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# Endothelin and Gelatinases in Renal Changes Following Blockade of Nitric Oxide Synthase in Hypertensive Rats

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## Abstract

**We investigated the involvement of matrix metalloproteinases (MMPs), tissue inhibitor (TIMP) and endothelin-1 (ET-1) in the renal damage in spontaneously hypertensive rats (SHR) following nitric oxide (NO) deprivation. SHR received $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME) from 5 wk-old for a period of 30 days. An $ET_A$ antagonist, FR139317 was used. We gave SHR FR139317 alone and cotreatment with L-NAME. L-NAME caused systemic hypertension, decrease in plasma nitrate/nitrite, increases in blood urea nitrogen and creatinine, impairment of glomerular dynamics. NO deprivation reduced the renal tissue cGMP, but it increased the collagen volume fraction, number of sclerotic glomeruli, arteriolar injury score and glomerular injury score. In addition, L-NAME elevated the plasma ET-1 at day 5. Cotreatment with FR139317 alleviated the L-NAME-induced functional and structural changes of renal glomeruli. L-NAME administration for 5 to 10 days resulted in decreases in $MMP_2$ and $MMP_9$ with increasing $TIMP_2$. After L-NAME for 15 days, opposite changes (increases in $MMP_2$ and $MMP_9$ with a decrease in $TIMP_2$) were observed. FR139317 cotreatment ameliorated the L-NAME-induced changes in $MMP_2$ and $MMP_9$ throughout the 30-day observation period. The $ET_A$ antagonist cotreatment attenuated the L-NAME-induced increase in $TIMP_2$ before day 15, but not after day 20. The results indicate that ET-1, MMPs and TIMP are involved at the early stage (before 10 days) of glomerular sclerosis and arteriosclerosis with functional impairment following NO deprivation. The changes in MMPs and TIMP at the late stage (after 20 days) may be a compensatory response to prevent further renal damage.**

**Key Words: glomerular sclerosis, arteriosclerosis, nitric oxide, matrix metalloproteinases, endothelin**

## Introduction

Hypertension of genetic origin and that caused by chronic nitric oxide (NO) deprivation are two different types of hypertension (7, 17, 18, 45). Acute or chronic administration of nitric oxide synthase (NOS) inhibitors caused systemic hypertension, ventricular hypertrophy, increased total peripheral

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resistance, and arterial impedance (6, 17-20). Long-term NO deprivation in normotensive and hypertensive rats caused not only hypertension, but also vascular changes, glomerular damage, stroke, and sudden death (17, 18, 24, 45, 48). The continuous formation of NO in either normotensive or hypertensive animals is important for homeostasis in vascular and organ functions (2, 7, 16).

Matrix metalloproteinases (MMPs) is a family of zinc-dependent proteinases or gelatinases created from a variety of cells in many organs (34, 41). Recent studies suggest that MMPs are involved in vascular changes such as fibrosis, inflammation, vascular reactivity and remodeling in a number of disorders such as deoxycorticosterone acetate (DOCA)-salt and stress-induced hypertension, as well as systolic hypertension, arterial stiffness, pregnancy and other conditions (1, 11, 22, 35, 43). There is also evidence indicating interactions among NO, endothelin (ET) and MMP systems in the pathogenesis of vascular or tissue damage (4, 8, 12, 21, 42, 46). However, the role of MMPs and ET in cardiovascular and renal damage following hypertension induced by chronic NO deprivation has not yet been fully explored (5, 8, 27, 38, 39).

In the present study, we used $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME) for chronic NO deprivation in spontaneously hypertensive rats (SHR) from a young age (5-wk-old). Renal functions and pathology were assessed, collagen volume fraction and plasma endothelin-1 (ET-1) were determined. Expression of matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinase (TIMP) was observed. An ET-1 antagonist (FR139317) was used to elucidate the role of ET in the renal changes (42).

## Materials and Methods

*Animals*

Inbred strains of SHR originated from the Charles River (USA) were obtained from the National Animal Center. All study procedures for animal experiments were reviewed and approved by the University Committee of Laboratory Animal Research Center and were designed in accordance with the National Animal Center Guidelines. Rats were housed at 22 ±1 °C under a 12/12 h light/dark regimen. Food and water were supplied *ad libitum*. Standard rodent diet in pellet (NaCl content < 1%) was purchased from PMI Feeds, Inc. (Richmond, IN, USA). The tail cuff pressure (TCP) was measured at conscious state with a photoelectric sphygmomanometer (UR-5000, Tokyo, Japan).

*Drugs*

L-NAME, a non-selective NOS inhibitor (Sigma Chemical Co., St. Louis, MO, USA) and FR139317, an endothelin A ($ET_A$) receptor antagonist (Fujisawa Pharmaceutical, Osaka, Japan) were dissolved in physiological saline solution (PSS) immediately before administration.

*Determinations of Plasma Nitrate/Nitrite, Creatinine (Cr), Blood Urea Nitrogen (BUN), and Endothelin-l (ET-1)*

Blood samples (0.5 ml) were collected. Plasma nitrate/nitrite (the metabolites of NO) was measured with a high-performance liquid chromatography (HPLC) (26). The concentration of Cr and BUN was determined with an autoanalyzer (Vitros 750, Johnson & Johnson, Rochester, NY, USA). ET-1 concentration was measured with an ELISA kit designed for direct determination of plasma ET-1 (American Research Products, Belmont, MA, USA).

*Glomerular Dynamics*

The effective renal plasma flow (ERPF), glomerular filtration rate (GFR), and filtration fraction (FF) were determined following standard clearance formulae (p-aminohippuric acid and inulin). The filtration fraction (FF) were calculated by the equation FF=GFR/ERPF (14).

*Tissue Cyclic Guanosine Monophosphate (cGMP), and Collagen Volume Fraction*

Measurements of cGMP and collagen volume fraction in the kidney were in accordance with the procedures described previously (23, 44). Collagen was stained with picrosirius red. Sections were analyzed under the microscope using a × 20 lens. A total of 100 fields was analyzed per section. The collagen content was determined by measuring the areas of stained tissue within a set field and expressed as a percentage of the total field. An image analysis system (Uni-Chemi 16 Images System, Tokyo, Japan) recorded images, excluding fields containing artifacts, vessels or damaged tissue.

*Histopathological Examinations*

Some rats died during the course of experimentation. A certain number were anesthetized with ether inhalation, decapitated, perfused, and fixed with 4% paraformaldehyde in phosphate buffer solution. The kidney was removed. For light microscopic examination, tissue specimens were embedded in paraformaldehyde, sectioned at 5 μm thickness, and stained with hematoxylin and eosin. The glomerular injury score (GIS) was determined by a method described by Raij (36). The arteriolar injury score (AIS) of the kidney was assessed

by the method provided by Mai *et al*. (30) and modified by Ono *et al.* (33). The number of sclerotic glomeruli was counted from 100 sections per kidney and expressed as a percentage of the total glomeri examined. The calculations were performed in a blind fashion by several laboratory assistants and medical students in order to minimize subjectivity.

*Detection of Matrix Metalloproteinases and Tissue Inhibitor*

Gelatinases *i.e.* matrix metalloproteinases ($MMP_2$ and $MMP_9$) and a tissue inhibitor ($TIMP_2$) activities in homogenated renal tissue were determined with commercially available ELISA assay kits according to the manufacturer's direction. The lower limit of detection was 1 ng/mg for $MMP_2$ and $MMP_9$, and 3 ng/mg for $TIMP_2$.

In addition, reverse-transcriptase polymerases chain reaction (RT-PCR) was used to quantify $MMP_2$, $MMP_9$ and $TIMP_2$ activities. In brief, total RNA was extracted from the kidney tissue using a RNA STAT kit (Tel-Test B, Friendswood, TX, USA). Appropriate primers were used for PCR, Glyceraldehydehyde-3-phosphate dehydrogenase (GAPDH) was used for contrast. We performed the PCRs for 30 cycles using the following condition: denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 1 min. Band intensities of the amplified DNA were compared after visualization on a UV transilluminator. Scanning densitometry was performed with Image Scan and Analysis System (Alpha-Innotech Copr., San Landro, CA, USA). The relative optical density was calculated and expressed as $MMP_2$, $MMP_9$ and $TIMP_2$ to GAPDH ratios.

*Experimental Protocols*

SHR (4-wk-old, male and weighing 120-130 g) underwent a one-wk observation period before they were given L-NAME and/or FR139317. During the experiment, TCP was measured every 2 to 3 days. L-NAME (a daily dose of 50 mg/kg) with or without FR139317 (daily dose of 40 mg/kg) was given orally *via* a gastric tube twice a day (half daily dose at 8 am and 8 pm) (6). The animals received L-NAME for a period of 30 days. The number of SHR was initially more than 10. Certain rats died during the course, and some were euthanized by an over dose of sodium pentobarbital (100 mg/kg, intraperitoneal injection) for biochemical and histopathological examinations at 5, 10, 15, 20 and 30 days after drug administration.

*Statistical Analysis*

The data were expressed as mean ± SEM. Statistical evaluations of the differences among and between groups were performed with one-way analysis of variance with repeated measures followed by *post hoc* comparison with Newman-Keul's test. Differences were considered significant at $P$ value < 0.05.

## Results

*Changes in TCP*

Fig. 1A displays the changes in TCP in SHR treated with vehicle, L-NAME, FR139317 alone, and L-NAME with FR139317. FR139317 alone did not significantly affect the time course in TCP changes in SHR. Chronic administration of L-NAME caused rapid elevation of TCP above 250 mmHg in 25 days. After L-NAME administration for 22-30 days, spontaneous mortality occurred with continuous L-NAME treatment. Cotreatment with FR139317 significantly attenuated the systemic hypertension following L-NAME treatment for 10-12 days. Thereafter, the TCP increased slightly with continuous L-NAME and FR139317 administration. FR139317 cotreatment also prevented the mortality during the 30-day observation period.

*Plasma ET-1*

L-NAME caused an increase in ET-1 at day 5. The ET-1 concentration reached a maximum at day 10. After that, ET-1 was only slightly increased. Cotreatment with FR139317 did not affect the L-NAME-induced changes in ET-1 (Fig. 1B).

*Plasma Nitrate/Nitrite, Creatinine (Cr) and Blood Urea Nitrogen (BUN)*

Treatment of L-NAME in SHR for 30 days significantly decreased plasma nitrate/nitrite, but increased Cr and BUN. Compared to the values in Vehicle group, FR139317 alone slightly but significantly reduced the concentrations of these factors. Cotreatment of L-NAME with FR139317 reduced the nitrate/nitrite, Cr, and BUN (Table 1).

*Effective Renal Plasma Flow (ERPF), Glomerular Filtration Rate (GFR) and Filtration Fraction (FF)*

The glomerular dynamics was severely suppressed after L-NAME administration. FR139317 did not affect the basal glomerular dynamics compared to those in Vehicle group. Cotreatment of FR139317 with L-NAME significantly improved the ERPF, GFR and FF. However, the values were still lower than the basal levels obtained in the Vehicle group (Table 1).

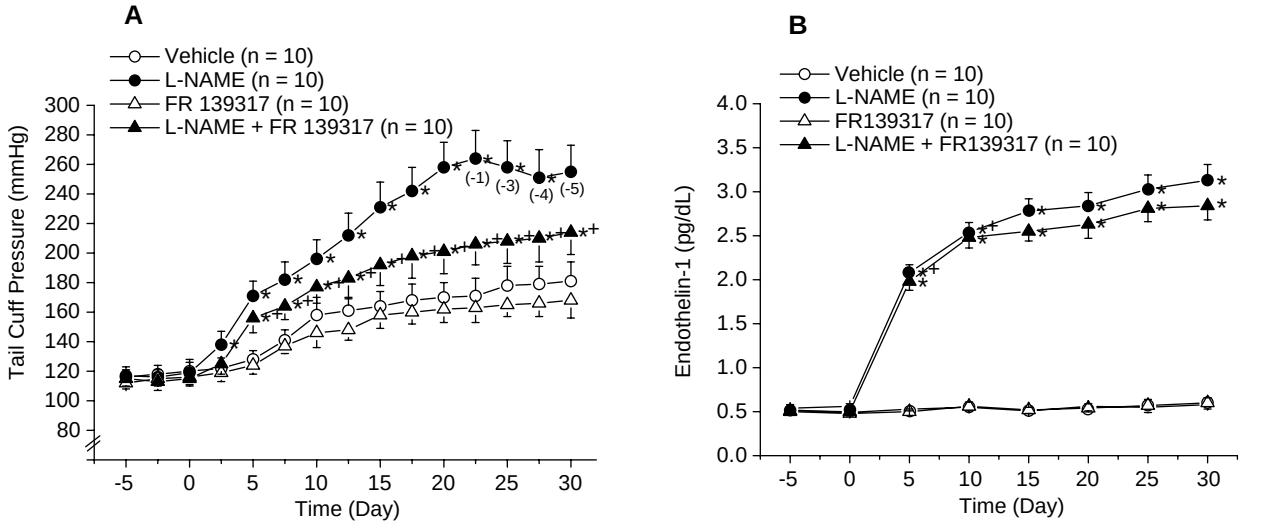


Fig. 1. Time course of changes in tail cuff pressure (A, TCP) and endothelin-1 (B, ET-1) in spontaneously hypertensive rats (SHR) receiving Vehicle, L-NAME, FR139317 and L-NAME with FR139317. Note the slight decrease in TCP with FR139317 alone and the rapid rise following L-NAME. Cotreatment of FR139317 with L-NAME significantly attenuated the L-NAME-induced hypertension throughout the entire course. In the group of SHR receiving L-NAME + FR139317, the TCP after day 10 increased only slightly in contrast to the rapid rise in the group receiving L-NAME alone. FR139317 alone did not affect the ET-1. L-NAME elevated the ET-1 at day 5 and reached a maximum at day 10. Thereafter, the ET-1 concentration stayed in a relatively steady level. Cotreatment of FR139317 with L-NAME essentially did not affect the increase in ET-1 caused by L-NAME. *$P$ < 0.05 *vs.* Vehicle group; +$P$ < 0.05 *vs.* L-NAME group. The number in parentheses with a minus sign signifies the rats that died during the observation period.

**Table 1. Plasma nitrate/nitrite (NOx), creatine (Cr), blood urea nitrogen (BUN), effective renal plasma flow (ERPF), glomerular filtration rate (GFR), and filtration fraction (FF).**

| | NOx (mmol/L) | Cr (mg/dL) | BUN (mg/dL) | ERPF (mL/min/g) | GFR (mL/min/g) | FF (%) |
|---|---|---|---|---|---|---|
| Vehicle | 974 ± 47 | 1.24 ± 0.07 | 1.84 ± 0.04 | 1.68 ± 0.19 | 0.48 ± 0.16 | 28.6 ± 3.7 |
| L-NAME | 54 ± 16$^{*}$ | 4.38 ± 0.16$^{*}$ | 3.96 ± 0.12$^{*}$ | 0.64 ± 0.08$^{*}$ | 0.11 ± 0.05$^{*}$ | 17.2 ± 1.8$^{*}$ |
| FR139317 | 634 ± 32$^{*+}$ | 1.02 ± 0.06$^{*+}$ | 1.26 ± 0.16$^{*+}$ | 1.63 ± 0.18 | 0.46 ± 0.16 | 28.2 ± 2.8 |
| L-NAME + FR139317 | 36 ± 12$^{*+}$ | 1.96 ± 0.14$^{*+}$ | 2.16 ± 0.11$^{*+}$ | 1.24 ± 0.09$^{*+}$ | 0.28 ± 0.11$^{*+}$ | 22.6 ± 1.9$^{*+}$ |

Values are means ± SEM (n = 10 in each group). $^{*}P$ < 0.05 *vs.* Vehicle; $^{+}P$ < 0.05 *vs.* the corresponding values in L-NAME group.

*Cyclic Guanosine Monophosphate (cGMP), Collagen Volume Fraction (CVF), Number of Sclerotic Glomeruli (NSG), Arteriolar Injury Score (AIS) and Glomerular Injury Score (GIS)*

In the kidney, L-NAME treatment reduced the tissue cGMP, while increasing the CVF, NSG, AIS and GIS. FR139317 alone essentially did not alter the basal values in the Vehicle group. Cotreatment with FR19317 significantly alleviated the L-NAME-induced changes in CVF, NSG, AIS and GIS, but did not affect the cGMP level (Table 2).

*Pathological Findings of the Kidney*

Fig. 2 illustrates the pathological changes of the kidney in rats receiving L-NAME for 30 days. There were abundant sclerotic glomeruli in the subcapsular and jexamedullary tissues. The lesions were characterized by fibrin deposition in the capsular epithelium, adhesion of the capillary tuft to Bowman's capsule, arteriolar hyalinosis, medial hypertrophy and luminal obliteration. Tubulointerstitial fibrosis with inflammatory cell aggregation was also observed and the vascular wall was altered with eosinophilic, fibrin-rich deposit, luminal narrowing and encroachment. Cotreatment with FR139317 attenuated the glomerular damage.

*Metalloproteinases and Tissue Inhibitor of Matrix Metalloproteinase*

Fig. 3 shows the RT-PCR detection of time-

**Table 2. Renal tissue cyclic guanosine monophosphate (cGMP), collagen volume fraction (CVF), number of sclerotic glomeruli (NSG), arteriolar injury score (AIS), and glomerular injury score (GIS).**

| | cGMP (fmol/mg) | CVF (%) | NSG (%) | AIS | GIS |
|---|---|---|---|---|---|
| Vehicle | 4998 ± 136 | 1.74 ± 0.36 | 4.7 ± 0.6 | 46 ± 5 | 48 ± 6 |
| L-NAME | 2012 ± 127* | 4.69 ± 0.86* | 58.9 ± 5.8* | 249 ± 20* | 228 ± 18* |
| FR139317 | 4880 ± 132 | 1.62 ± 0.28 | 4.2 ± 0.5 | 44 ± 5 | 42 ± 7 |
| L-NAME + FR139317 | 1994 ± 128* | 2.26 ± 0.48*+ | 21.8 ± 3.9*+ | 103 ± 16*+ | 96 ± 11*+ |

Values are means ± SEM (n = 10 for each group). *$P < 0.05$ *vs.* Vehicle; +$P < 0.05$ *vs.* the corresponding values in L-NAME group.

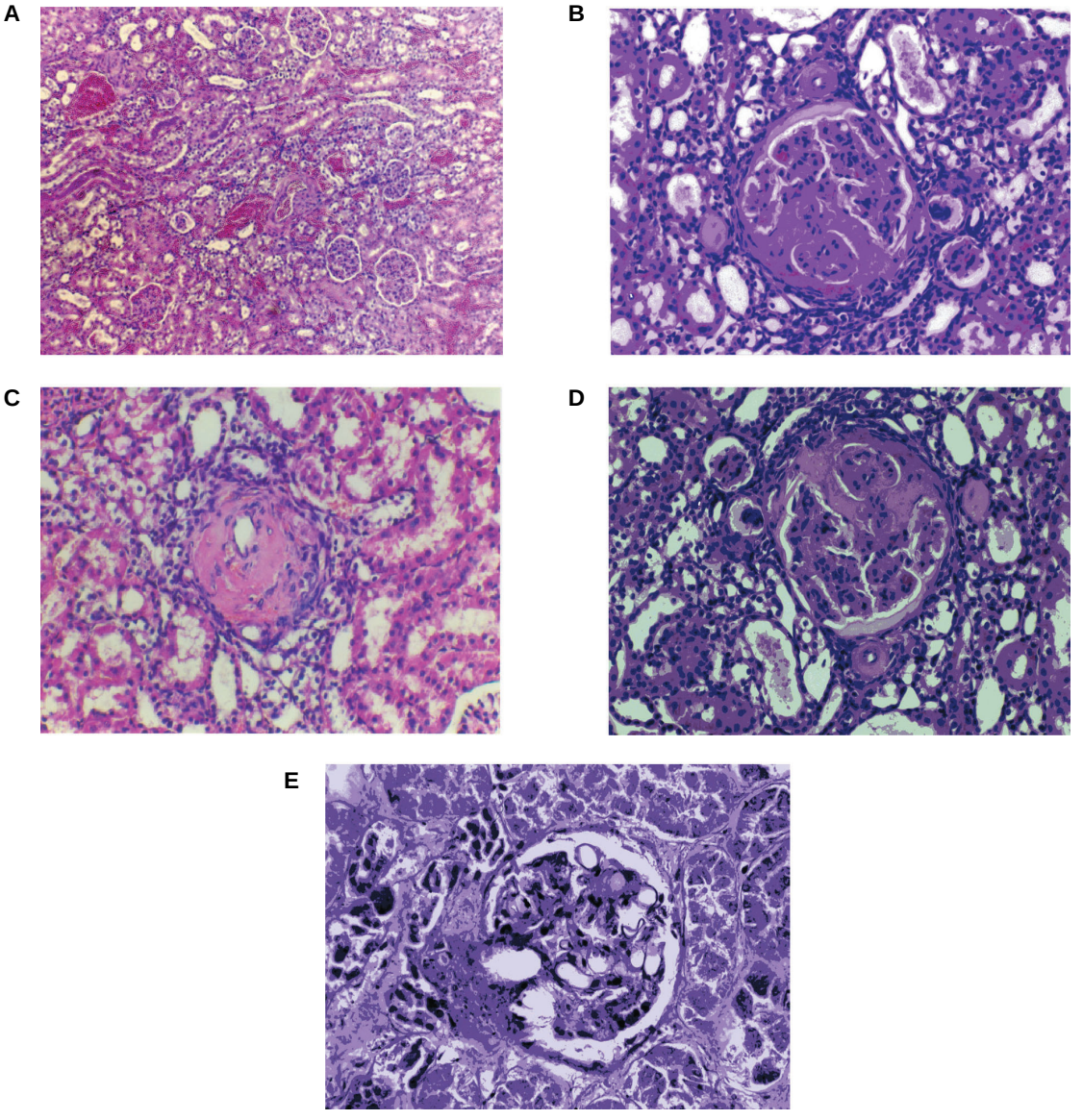


Fig. 2. Histopathological micrography of renal tissue in SHR following L-NAME administration for 30 days. Note the numerous sclerotic glomeruli with interstitial fibrosis and inflammatory cells infiltration (A). There are fibrin depositions in the capsular epithelium, adhesion and encroachment of the capillary tuff to Bowman's capsule (B). Severe fibrinoid sclerosis and complete obliteration of arterioles. The structure of the vessel wall is altered with eosinophilic and fibrin-rich deposits and severe luminal narrowing (C). Comparison of nephroglomerulosclerosis in L-NAME treated SHR (D) and L-NAME cotreated with FR139317 (E). Note the severe glomerulosclerosis in D compared to the mild changes in E. FR139317 reduced the nephrosclerosis caused by NO deprivation (Hematoxylin and eosin stain, original magnification, ×100 for A and ×200 for B-E).

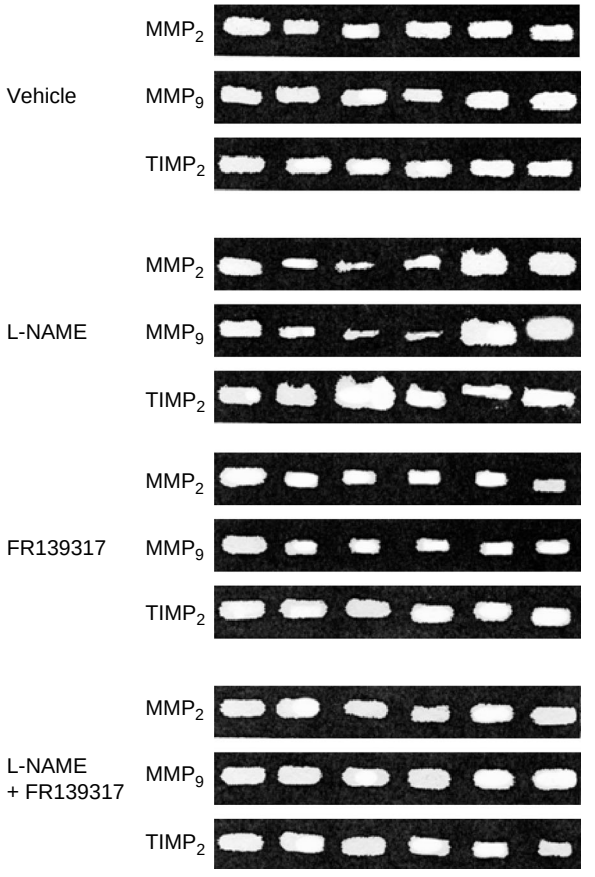


Fig. 3. Time course of matrix metalloproteinases ($MMP_2$ and $MMP_9$) and tissue inhibitor ($TIMP_2$) in various groups. Reverse-transcriptase polymerase chain reaction was used for the detection of mRNA expression. GAPDH served for contract. For clarity, GAPDH bands were not shown.

dependent changes in metalloproteinases ($MMP_2$ and $MMP_9$) and tissue inhibitors of MMPs ($TIMP_2$) mRNA expression at different days in various treatment groups. Table 3 contains the concentration of $MMP_2$, $MMP_9$ and $TIMP_2$ obtained with ELISA at various days. Both RT-PCR and ELISA data revealed that FR139317 alone slightly decreased the $MMP_2$, $MMP_9$, while increased the $TIMP_2$ after day 5. L-NAME administration for 5-10 days caused significant decreases in $MMP_2$ and $MMP_9$ with an increase in $TIMP_2$. The changes reached a maximum at day 10 and were not further enhanced after day 15. Following 15-20 days of L-NAME administration, the opposite changes occurred. There were increases in $MMP_2$ and $MMP_9$. At day 30, the activities of MMPs and TIMP were similar to the values at day 20. Cotreatment of FR139317 with L-NAME significantly alleviated the L-NAME-induced changes in $MMP_2$ and $MMP_9$ throughout the whole 30-day observation period. The $ET_A$ antagonist cotreatment abrogated the L-NAME-induced increase in $TIMP_2$ from day 5 to 15. After day 20, FR139317 cotreatment exerted little effects on the MMPs and inhibitor activities compared to the values at day 10.

## Discussion

Experimental and clinical studies have suggested that matrix metalloproteinases and the tissue inhibitors are involved in the extracellular matrix metabolism and vascular remodeling in hypertension (1, 11, 25, 29, 47, 48). Earlier studies have revealed that NO deprivation with L-NAME caused hypertension, arteriosclerosis, cardiovascular remodeling and glomerular damage (17, 18, 30, 33). In the present investigation, we found that L-NAME administration to SHR for 30 days caused severe hypertension and glomerular sclerosis. The renal pathology was accompanied by an elevation in plasma ET-1, impairment of glomerular dynamics, reduction in plasma nitrate/nitrite, and increases in blood urea nitrogen and creatinine. In the kidney, the cGMP was depressed, while collagen content, number of sclerotic glomeruli, the arteriolar and glomerular injury scores were remarkably increased. An $ET_A$ receptor antagonist (FR139317) alone exerted slight effects on these changes. However, cotreatment of FR139317 with L-NAME tended to improve most L-NAME-induced functional and morphological changes of the kidney.

The histopathological micrography displayed an increase in fibrin deposition, and the renal collagen content was augmented. The observations suggest an enhanced extracellular matrix. Detection of $MMP_2$, $MMP_9$ and $TIMP_2$ with ELISA and RT-PCR illustrated the time course of changes in gelatinases and tissue inhibitors. Interestingly, L-NAME treatment reduced $MMP_2$ and $MMP_9$, but it enhanced the $TIMP_2$ after L-NAME for 5 to 10 days. After day 20, opposite changes were observed. There were increases in $MMP_2$ and $MMP_9$ with a decrease in $TIMP_2$. The time-dependent changes in MMPs and inhibitors coincided with the plasma ET-1 concentration (Fig. 1B). Furthermore, the effects of $ET_A$ antagonism with FR139317 on the L-NAME-induced changes suggest that ET-1 is involved in the glomerular alterations following L-NAME treatment.

Several studies have implicated the involvement of ET-1 in many hypertensive models including deoxycorticosterone (DOCA)-salt, salt-sensitive and hydroxysteroid dehydrogenase inhibition-induced hypertension (1, 10, 21, 28, 32, 37, 38). These studies focused mainly on the role of ET in the regulation of cardiac function and structure, blood pressure and vascular reactivities. In DOCA-salt hypertensive rats with cardiac fibrosis, blockades of $ET_A$ receptor reduced $MMP_2$, and $MMP_9$ with concomitant improvement of myocardial fibrosis, indicating ET-1 was responsible

**Table 3. Changes in matrix metalloproteinase ($MMP_2$ and $MMP_9$) and tissue inhibitor ($TIMP_2$) in renal tissue at different days.**

| Day | 0 | 5 | 10 | 15 | 20 | 30 |
|---|---|---|---|---|---|---|
| $MMP_2$ (ng/mg) | | | | | | |
| Vehicle | 138.2 ± 7.4 | 137.3 ± 6.2 | 140.1 ± 7.4 | 146.3 ± 7.6 | 139.6 ± 6.8 | 146.0 ± 7.6 |
| L-NAME | 136.4 ± 8.9 | 96.2 ± 5.8$^{*+}$ | 64.2 ± 4.6$^{*+}$ | 98.8 ± 6.5$^{*+}$ | 198.4 ± 10.3$^{*+}$ | 188.6 ± 9.4$^{*+}$ |
| FR139317 | 129.8 ± 6.2 | 101.2 ± 6.4$^{*+}$ | 84.6 ± 5.1$^{*+}$ | 139.4 ± 6.8 | 134.6 ± 5.4 | 130.3 ± 4.6 |
| L-NAME + FR139317 | 139.6 ± 8.9 | 129.8 ± 6.8$^{\S}$ | 134.6 ± 7.3$^{\S}$ | 116.4 ± 4.9$^{\S}$ | 128.8 ± 3.4$^{\S}$ | 129.4 ± 4.4$^{\S}$ |
| $MMP_9$ (ng/mg) | | | | | | |
| Vehicle | 106.4 ± 7.6 | 107.2 ± 8.8 | 109.2 ± 7.4 | 113.6 ± 6.8 | 108.4 ± 6.6 | 110.2 ± 7.0 |
| L-NAME | 103.7 ± 8.4 | 88.4 ± 6.6$^{*+}$ | 66.6 ± 4.2$^{*+}$ | 74.2 ± 3.8$^{*+}$ | 148.2 ± 6.4$^{*+}$ | 142.6 ± 5.8$^{*+}$ |
| FR139317 | 101.4 ± 9.2 | 112.4 ± 7.4 | 104.8 ± 6.2 | 106.9 ± 3.4 | 110.2 ± 4.8 | 109.8 ± 3.9 |
| L-NAME + FR139317 | 105.6 ± 8.8 | 99.6 ± 4.8$^{\S}$ | 98.4 ± 3.8$^{\S}$ | 78.4 ± 3.9$^{+}$ | 80.2 ± 4.2$^{\S}$ | 84.3 ± 3.2$^{\S}$ |
| $TIMP_2$ (ng/mg) | | | | | | |
| Vehicle | 26.6 ± 2.4 | 27.2 ± 2.6 | 28.4 ± 3.2 | 26.3 ± 2.2 | 2.5 ± 2.4 | 26.9 ± 2.3 |
| L-NALE | 25.8 ± 2.1 | 40.6 ± 3.2$^{*+}$ | 48.4 ± 4.0$^{*+}$ | 53.6 ± 4.2$^{*+}$ | 26.7 ± 2.3 | 29.6 ± 2.4 |
| FR139317 | 25.2 ± 1.8 | 26.7 ± 2.2 | 25.6 ± 2.4 | 26.2 ± 3.1 | 24.6 ± 2.4 | 25.1 ± 2.6 |
| L-NAME + FR139317 | 25.6 ± 2.2 | 30.2 ± 2.8$^{\S}$ | 27.8 ± 2.6$^{\S}$ | 25.3 ± 2.4$^{\S}$ | 26.8 ± 3.2 | 25.4 ± 2.8 |

Values are means ± SEM (n = 10 in each group). $^{*}P < 0.05$ *vs.* Vehicle; $^{+}P < 0.05$ *vs*. the values at day 0 (no treatment); $^{\S}P < 0.05$ *vs*. the corresponding values obtained from L-NAME group.

for cardiac metalloproteinase regulation and matrix remodeling in DOCA-salt hypertension (1).

Our present investigation of L-NAME-induced hypertension and the associated renal changes are in agreement with earlier studies in normotensive rats and transgenic mice harboring the luciferase gene (3, 39). In these animal models, L-NAME treatment caused hypertension and renal vascular fibrosis with collagen 1 gene expression and synthesis. The renal ET-1 content was markedly increased. The L-NAME-induced changes were prevented by bosentan, a non-selective ET-1 ($ET_A$ and $ET_B$) receptor antagonist. These findings suggest that ET-1 is a major activator of collagen 1 formation and participates in renal vascular fibrosis. The studies were carried out in animal models different from that used in our experimentation. In addition, they did not investigate the role of specific ET-1 receptor and the changes of MMP/TIMP system.

Matsumura *et al*. (32) used spotting lethal rats carrying a deletion in the ETB receptor gene and revealed that DOCA-salt caused systemic hypertension, vascular hypertrophy, renal dysfunction and glomerular damage. The DOCA-salt effects developed earlier and higher in ETB receptor deficient rats than wild-type rats. Furthermore, chronic treatment with $ET_A$ receptor antagonist suppressed the DOCA-salt-induced alterations in both $ET_B$ deficient and wild-type rats. These results suggest that $ET_B$-mediated actions are protective in the pathogenesis of DOCA-salt-induced hypertension and renal dysfunction. On the other hand, enhanced ET-1 production and $ET_A$-mediated action are responsible for the increased susceptibility and severity in DOCA-salt hypertension. In rats subjected to chronic stressful condition, the expression of $MMP_2$ and $MMP_9$ was enhanced before the development of hypertension. $ET_A$ receptor antagonism abolished the MMPs expression. The study indicates that ET-1 mediates the early events in gelatinases activity and vascular reactivity changes due to stress (11).

The effects of ET-1 on the MMP system in DOCA-salt and stress-induced hypertension may explain the early stage (5-10 days) changes in $MMP_2$, $MMP_9$, $TIMP_2$, ET-1 and renal morphology in NO-deprivated hypertension. Our results demonstrated that the plasma ET-1 was raised following L-NAME administration for 5 days and reached a maximum at day 10. Blockade of $ET_A$ receptor with FR139317 attenuated the L-NAME-induced hypertension and improved the functional and histological changes in the kidney. The findings indicate that $ET_A$-mediated effects play detrimental role in the L-NAME-induced hypertension and renal damage. In certain aspects, our results are in agreement with the involvement of $ET_A$ receptor in the DOCA-salt hypertension.

It has been proposed that ET-1 *via* $ET_A$ receptor exerts pro-fibrotic action. On the other hand, NO acting through the soluble guanylate cyclase exerts anti-fibrotic effect (40). Accordingly, it is likely that the early elevation of ET-1 following L-NAME contributes to the changes in $MMP_2$, $MMP_9$, $TIMP_2$, and renal morphology. We observed in some SHRs that glomerular lesions had already developed following L-NAME administration for 10 to 15 days (not shown). In a previous study from our laboratory, we also found severe macrophage/monocyte infiltration in the adventitial layer of the middle cerebral artery in SHR as early as 2 weeks after L-NAME treatment (17). Mutual interaction between ET and MMP system has been demonstrated in various experimental conditions. In cultured mesangial cells, exogenous ET-1 reduced $MMP_2$ but increased $TIMP_2$. Furthermore, $ET_A$ antagonism with FR139317 restored the $MMP_2$ secretion and activation. These findings suggest that ET-1 is a $MMP_2$ inhibitor (42).

Although ET-1 may exert inhibitory action on MMP systems, interaction and coexistence between ET-1 and MMP systems have been demonstrated. The biosynthesis of mature and active ET-1 requires the conversion of big ET to ET-1 *via* the catalytic actions of one or more MMPs or ET converting enzymes (27). In patients with plexogenic pulmonary arteriopathy, immunohistochemical staining revealed colocalization of $MMP_2$ and ET in various lesions in the pulmonary arterial bed (31). The effects of NO on the MMP/TIMP system are still controversial. In rats' cultured mesangial cells, NO reduced the interleukin-1 stimulated enhancement in $MMP_9$ and $TIMP_1$ (9). In endothelial NOS gene transferred mice compared to wild-type, inhibition of $MMP_2$ and $MMP_9$ with activation of $TIMP_2$ was observed (15).

Fernandez-Patron and coworkers reported that $MMP_2$ cleaved big ET-1 to active form of ET-1 in perfused rat mesenteric arteries and cultured coronary endothelial cells (12, 13). The effect of gelatinase on ET-1 cleavage contributes to vasoconstriction and neutrophil-endothelial cell adhesion. Thus, the generation of ET might directly and/or reciprocally activate or enhance the proteolytic activities of MMP system. In the present study, we observed increases in $MMP_2$ and $MMP_9$ with a decrease in $TIMP_2$ in the late stage (20-30 days) of chronic NO deprivation. The opposite changes in MMP/TIMP systems may be attributable to a compensatory response that prevents further structural alterations in the kidney. In connection to the findings by Fernandez-Patron *et al.* (12, 13), it appears that ET-1 may contribute only slightly to the increases in MMPs with a decrease in $TIMP_2$ in SHR following L-NAME treatment for 20-30 days, since the plasma ET-1 increased only slightly after day 15. The ultimate mechanism requires further investigation.

It has been known that the kidney contains high concentration of ET-1 and is a major target organ of this peptide. Aberrations in the renal ET system may lead to renal function impairment and subsequent development of hypertension (8). Renal damage due to long-term hypertension further exacerbates hypertension, thereby creating a vicious cycle. L-NAME-induced hypertension has been used as a hypertensive model with renal damage. However, the information on the role of ET and gelatinases in cardiovascular and renal functions associated with chronic NO deprivation has not been clarified. The results of the present investigation may provide information on the regulation of matrix metabolism and the involvement of ET and gelatinases. Advanced experimental and clinical studies are necessary for elucidating the interaction between ET and MMP system in renal functions and morphology at various stages of different hypertensive models.

In conclusion, the results of the present study demonstrated that $ET_A$ receptor antagonism with FR139317 slightly decreased the basal arterial pressure, thus indicating that ET-1 plays a little role in the basal arterial pressure. In addition, L-NAME caused a rapid rise in arterial pressure and the hypertension was greatly alleviated by cotreatment with $ET_A$ receptor antagonist following L-NAME treatment for 10 to 15 days. The effects of the $ET_A$ receptor blockade were concomitant with the time course of change in plasma ET-1 following L-NAME administration. The findings imply that ET-1 was involved in the early stage of NO-deprivated hypertension. ET-1 might also be responsible for the changes in gelatinases and tissue inhibitors. At the early stage, decreases in MMPs with increase in $TIMP_2$ possibly accounted for the renal functional and morphological alterations. The opposite changes of MMPs/TIMP in the late stage might be explained by a compensatory mechanism to prevent further advance in renal function impairment and matrix accumulation leading to severe glomerular sclerosis.

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