more severe in juxtamedullary nephrons.

The WTR of the afferent arterioles was increased significantly by L-NAME dose dependently ($P<0.01$). TUNEL staining expressed smooth muscle cells in afferent arterioles, but electron microscopic examination also revealed fibrinoid necrosis with smooth muscle cellular swelling (Figure 1). On the other hand, the WTR of interlobular arteries was significantly increased in both L-NAME groups ($P<0.01$) (Table 2).

Compared with the controls, the mean glomerular tuft area ($A_T$) of both L-NAME–treated SHR was reduced significantly by $\approx 25\%$, and this was unassociated with a reduced glomerular area ($A_G$) in either the subcapsular or juxtamedullary glomeruli (Table 3). Although the ERPF was reduced significantly in a dose-dependent manner by L-NAME, the $A_G$ remained unchanged.

The intraglomerular cell numbers ($N_{GC}$) were significantly reduced in both L-NAME–treated SHR, but more so in the group receiving the 80 mg/L dose of L-NAME (Table 3). L-NAME produced at least a 3-fold increase of the apoptotic and PCNA indices ($P<0.01$) (Table 3, Figure 2).

In L-NAME rats, TUNEL staining regularly expressed 3 types of cells (ie, mesangial, endothelial, and epithelial). Apoptosis of nonsclerotic glomeruli was observed in the endothelial cells of the focal segmental ischemic and sclerotic glomeruli. The apoptosis was also observed in epithelial cells by electron microscopy (Figure 3). The PCNA staining revealed a decreased expression of endothelial cells in the high-dose L-NAME group (Table 3).

**Discussion**

The results of this study demonstrated the dramatic effects of prolonged L-NAME administration to 20-week-old SHR. Thus, NOS inhibition induced not only increased glomerular apoptosis but also decreased intraglomerular cell numbers. These changes were associated with increased urinary protein excretion and histological nephrosclerosis scores associated with reduced urinary NO excretion and increased cortical tissue Ang II levels. L-NAME also adversely affected renal function (eg, reduced ERPF and GFR, elevated RVR) that was associated with severe histological changes (eg, reduced $A_T$ and increased wall/lumen ratio of afferent and interlobular arteries).

Glomerular size and cell number are regulated with programmed cell death (apoptosis) and cell proliferation. Apoptosis has been observed in glomeruli from patients with proliferative glomerulonephritis and glomerular sclerosis. Recently, increased apoptosis has been reported in the normotensive and SHR with a remnant kidney. In the nonsclerotic glomerulus of the L-NAME–treated SHR, which we report herein, the apoptosis labeling index was also

**TABLE 3. Morphometrical Analysis of Glomerulus in Minor Glomerular Abnormalities**

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<tr>
<th>Index</th>
<th>Control</th>
<th>L-NAME 50 mg/L</th>
<th>L-NAME 80 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular area: $A_G$ ($\times 103 \mu m^2$)</td>
<td>10.2±0.3</td>
<td>10.2±0.4</td>
<td>9.5±0.3</td>
</tr>
<tr>
<td>Glomerular tuft area: $A_T$ ($\mu m^2$)</td>
<td>115±4</td>
<td>82±2*</td>
<td>88±3*</td>
</tr>
<tr>
<td>Tuft area variation, % SD</td>
<td>70.5±5.8</td>
<td>50.2±2.8†</td>
<td>63.5±5‡</td>
</tr>
<tr>
<td>Cell numbers of glomerulus: $N_{GC}$ (/Glo)</td>
<td>35±1.0</td>
<td>31.7±0.8*</td>
<td>29.7±0.8*</td>
</tr>
<tr>
<td>Apoptosis index, %</td>
<td>0.99±0.12</td>
<td>3.81±0.32*</td>
<td>3.20±0.5*</td>
</tr>
<tr>
<td>PCNA index, %</td>
<td>5.5±1.3</td>
<td>27.7±2.2*</td>
<td>9.3±1.1§</td>
</tr>
</tbody>
</table>

Values are mean±1 SEM. *$P<0.01$, †$P<0.05$ vs control; §$P<0.01$; ‡$P<0.05$ vs L-NAME.

![Figure 1](image-url)
increased in association with a reduced glomerular cell number. Although the GIS increased with the higher dose of L-NAME, apoptosis was not associated with a reduced PCNA index. This induced apoptosis may be explained on the basis of 3 possibilities: (1) glomerular hypertension, as we previously reported; (2) stimulation of the local renal renin-angiotensin system (ie, Ang II–induced apoptosis) as was demonstrated in the heart and aorta; and (3) inhibition of NO synthesis which, in turn, has been shown to inhibit apoptosis as well as caspase activity.

Apoptosis and reduced intraglomerular cell number result from prolonged NO synthesis inhibition in addition to minimally altered glomeruli with increased PCNA expression. Interestingly, apoptosis and cell loss were produced with the higher L-NAME dose even though the PCNA was downregulated. In the present study, the L-NAME–induced glomerular cell apoptosis was compared with glomeruli of control SHR demonstrating only minor glomerular abnormalities. Han et al described the expression of PCNA and TUNEL staining in coronary artery injury rats, also suggesting that the downregulation of PCNA preceded the decreased apoptosis. In the present study, the changes of the glomerular cell PCNA also preceded the apoptosis cell expression. Activation of renin-angiotensin system has been suggested recently for the model of chronic NOS inhibition–treated SHR or normotensive rats. Li et al reported that plasma renin activity was elevated in L-NAME–treated SHR in which the L-NAME–exacerbated hypertension was unaccompanied by a further increase in left ventricular mass or of vascular hypertrophy in resistance-sized renal, mesenteric, and coronary arteries. However, Kashiwagi et al reported that adrenocortical Ang
II levels in normotensive rats treated with L-NAME (250 mg/L in drinking water for 12 weeks) were about twice as great as those in control Wistar rats (263±49 versus 533±80 pg/mg weight). In the present study, the cortical Ang II tissue levels of SHR, administered a low dose of L-NAME (50 mg/L) for a shorter period (3 weeks), were increased by ≈3-fold compared with the control SHR. It is noteworthy that the Ang II levels of the Wistar rats and SHR were very similar.21

The relationship between Ang II and apoptosis was investigated by Ravassa et al.,36 using in vitro SHR and Wistar-Kyoto myocytes. In that report, the Ang II infusion increased cardiomycocyte apoptosis, and this effect was greater in SHR than in WKY cells and was associated with increased ratio of caspase-3/procaspase-3 in both strains. Furthermore, Anversa and colleagues also reported Ang II induced myocyte apoptosis with cytosolic Ca2+ elevations33 by activating p3837,40 using cardiac myocytes of normotensive rats in vitro. Our present study indicated that glomerular hypertension with intrarenal tissue Ang II elevation induced glomerular cell apoptosis in vivo in NOS-blockaded SHR. Clearly, this information would be valuable in understanding the Ang II in L-NAME-treated groups. Finally, Diep and Schiffrin41 reported both angiotensin type 1 (AT1) and type 2 (AT2) receptor activation mediated apoptosis in vivo in the vasculature of rats receiving Ang II infusions and with an AT1 (losartan) and an AT2 (PD 123,319) receptor antagonist.

With respect to the morphometric analysis of the L-NAME rats, glomerular tuft size was markedly decreased in association with the marked reduction in ERPF. These changes suggest that glomerular function was markedly diminished by L-NAME. Furthermore, the nephrosclerosis, produced by the higher dose of L-NAME, decreased body weight, absolute kidney weight, and A1. The A2 variation (as indicated by the SEM of A1) might be associated with mesangial dysfunction (or mesangiolysis), and the irregular glomerular capillary tuft area could be considered the important factor of hypertensive glomerular injury resulting from the increased glomerular hydrostatic pressure.18,22 As we previously reported in aged 73-week-old SHR (without L-NAME treatment), P0 increased in association with an elevated arterial pressure and severe nephrosclerosis.43 More recently, even more severe nephrosclerosis in 85-week-old SHR was associated with increased A0 and A1 variation compared with young SHR.25

The glomerular injuries with hypertension were initiated most likely by glomerular endothelial cell injury and cellular proliferation as shown by PCNA-positive cells and TUNEL-positive cell loss (probably apoptosis). Thus, these apoptotic changes were most likely induced by factors such as induction of the local renin-angiotensin system with increased glomerular capillary wall pressure and production relative oxygen species.12,13 In conclusion, severe hypertensive nephrosclerosis, exacerbated by prolonged NO synthetase inhibition, was associated with increased mesangial matrix, glomerular sclerosis, and glomerular cell loss. In addition, glomerular endothelial cell loss was also related to glomerular capillary thrombosis and mesangiolysis associated with altered renal and intraglomerular hemodynamics and, structurally, by diminished glomerular capillary lumina.

References
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