Introduction

Cerebrovascular and coronary arterial diseases are two major cardiovascular complications in patients with long-term hypertension (1-3). In elderly hypertensive patients, stroke of either thrombotic or haemorrhagic origin remains the main cause of morbidity and mortality (1-4). Chronic nitric oxide (NO) deprivation (5-11) and spontaneous hypertension (12-18) are two different models of arterial hypertension. In spontaneously hypertensive rats (SHR), several laboratories have found that hypertrophic vascular remodelling (increased media thickness and cross-section area) (6, 12, 14, 16-18) and/or eutrophic remodelling (decreased lumen and external diameters with unaltered media cross-section area) (6, 13, 15, 16, 18) developed in arteries.
Chronic inhibition of NO synthesis with Nω-nitro-L-arginine methyl ester (L-NAME) induced endothelial dysfunction in a rabbit aorta (7, 10). Chronic L-NAME administration in rats caused inflammatory changes such as monocyte/macrophage infiltration (Mc/Mphl), myofibroblast formation and monocyte chemoattractant protein-1 (MCP-1) in resistant arteries (5, 7, 10, 11, 16, 19), pial arterioles and posterior cerebral arteries (17). We have previously reported that Windkessel functions of large arteries are less affected after acute NO blockade (20). Wang et al. found that chronic hypertension caused structural adaptations of cerebral arteries (15). Vascular remodelling in hypertension involves two processes: eutrophic and hypertrophic remodelling (6, 13, 21). In eutrophic remodelling, the carotid arterial wall matrix is rearranged with a reduced lumen; however, there is no evidence of net growth or changes in media cross-section area of the artery. In hypertrophic remodelling, an increase in media cross-section area and encroachment on the carotid arterial lumen occurs, indicating growth (13, 21). An increase in cross-section and intima-media thickness of carotid arteries is associated with cardiovascular and cerebrovascular risk factors (13, 22). However, the mechanism of vascular remodelling (6, 13-19, 21, 23), inflammatory cell formation and migration (5-7, 10, 11, 16, 17, 24, 25), AIS (26, 27), remodelling index and growth index (6, 21) of cerebral arteries are uncertain. The protective risk factors of the vascular wall in early hypertensive change remain undetermined. In this study, we have attempted to examine the structural changes of cerebral arteries in normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) after long-term NO inhibition. The vascular remodelling and/or injury in various beds of cerebral arteries were examined. We found significant structural changes in cerebral arteries following chronic NO deprivation with L-NAME. These results may provide important information for potential benefits in preventive medicine and anti-hypertensive therapy.

Materials and methods

Animal preparation and procedure

Five-week-old, male SHR and WKY were obtained from the National Animal Center and housed one per cage. They were fed with standard rat chow and tap water, and kept in an air-conditioned animal room (temperature: 22-24°C, lighting: 0800-2000 hr). Experimental rats were fed L-NAME (Sigma) dissolved in drinking water (1 mg·ml⁻¹) from 5 to 7 or 9 weeks of age. Control rats were given water without L-NAME. The volume of drinking water consumed with L-NAME was measured daily to calculate the amount of L-NAME intake (60-80 mg·kg⁻¹). Tail cuff pressure (TCP), which represents the systolic arterial pressure, was measured using a photoelectric volume oscillometer device (UR-5000, Ueda, Tokyo, Japan). Three measurements of TCP were made and the average was taken for an individual data point. TCP was measured before L-NAME administration, and at 2-3 day intervals during the experiment. At week 7 or 9, rats were sacrificed by deep anesthesia with intraperitoneal pentobarbital (65 mg·kg⁻¹).

Morphometric measurements and immunohistochemical stainings

Rats were fixed by transcardial perfusion with 4% paraformaldehyde. Several brain regions and arteries of circle of Willis including internal carotid arteries (ICA) and middle cerebral arteries (MCA) were rapidly excised. The tissues were stored in phosphate buffered saline containing 2.5% sucrose. The specimens were then dehydrated, embedded in paraffin, cut into 5µm sections and mounted on slides. For histopathological measurements, sections were stained with haematoxylin-eosin (HE) and periodic acid-Schiff (PAS). For immunohistochemical examinations, sections were deparaffinized in xylene and ethanol and rehydrated three times for 5 min each in solution containing 0.02% Triton X-100 and 0.01% bovine serum albumin. Sections were preincubated with 2% horse serum to minimize nonspecific binding. They were incubated overnight at 4°C with mouse anti-rat macrophage/monocyte antibody (anti-ED1, Serotec, Kidlington, Oxford, UK) and anti-rat α-smooth muscle actin antibody (anti-α-actin, Sigma). The slides were washed and incubated with biotinylated rabbit anti-mouse IgG as secondary antibody. The bound primary antibody was visualized using avidin-biotin-peroxidase method (ABC Elite Kit; Vector Laboratories). These procedures were in accordance with those described by Dijkstra et al. (24). The number of ED1-positive cells was counted from 120 sections. The external and internal lumen diameters were measured for each segment of artery.

Vascular remodelling

The remodelling index is the ratio of calculated change in lumen diameter to the observed difference in lumen diameter (the difference in percentage of vessel lumen diameters between WKY and SHR). Growth index is the change (%) in media cross-section area as described by Mulvany (6) and Schiffrin et al. (21).

Arteriolar injury score (AIS)

AIS was assessed as described by Mai et al. (26) with modification by Ono et al. (27). The following equation was used for the calculation of AIS: [(1 × number of grade 2 arterioles) + (2 × number of grade 3 arterioles) + (3 × number of grade 4 arterioles)] × 100/(number of arterioles observed).

Statistical analysis

Data obtained from various artery segments from L-NAME treated, as well as untreated rats, and the differences of TCP and AIS were compared within strains by Student’s paired t test. Comparisons between and among strains were made using two-way analysis of variance (ANOVA) and Bonferroni test.
Student’s unpaired t test and one way ANOVA were used to compare the changes in body weight, heart weight, nuclei density, and other parameters between treated and untreated WKY and SHR. Data were presented as mean ± SEM. The significance for student’s unpaired t test and for Bonferroni test was set at $P < 0.05$ and $P < 0.001$, respectively. For comparisons between two means of various strains, a $P < 0.01$ was considered to be significant.

**Results**

**Body weight (BW), heart weight (HW), heart rate (HR) and blood pressure (BP)**

Chronic NO blockade with L-NAME for 4 weeks (from week 5 to 9) resulted in time-dependent elevation in TCP in both WKY and SHR compared with control groups (Fig. 1). The TCP in SHR treated with L-NAME soon reached a level above 250 mmHg. Thereafter, SHR rats with malignant hypertension displayed signs of, e.g. stroke, motor disorders, and eye bleeding. Some rats died within 24 to 27 days. Table 1 summarizes the changes in BW, HW, HR, and TCP in WKY and SHR with and without L-NAME treatment. After 5 days following L-NAME treatment, the magnitude of TCP increase was much greater in SHR than WKY (+74.0±3.2 mmHg vs. +27.0±2.2 mmHg, $P < 0.001$). The increase in TCP was accompanied by an increase in HW, and a decrease in BW in L-NAME treated SHR. Inhibition of NO with L-NAME in normotensive WKY produced significant TCP elevation with little changes in BW and HW.

**Morphometric and immunohistochemical changes**

Reductions in lumen diameter, external diameter, and media cross-section area were observed in ICA of all hypertensive rats (untreated SHR, treated WKY, and treated SHR), indicating that hypertension and L-NAME treatment reduced lumen diameter and media cross-section area in both WKY and SHR (Table 2). L-NAME did not affect the media thickness of ICA in WKY, but it caused a significant increase in media thickness (7-8%) in SHR. L-NAME reduced the media cross-section area of the vascular smooth muscle cell (VSMC) layer, but increased the cell volume density in both WKY and SHR. This finding suggests that medial hypotrophy in SHR with NO inhibition is primarily a result of VSMC hypertrophy rather than hyperplasia. The media/lumen ratio was greater in SHR than in WKY (7.45±0.11% vs. 5.50±0.02%, $P < 0.05$). L-NAME treatment further increased the media/lumen ratio in WKY and SHR (8.54±0.02% vs. 5.50±0.02% in WKY, and 9.30±0.29% vs. 7.45±0.11% in SHR, $P < 0.05$). Since L-NAME decreased the media cross-section area, the growth index became negative in treated WKY and SHR. The growth index was also negative in untreated SHR. The negative growth index associated with hypertension resulted in medial hypotrophy with VSMC hypertrophy.

L-NAME and hypertension did not significantly affect the number of layers and nuclei density of VSMC in each group of WKY and SHR. L-NAME increased the AIS in WKY and SHR (Table 2 and Fig. 2). The magnitude of the increase in AIS did not significantly differ between SHR (+116±14 ) and WKY (+91±22 ) ($P > 0.05$).

**Effects of NO-deprivation on inflammatory and VSMC hypertrophy with medial hypotrophy**

ED1 stain revealed the invasion of inflammatory cells (Mc/MpH1) to the external layers of arterial walls. Sections of ICA from L-NAME treated SHR and treated WKY exhibited marked infiltration of monocytes and fibroblast-like cells in perivascular areas as demonstrated by HE stain (Fig. 2). More arteriolar hyalinosis in the perivascular area and fewer nucleoli
of VSMC in ICA in L-NAME treated SHR and treated WKY were observed by PAS stain. Surprisingly, ED1-positive cells were found in the adventitial layer, but not in the medial layer of ICA. L-NAME significantly enhanced the extent of Mc/MphI in WKY and SHR (Table 2 and Fig. 2). Anti-α-actin positive cells of spindle shape (myofibroblasts are usually transformed from fibroblasts or pericytes) were another major cell type that appeared with inflammatory lesions in L-NAME treated rats. Although anti-α-actin is used as a marker for myofibroblasts, the antibody against rat α-actin can also recognize VSMC. Therefore, it is possible that these immunopositive cells had been derived from VSMC. These cells then migrated into the perivascular and interstitial spaces to form inflammatory lesions. In control WKY and SHR, inflammatory and proliferative cells were essentially absent (Fig. 2).

Compared with untreated rats, the number of Mc/MphI was significantly greater in L-NAME treated groups, while anti-α-actin-positive cells were significantly lower. The number of

### Table 1: Body weight, heart weight, heart rate and blood pressure.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated SHRs (n=12)</th>
<th>Untreated WKYs (n=12)</th>
<th>L-NAME Treated SHRs (n=8)</th>
<th>L-NAME Treated WKYs (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>8±3</td>
<td>78±3</td>
<td>80±2</td>
<td>78±2</td>
</tr>
<tr>
<td>BW, g</td>
<td>24±6</td>
<td>25±12</td>
<td>22±15</td>
<td>190±12†</td>
</tr>
<tr>
<td>HW, g</td>
<td>0.96±0.03</td>
<td>1.06±0.02</td>
<td>1.00±0.02</td>
<td>1.29±0.05††</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>396±14</td>
<td>446±19</td>
<td>376±17</td>
<td>409±12</td>
</tr>
<tr>
<td>TCP, mmHg</td>
<td>110±2</td>
<td>179±2*</td>
<td>140±3*</td>
<td>260±5†</td>
</tr>
<tr>
<td>Increase in TCP</td>
<td>15±1</td>
<td>66±2*</td>
<td>42±2*</td>
<td>140±4†</td>
</tr>
</tbody>
</table>

Values are mean±SEM, BW, body weight; wk, week; HW, heart weight; HR, heart rate; TCP, tail cuff pressure; L-NAME, Nω-nitro-L-arginine methyl ester. * P<0.01 vs. untreated WKY, †P<0.01 vs. corresponding untreated SHR. The abbreviations in Table 2 are the same as those in this Table. The BW in the first row is the value at 5-weeks-old. Otherwise, BW, HW, HR, and TCP are values at 9 weeks-old.

### Table 2: Morphological measurements and media composition in internal carotid arteries.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated SHRs (n=12)</th>
<th>Untreated WKYs (n=12)</th>
<th>L-NAME Treated SHRs (n=8)</th>
<th>L-NAME Treated WKYs (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen diameter, μm</td>
<td>846.6±26.8</td>
<td>668.2±38.7</td>
<td>581.2±27.7*</td>
<td>570.9±38.9*</td>
</tr>
<tr>
<td>External diameter, μm</td>
<td>1053.2±33.9</td>
<td>854.9±24.4*</td>
<td>761.6±39.2*</td>
<td>831.3±40.1*</td>
</tr>
<tr>
<td>Media thickness, μm</td>
<td>46.6±1.6</td>
<td>49.8±2.0</td>
<td>49.6±1.8</td>
<td>53.1±1.4*</td>
</tr>
<tr>
<td>MCSA, μm²</td>
<td>13265±2736</td>
<td>11530±2639*</td>
<td>10040±2196*</td>
<td>10731±1706*</td>
</tr>
<tr>
<td>Growth index, %</td>
<td>0±5.4</td>
<td>-14.1±2.9</td>
<td>-24.8±2.6*</td>
<td>-20.4±2.9*</td>
</tr>
<tr>
<td>Monocyte/macrophage</td>
<td>1.3±0.9</td>
<td>2.1±0.9</td>
<td>7.9±0.6*</td>
<td>13.6±2.7††</td>
</tr>
<tr>
<td>Layers of VSMC</td>
<td>5-6</td>
<td>5-6</td>
<td>5-6</td>
<td>5-6</td>
</tr>
<tr>
<td>Nuclei density</td>
<td>24.6±1.6</td>
<td>28.2±3.4</td>
<td>19.8±2.4</td>
<td>19.5±2.1</td>
</tr>
<tr>
<td>Cell volume density, %</td>
<td>100±4.4</td>
<td>93.7±6.2</td>
<td>132.9±9.3*</td>
<td>143.5±11.3*†</td>
</tr>
<tr>
<td>Media/lumen ratio, %</td>
<td>5.5±0.02</td>
<td>7.45±0.11*</td>
<td>8.54±0.02*</td>
<td>9.30±0.29††</td>
</tr>
<tr>
<td>Remodelling index, %</td>
<td>100±3</td>
<td>110±3</td>
<td>114±5</td>
<td>111±6</td>
</tr>
<tr>
<td>Arteriolar injury score</td>
<td>46±10</td>
<td>53±6</td>
<td>137±28*</td>
<td>169±18††</td>
</tr>
</tbody>
</table>

Values are mean±SEM. VSMC, vascular smooth muscle cell. MCSA, media cross-section area

* P<0.05 vs. untreated WKY, †P<0.05 vs. corresponding untreated SHR.
Mc/MphI in untreated SHR and WKY was significantly decreased. The appearance of anti-α-actin-positive cells was more obvious in L-NAME treated WKY and SHR than untreated controls. Significantly higher Mc/MphI and cellular hypertrophy of medial layer were present in treated SHR and WKY. The appearance of arteriolar hyalinosis and AIS was also increased in the treated rats. Encroachment of endothelial cells to vessel lumen also developed (Fig. 2). L-NAME caused monocytes infiltration in ICA of WKY and SHR (Fig. 3A and B). There were not many Mc/MphI changes in L-NAME treated WKY (Fig. 4A and C). Mc/MphI in the adventitia of middle cerebral artery was remarkably observed in SHR as early as 2 weeks after L-NAME treatment (Fig. 4B and D).

Immunohistochemistry of ED1 in SHR and WKY with motor disorder, following L-NAME treatment, demonstrated that ED1-positive cells were found around the perivascular area of vessels (Fig. 3A and B). In ICA, the number of Mc/MphI was 7.9±0.6 per section in treated WKY and 13.6±2.7 per section in treated SHR (*P <0.05). The remodelling scheme of ICA was used for graphic representation of lumen (inner circle) and medial (outer circle) layer dimensions. To our knowledge, this study is the first to report eutrophic VSMC and hypotrophic medial changes observed in untreated SHR vs. WKY. Hypertrophic cellular and eutrophic medial changes occurred in SHR following L-NAME treatment. Hypertrophic cellular and hypotrophic medial changes were found in L-NAME treated rats, but not in untreated WKY (Fig. 5). Vascular endothelium in SHR and WKY, before the appearance of stroke signs, was almost intact.

Discussion

This study reports on the effects of L-NAME treatment in 5-week-old WKY and SHR over a period of four weeks. Both WKY and SHR developed severe hypertension. Cardiac hypertrophy and body weight loss were only observed in SHR treated with L-NAME. A previous study also found that L-NAME reduced the body weight (28). The decreased body weight associated with L-NAME treatment may be attributed to the
increase in plasma renin activity (29). L-NAME decreased proximal tubular resorption leading to increased diuresis and decreased expansion of extracellular fluid volume (30). In the present study, we found that heart weight increased in L-NAME treated WKY and SHR. Similarly, Raij (31) had also reported an increase in cardiac weight following chronic L-NAME treatment. Cardiac hypertrophy may result from an increase in systolic blood pressure, a decrease in plasma renin activity, or a decrease in cardiac angiotensin converting enzyme activity following L-NAME treatment (32). Here we found that NO deprivation also induced vascular inflammatory responses as indicated by Mc/MphI and myofibroblast formation in ICA. A combination of negative growth indices, increased vascular cell volume density, and increased AIS was seen in SHR with 4 weeks of L-NAME treatment. In L-NAME treated animals, remodelling and growth indices were 114±5% (–24.8±2.6%) in WKY, and 111±6% (–20.4±2.9%) in SHR (P<0.001). L-NAME treatment in both WKY and SHR induced a remodelling with medial hypotrophy and VSMC hypertrophy. Nitric oxide appeared to be crucial in outward remodelling. However, the reduction in lumen diameter and external diameter (Table 2) in our study indicates that NO plays a role in the inward remodelling.

The media thickness did not differ among untreated WKY, untreated SHR and L-NAME treated WKY. We observed a significant increase in media thickness in L-NAME treated SHR (a 7-8% increased over untreated SHR). These data indicate that hypertension itself is not the only factor for the change in media
thickness. Figure 2 illustrates a higher extent of arteriolar hyla-
linosis and inflammatory cell infiltration in L-NAME treated
WKY than those in untreated SHR. At this time, the TCP in
treated WKY was lower than that in untreated SHR. This find-
ing indicates that L-NAME causes vascular changes prominent-
ly in ICA. As depicted in Figure 3, the number of ED1-positive
cells was greater in SHR receiving L-NAME for four weeks
compared to the corresponding treated WKY. The finding sug-
gests that hypertension triggered the vascular change in ICA.
Both groups received L-NAME treatment, but the increase in
blood pressure in SHR was much higher than that in WKY. On
the other hand, treatment of L-NAME for only two weeks
induced infiltration of inflammatory cells in right and left mid-
dle CA of SHR, but not in WKY (Fig. 4). These results suggest
that NO inhibition by L-NAME is the culprit for the inflammat-
ory vascular change. Accordingly, hypertension and NO deple-
tion play an important role in vascular remodelling and
inflammatory cell infiltration, depending on the time and
vessels. In contrast, we observed a marked difference in
media/lumen ratio not only between untreated WKY and SHR,
but also between untreated and treated WKY and SHR. The
ED1 antibody recognizes most lysosomal enzyme in macro-
phage and monocytes (11, 24, 26). Increased ED1-positive cells
in the adventitial layers of L-NAME treated WKY or SHR indi-
cate an increased invasion of inflammatory cells. Although
many investigators have addressed the pathophysiological sig-
ificance of these ED1-positive cells (24, 26, 27, 33-38), there
are still arguments regarding the cause of inflammatory cell
infiltration (34-37). In the present study, the number of
Mc/Mphl did not differ between untreated WKY and SHR, but
increased following L-NAME treatment. These results suggest
that L-NAME, rather than hypertension, is the major cause of
vascular inflammatory changes. Profibrotic activity of TGF-β1
has been shown to cause differentiation of interstitial fibroblast
and/or pericyte to myofibroblast (35, 37-40). Myofibroblasts
produce extracellular matrix proteins via action of TGF-β1 and
are responsible for tissue fibrosis/remodelling (35, 37-40).

Our results have provided evidence for vascular remodelling
in genetic or NO-deprived hypertension. The present study
revealed decreases in lumen diameter, external diameter, and
nuclei density of VSMC in ICA of treated SHR and WKY. The
higher remodelling index indicates vascular changes, and the
negative growth index suggests partial atrophy of VSMC in
chronic hypertension with NO deprivation (6, 13, 22, 40, 41).
Our results also demonstrated the involvement of a genetic
factor and L-NAME induced endogenous NO deficiency in the
development of hypertension (5, 6, 8-11, 20).

The hypertrophy with hyalinosis of VSMC, hypertrophy of
media, inflammatory changes of the vascular wall, and the
decrease of media cross-section area in hypertensive rats were
important findings (12, 19). These results were in agreement
with those from other studies (17, 26, 39, 40).

With respect to haemorrhagic and thromboembolic types of
stroke in hypertensive patients (7, 10), our results suggest that
increases in Mc/Mphl and AIS are likely to be the risk factors
for early-stage hypertensive cerebrovascular diseases. L-NAME
induced hypertrophy of VSMC and hyalination of media are
due to increased cell volume density, decreased medial area, and
negative growth index. Medial hyalinosis was observed in
ICA, but eutrophic changes in VSMC developed in untreated
SHR. We also found that L-NAME increased the Mc/Mphl and
AIS in SHR and WKY; yet, the increase in AIS was not merely
the result of hypertension because blood pressure was also elev-
ed in untreated SHR, treated WKY, and treated SHR. The
presence of abundant glycoprotein in PAS-positive cells in L-
NAME treated rats might have resulted from NO deprivation
and inflammatory cells induced by proinflammatory cytokines
(3, 38, 40). Mai et al. (26) and Pockley (3) speculated that arte-
riolar hyalinosis might be caused by changes in myeloid cells
after long-term hypertension. Migration of inflammatory cells
into the perivascular space then caused arteriolar hyalinosis and
further enhanced the hypertension of either genetic origin or of
NO blockade. Under our experimental conditions, it was not
possible to discriminate primary and secondary actions of L-
NAME because L-NAME produced either direct inflammatory
changes (10, 11, 19, 35) or indirect haemodynamic-induced
inflammation-promoting (40) hypertrophic molecules (39). The
formation of reactive oxygen species (ROS) and cytokines is
likely a key factor for the generation and migration of inflam-
matory cells. An in vivo study by Niu et al. (42) revealed that in
cultured endothelial cells, NO inhibition by L-NAME caused
neutrophil adhesion. The adhesion of endothelial cells with neu-
rophil was prevented by platelet activating factor (PAF) inhib-
itor and intracellular ROS scavengers. They suggested that ROS
and PAF contributed to the adhesion on the surface of endothe-

tlial cells for neutrophil. Although we did not use PAF inhibitor
and ROS scavengers in this in vitro study, the inflammatory
cells were observed in the adventitial layer of cerebral arteries
in WKY and SHR following L-NAME treatment for only 4
weeks. The results may imply that NO inhibition with L-NAME
in vitro caused formation of ROS, PAF, inflammatory cytokines
and possibly other factors for the generation and migration of
neutrophils, monocytes and macrophages into the vascular wall.
These changes are responsible for the atherosclerotic lesion in
the cerebral arteries.

In the present study, we showed hypertrophy of VSMC,
increased AIS, and Mc/Mphl of vessels in rats treated with
L-NAME. Immunohistochemical stain with ED1 and PAS
revealed that Mc/Mphl or myofibroblasts increased in SHR and
WKY following L-NAME treatment for only 4 weeks; howev-
er, Mc/Mphl was observed in the adventitia of middle cerebral
arteries of treated SHR as early as 2 weeks (Fig. 4B and D). Our
data showed that most Mc/Mphl of ED1-positive stain around
the perivascular area of ICA in SHR and WKY were associated
with signs of stroke following L-NAME (Fig. 3A and B). The results of the present study also suggest that AIS or Mc/MphI be used as a predictor or marker for cardiovascular risk. Figure 5 illustrates the interaction of genetic factor and NO deprivation on the remodelling scheme of ICA.

In conclusion, our major findings are: 1) L-NAME rapidly elevates arterial pressure in SHR and WKY, with a much greater magnitude in SHR than WKY. We noted significant increases in heart weight with decreases in body weight in treated SHR. 2) L-NAME causes hypertrophy of vascular smooth muscle cells with hypertrophy of media, negative growth index, higher Mc/MphI, media/lumen ratio and greater AIS. 3) Mc/MphI in adventitia of middle cerebral arteries in SHR is observed following L-NAME treatment for only two weeks. 4) Morphological changes dramatically vary between SHR and WKY with NO deprivation. 5) Mc/MphI or AIS in vascular wall is likely the early marker for stroke risk prediction. 6) Late changes in chronic hypertension include hyalinosis in whole layers, increased Mc/MphI cells, hypertrophy of VSMC, and medial hypotrophy.

Acknowledgments
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References