p38 MAPK Inhibitors Ameliorate Target Organ Damage in Hypertension: Part 1. p38 MAPK-Dependent Endothelial Dysfunction and Hypertension

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ABSTRACT
Numerous mediators, believed to play a role in endothelial dysfunction (e.g., neurohormones, cytokines, hypoxia, and stretch), have been shown to activate p38 mitogen-activated protein kinase (MAPK) in a variety of cell types. The purpose of the present study was to examine the regulation of p38 MAPK in endothelium and its role in endothelial dysfunction and salt sensitivity. In cultured human umbilical vein endothelial cells (HUVECs), tumor necrosis factor-α and lipopolysaccharide increased phosphorylation of p38 MAPK (P-p38 MAPK) and increased ICAM-1 expression. Preincubation with highly selective p38 MAPK inhibitors, 1-(1,3-dihydroxyprop-2-yl)-4-(4-fluorophenyl)-5-[2-phenoxypyrimidin-4-yl] imidazole (SB-239063AN) or SB-239063, dose dependently reduced intercellular adhesion molecule-1 expression in HUVECs. In spontaneously hypertensive-stroke prone rats (SHR-SP), P-p38 MAPK was localized by immunohistochemistry to the aortic endothelium and adventitia but was undetectable in aortae from normotensive rats. Introduction of a salt/fat diet (SFD) to the SHR-SP strain induced endothelial dysfunction (ex vivo vascular reactivity analysis), albuminuria, and an increase in blood pressure within 4 weeks. Chronic dietary dosing (approx. 100 mg/kg/day) with SB-239063AN inhibited the SFD diet-induced hypertension. In addition, delayed treatment also significantly improved survival and restored nitric oxide-mediated endothelium-dependent relaxation in SFD-SHR-SPs with established endothelial dysfunction. These results suggest an important role for p38 MAPK in endothelial inflammation and dysfunction as well as providing the first evidence for p38 MAPK-dependent hypertension.

Endothelial-derived factors normally play an important role in maintaining vascular homeostasis by regulating vasomotion, inflammation, thrombosis, and smooth muscle proliferation (Kinlay et al., 2001; Schiffrin, 2002). In contrast, endothelial dysfunction has been implicated in the pathogenesis and clinical course of major cardiovascular disease (Verma and Anderson, 2002; Schiffrin, 2002). In particular, endothelial dysfunction is associated with atherosclerosis and hypertension as well as diabetes and dyslipidemia (Ross, 1999; Shimokawa, 1999).

The mechanisms underlying endothelial dysfunction are not well understood but evidence suggests that growth factors, angiotensin II, oxidative stress, elevated low-density lipoprotein, and cytokines are contributing factors. All have been shown to activate p38 MAPK signaling in a variety of cell types with only limited supporting evidence in endothelial cells and endothelial dysfunction (Huot et al., 1997; Pietersma et al., 1997; Read et al., 1997; Tamura et al., 1998; Raitakari and Celermajer, 2000; Touyz et al., 2001; Zhu et al., 2001).

The first part of this two-part study examined the hypothesis that p38 MAPK plays an important role in endothelial dysfunction associated with salt-sensitive hypertension and that treatment with a selective p38 MAPK inhibitor would restore endothelial-dependent vasorelaxation. Specifically, the regulation of p38 MAPK and the downstream expression of adhesion molecules were examined in human umbilical vein endothelial cells (HUVECs). In addition, the effects of treatment with selective p38 MAPK inhibitors (SB-239063 and SB-239063AN) on endothelial-dependent vasorelaxation, blood pressure, microalbuminuria, and survival were determined in the spontaneously hypertensive stroke prone rat (SHR-SP).

ABBREVIATIONS: HUVEC, human umbilical vein endothelial cell; SHR-SP, spontaneously hypertensive-stroke prone; TNF, tumor necrosis factor; MAPK, mitogen-activated protein kinase; LPS, lipopolysaccharide; ICAM-1, intercellular adhesion molecule-1; PECAM-1, platelet-endothelial cell adhesion molecule-1; WKY, Wistar-Kyoto; SD, Sprague-Dawley; SFD, salt/fat diet; ND, normal chow diet; SNP, sodium nitroprusside; P-p38, phospho-p38; MAP, mean arterial pressure; BP, blood pressure; NO, nitric oxide; ROS, reactive oxygen species.
rat (SHR-SP). A separate second part of this study examined the role of p38 MAPK in renal dysfunction associated with hypertensive salt sensitivity (Lenhard et al., 2003).

Materials and Methods

Endothelial Cell Culture and Treatment. HUVECs were purchased from Cambrex Bio Science Walkersville, Inc. (Walkersville, MD) and were cultured in EGM-2 basal medium supplied with 2% fetal bovine serum and growth factors (as recommended by Cambrex Bio Science Walkersville) in a humidified incubator at 37°C with 5% CO2. HUVECs were grown in 100-mm culture dishes, passaged using 0.05% trypsin/1 mM EDTA solution, and experiments were performed in confluent HUVECs, passage 5 to 6, maintained in EGM-2 basal medium supplied with 0.5% fetal bovine serum without growth factors. Temporal p38 MAPK activation studies were performed in HUVECs stimulated with TNF-α at a final concentration of 1 ng/ml for 5 to 60 min. When examining expression of adhesion molecules, HUVECs were pretreated with various concentrations of a selective p38 MAPK inhibitor (SB-239063AN, for 1 h before stimulation with TNF-α (1 ng/ml) or 100 ng/ml LPS 011:B4 (Sigma-Aldrich, St. Louis, MO) for 6 h (Kim et al., 2001). HUVECs were then washed in cold phosphate-buffered saline and disrupted using lysis buffer containing 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na3VO4, and 1 μg/ml leupeptin (Cell Signaling Technology Inc., Beverly, MA). Cell lysates were used for Western blot analysis. All HUVEC experiments were repeated at least three times, and representative blots are presented.

Western Blotting. Harvested HUVECs were sonicated twice for 5 s and were centrifuged at 14,000g for 10 min. The resulting supernatant was used for protein determination (DC protein assay; Bio-Rad, Hercules, CA) and subsequent Western blot analysis. Briefly, 30 μg of protein was resolved on 8 to 16% precasted SDS-PAGE tris-glycine gel (Invitrogen, Carlsbad, CA), and protein was transferred to a polyvinylidene difluoride membrane. Nonspecific binding was blocked by incubation of the membrane with blocking solution (Zymed Laboratories, San South Francisco, CA) at room temperature for 1 h. The membrane was then incubated with primary antibodies in blocking solution overnight at 4°C followed by incubating with horseradish peroxidase-conjugated secondary antibodies at room temperature for 1 h. Primary antibodies used in the current study were P-p38 MAPK (Cell Signaling Technology Inc.), p38 MAPK (Cell Signaling Technology Inc.), ICAM-1 (Santa Cruz Biotechnology Inc., Stan Cruz, CA), and PECAM-1 (Santa Cruz Biotechnology Inc.) in 1:500 to 1:1000 dilution. Immunoreactive bands were detected using chemiluminescence detection reagent (Amersham Biosciences Inc., Piscataway, NJ).

SHR-SP Studies. SHR-SP rats, obtained from the National Institutes of Health (Bethesda, MD), were bred in the Department of Laboratory Animal Science at GlaxoSmithKline (King of Prussia, PA). Age-matched normotensive rats [Wistar-Kyoto (WKY) and Sprague-Dawley (SD)] were purchased from Charles River Laboratories, Inc. (Wilmington, MA). Experiments were conducted in accordance with the Guide for Care and Use of Laboratory Animals (NIH Publication 85-23), and experimental protocols were reviewed and approved by the GlaxoSmithKline Animal Care and Use Committee.

Endothelial Dysfunction and Albuminuria Time-Course Study. Vascular reactivity was examined in SHR-SPs on a normal chow diet (n = 18) and in normotensive WKY rats (n = 18) in subsets (n = 6) at 0 (10–12 weeks of age), 4, and 8 weeks. SHR-SPs on a salt/fat diet (SFD) were examined at 4 and 8 weeks after starting the diet (n = 5–6/group). The SFD has been described previously and contains 24.5% fat in the food and 1% NaCl in the drinking water provided ad libitum (Barone et al., 1996). A 24-h urine collection was obtained weekly from each SHR-SP animal and albumin excretion (milligrams per day) was determined using an immunoturbidimetric assay optimized for the determination of rat urinary albumin in an AutoAnalyzer format (KRA-010/020, Kamiya Biomedical, Seattle, WA).

Treatment Study. A colony of 62 male SHR-SPs, aged 9 to 12 weeks, was divided and assigned to one of three groups. All animals were placed on the normal chow diet (NIH-07 diet) while baseline/entry monitoring was completed and then assigned to diet or treatment groups. One SHR-SP group was maintained on the normal chow diet (ND) throughout the study (n = 10). The other two groups received SFD for a “run-in” period until 10% mortality (about 6 weeks). Then, 18 SHR-SPs in the SFD group received diet containing 1500 ppm of SB-239063AN (see Fig. 1 for chemical structure), a highly selective p38α/β MAPK inhibitor, for the duration of the study (12 weeks). The remaining SHR-SPs in the SFD group continued to receive the SFD until the end of the study (n = 28). Mean age and body weight were consistent in all groups. SFD-SHR-SPs have an accelerated mortality rate and were promptly euthanized when signs of morbidity were noted. These signs may include piloerection, lack of grooming, hypersensitivity to sound or touch, loss of appetite in the setting of cachexia, ataxia, decreased movement, and convulsive movements (Behr et al., 2001).

Assessment of Vascular Reactivity. All animals were randomly selected from the time course or treatment groups (n = 5–6/group) and anesthetized with 5% isoflurane in O2, and their proximal aorta movements (Behr et al., 2001). Accelerated mortality rate and were promptly euthanized when signs of morbidity were noted. All animals were randomly selected from the time course or treatment groups (n = 5–6/group) and anesthetized with 5% isoflurane in O2, and their proximal aorta movements (Behr et al., 2001). Accelerated mortality rate and were promptly euthanized when signs of morbidity were noted. Changes in isometric force were measured under a resting tension (1 g) using FT03 force-displacement transducers (Grass Instruments, Quincy, MA) coupled to model 7D polygraphs. After a 60-min equilibration period, the vessels were treated with standard concentrations of KCl (60 mM) and norepinephrine (1 μM). Vascular reactivity was evaluated by examining the cumulative concentration-concentration relationship elicited by comparing half-log increments (0.1 nM–10 μM) of norepinephrine to the tissue baths. Endothelium-dependent vasorelaxation was assessed in vessels precontracted with an approximate EC80 norepinephrine (100 nM) by comparing concentration-related carbachol-induced relaxation. Cumulative concentration-response curves to carbachol were obtained by adding half-log increments (1 nM–100 μM) of the relaxant to the tissue. In a similar manner, endothelium-independent vasorelaxation was demonstrated using sodium nitroprusside (SNP) at 0.1 nM to 10 μM. Norepinephrine contraction was expressed as a percentage of the contractile response to 60 mM KCl and carbachol and SNP relaxation was expressed as percentage of reversal of the norepinephrine precontraction response.

Blood Pressure Telemetry Studies. Male SHR-SP rats (5–6 weeks of age) maintained on a normal powdered diet (Purina Diet
were anesthetized with 2% isoflurane anesthesia and a telemetry transmitter (TA11PA-C40; Data Sciences International, St. Paul, MN) was implanted. The transmitter catheter was inserted into the femoral artery and advanced threaded to the lower abdominal aorta. At approximately 8 weeks of age, animals were fed one of three diets: 1) SFD composed of a high-fat diet (24.5% fat; Zeigler Bros., Gardner, PA) plus 1% NaCl drinking water (n = 10 rats); 2) High-fat diet containing trans-1-(4-hydroxycyclohexyl)-4-(fluorophenyl) methoxy pyridimidin-4-yl)limidazole (SB-239063) (Fig. 1) at 1200 ppm plus 1% NaCl drinking water (n = 6 rats); or 3) normal powdered diet plus tap water (n = 5 rats). Baseline measurements of systolic and diastolic blood pressure, heart rate, and activity were obtained 1 week before starting special diets. Recordings were obtained each week thereafter for a continuous period of 48 h with data acquisition of 10-s averages every 5 min. Body weight and fluid consumption were measured daily. All animals were monitored for 6 weeks.

Pilot studies were performed in SFD-SHR-SP to establish dosing regimens sufficient to attain plasma peak and trough concentrations of approximately 1.5 and 0.5 μM, respectively, for SB-239063AN and SB-239063. In the present chronic studies, plasma samples were prepared for drug analysis after 3 weeks of treatment (sampling time was 10:00–11:00 AM).

Phospho-p38 MAPK Immunohistochemistry. Thoracic aortae from age-matched (26–28-week) SHR-SP and SD rats (n = 5/group) were rapidly harvested and fixed in 10% formalin overnight and then kept in 70% ethanol until paraffin embedding, sectioning, and staining for phospho-p38 MAPK. Slides were deparaffinized, rehydrated, and placed in phosphate-buffered saline with 0.1% Tween 20. Sections were stained using streptavidin-horseradish peroxidase labeling system on the DAKO autostainer (DAKO, Carpinteria, CA). Briefly, the slides were exposed to 3% hydrogen peroxide for 15 min, and nonspecific binding was blocked with normal serum at 1:50. Sections were incubated with the primary antibodies for 45 min followed by a 30-min incubation with biotinylated secondary antibodies. Slides were incubated in 1:200 dilution of streptavidin-horseradish peroxidase (DAKO) for 15 min followed by incubation with substrate 3,3’-diaminobenzidine for 5 min. The slides were counterstained with hematoxylin, dehydrated, and coverslipped. A monoclonal antibody (1:10 dilution) was used for the localization of phospho-p38 MAPK (Sigma-Aldrich).

Drugs. Potency, selectivity, in vivo and in vitro efficacy profiles, and pharmacokinetic properties were similar for SB-239063 and SB-239063AN (Fig. 1). Both compounds have been cross-screened for activity in a panel of 56 protein kinases, and IC50 values have been generated where possible. SB-239063 and SB-239063AN are potent p38 inhibitors (IC50 = 46 and 36 nM, respectively) with selectivity of 25- and 4-fold versus c-Jun NH2-terminal kinase 2, respectively, and ≥600-fold versus all other kinases tested. In an LPS-stimulated human whole blood assay, SB-239063 and SB-239063AN inhibited TNF-α production with IC50 values of 1.0 and 0.3 μM, respectively.

Statistical Analysis. All summary values are expressed as the mean ± standard error of the mean. Chi-square analysis was performed for quantal responses at each time interval. Multiple comparisons of the means were made by analysis of variance followed by post hoc analysis with the Bonferroni correction for multiple comparisons. All statistical analyses were done using InStat (GraphPad Software Inc., San Diego, CA) and P ≤ 0.05 was considered to be significant.

Results

Activation of Endothelial p38 MAPK in Vitro and in Vivo. The activation of p38 MAPK was examined by Western blot analysis of phospho-p38 (P-p38) MAPK in cultured HUVECs. As shown in Fig. 2A, incubation of HUVECs with a proinflammatory cytokine, TNF-α (1 ng/ml), produced a rapid increase in p38 MAPK phosphorylation that persisted for approximately 30 min. LPS induced a similar temporal activation pattern in HUVECs (data not shown). In contrast, total p38 MAPK was not altered by TNF-α treatment.

To investigate endothelial p38 MAPK activation in situ, P-p38 MAPK immunohistochemistry was performed on thoracic aortae obtained from age-matched (16–18-week) SHR-SPs and normotensive Sprague-Dawley rats. Striking differences in phospho-p38 MAPK immunoreactivity were noted in the hypertensive and normotensive strains. P-p38 MAPK immunoreactivity was localized primarily to the endothelium and adventitial/medial boarder in the SHR-SP aortae (Fig. 2C). In contrast, little or no phospho-p38 MAPK was evident in aortae from normotensive SD and WKY rats (Fig. 2B).

Effect of p38 MAPK Inhibition on Adhesion Molecule Expression in HUVECs. The effects of a selective p38 MAPK inhibitor, SB-239063AN, on adhesion molecule expression induced by inflammatory mediators were examined in cultured HUVECs. Both LPS and TNF-α induced a dramatic increase in ICAM-1 expression after a 6-h incubation (Fig. 3, A and B). Preincubation with SB-239063AN (1–10 μM) produced a dose-dependent inhibition of HUVEC ICAM-1 expression induced by LPS or TNF-α stimulation (Fig. 3, A and B). In contrast, neither LPS nor TNF-α altered PECAM expression in HUVECs.

Endothelial Dysfunction and Albuminuria in SFD-SHR-SPs. To assess the time course of endothelial dysfunction in SFD-SHR-SPs, ex vivo vascular reactivity of the thoracic aorta was compared at 0, 4, and 8 weeks of study in WKY (normal diet) and SHR-SPs (normal or salt/fat diet). On study week 0, there was no significant difference in vascular reactivity in age-matched WKY or SHR-SP rats, i.e., the potency and efficacy of a-adrenoceptor-mediated contraction (phenylephrine), endothelial-dependent relaxation (carbachol) and endothelial-independent relaxation (SNP) were equivalent (Fig. 4). However, the introduction of the SFD to SHR-SPs resulted in a significant reduction in the efficacy of...
endothelial-dependent relaxation at 4 and 8 weeks compared with age-matched WKYS (Fig. 4). Little or no effect on endothelial-dependent relaxation was observed in the ND-SHR-SP or ND-WKY groups (Fig. 4). No significant changes in the phenylephrine and SNP efficacy ($R_{\text{max}}$) were noted in any of the groups. These results indicate that the introduction of a SFD induces significant endothelial dysfunction within 4 weeks.

The introduction of an SFD was also associated with a significant increase in albuminuria in SHR-SPs. Time-dependent increases in albuminuria were observed after 4 weeks on the SFD (Fig. 5). Only slight increases in albuminuria were observed in SHR-SPs on a normal diet, and no changes were noted in WKY (data not shown). The results indicate that endothelial dysfunction precedes the onset of detectable microalbuminuria in SFD-SHR-SPs and suggests a causal relationship. A more detailed description of changes in renal function can be found in the second part of this series in the current issue.

**Restoration of Endothelial Function and Survival Benefit by Chronic/Delayed Treatment with p38 MAPK Inhibitor.** A study was performed in the SFD-SHR-SP model of endothelial dysfunction to determine whether chronic treatment with a p38 MAPK inhibitor could restore endothelial function. SHR-SPs were placed on an SFD and randomized in 2:1 manner to no treatment or treatment with SB-239063AN (1500 ppm in the diet) when 10% mortality was observed (approximately 6 weeks after starting the SFD). This model exhibits severe endothelial dysfunction and target organ damage during this time period. The introduction of treatment with SB-239063AN induced a surprisingly rapid improvement in survival (Fig. 6). In addition, 12 weeks of treatment restored endothelial-dependent relaxation to levels comparable with SHR-SPs that had been maintained on a ND throughout the study (Fig. 7 and Table 1). Plasma concentrations of SB-239063AN after 3 weeks of treatment were 386 ± 25 ng/ml, well within the peak/trough range defined in pilot exposure studies.

In vascular reactivity studies performed as described above, SB-239063 and SB-239063AN had no significant effects on contraction or relaxation parameters when added directly to the tissue bath in concentration up to 10 μM (data not shown).

**SFD-Induced Hypertension Is Attenuated by Treatment with a Selective p38 MAPK Inhibitor.** Chronic blood pressure telemetry studies were performed to examine the effects of treatment with a p38 MAPK inhibitor on SFD-induced progressive hypertension in SHR-SPs. Introduction of the SFD increased mean arterial blood pressure (MAP) 45 to 50 mm Hg within 4 weeks. SB-239063 (1200 ppm) added to the diet significantly reduced SFD-induced hypertension,
and MAP in the treatment group was similar to SHR-SPs maintained on a ND (Fig. 8). The study was terminated at 5 weeks because of mortality in the untreated group. Plasma concentrations of SB-239063 after 3 weeks of treatment were 419 ± 56 ng/ml, well within the peak/trough range defined in pilot exposure studies. Similar chronic treatment with SB-239063 had no significant effect on MAP in the salt-insensitive SHR strain (data not shown).

### Discussion

The salt-sensitive phenotype in the SHR-SP relates to an enhanced BP response to salt and acceleration/potentiation of hypertension-induced target organ damage (Liu et al., 1999; Barone et al., 2001; Griffin et al., 2001; Ma et al., 2001). In the present study, the regulation of endothelial p38 MAPK and its role in BP regulation and endothelial dysfunction were examined in this model and in cultured HUVECs. Proinflammatory cytokines enhanced phosphorylation of endothelial p38 MAPK in HUVECs and phosphorylated p38 MAPK was localized to the endothelium, outer media, and adventitia in blood vessels from SHR-SPs. Treatment with selective inhibitors of p38 MAPK attenuated the expression of ICAM adhesion molecules in HUVECs. In SHR-SPs with established target organ damage (endothelial and renal dysfunction) induced with an SFD, chronic treatment with p38 MAPK inhibitors restored endothelial-dependent vasorelaxation and attenuated the morbidity/mortality. In addition, chronic treatment with a p38 MAPK inhibitor abolished the gradual increase in BP induced by an SFD. These results suggest, for the first time, a role for p38 MAPK in the pathogenesis of hypertension.

The SHR-SP strain exhibits differential salt-sensitivity (Griffin et al., 2001), and, when placed on a salt/fat diet, represents an aggressive hypertension model of accelerated target organ damage with characteristic pathology evident in the blood vessels, brain, heart, kidney, and retina (Barone et al., 2001). The introduction of the SFD induces a progressive increase in blood pressure in the SHR-SP within 2 weeks with concomitant depression of endothelial-dependent vasorelaxation, which is followed by evidence of renal dysfunction (albuminuria) and subsequent neurological deficits (Behr et al., 2001; Ma et al., 2001). Thus, it seems that endothelial dysfunction (loss of bioavailable endothelial NO) contributes to the progression and maintenance of chronic hypertension and is a likely determinant of the prothrombotic state and target organ damage observed in this model (Behr et al., 2001; Ma et al., 2001; Yamashita et al., 2002).

Endothelial dysfunction in the SHR-SP is associated with an accumulation of nitrotyrosine in the blood vessel wall, suggesting rapid inactivation of endothelial NO and peroxynitrite production by excessive generation of reactive oxygen species (ROS). The major source of vascular ROS in
Hypertension is via NAD(P)H oxidase activation by mechanical factors, vasoactive agents, cytokines, and growth factors (Landmesser and Harrison, 2001; Wilcox, 2002; Touyz, 2003). All of these stimuli activate p38 MAPK (kinase-dependent and redox-dependent signaling), which contributes to further generation of ROS via proinflammatory cytokine generation.

The present results suggest that inappropriate activation of the p38 MAPK pathway contributes to an excess ROS and subsequent reduction in NO bioavailability. Furthermore, the process is reversible by treatment with a selective p38 MAPK inhibitor.

In addition to the proposed role in NO scavenging, evidence suggests that redox-dependent and kinase-dependent signaling through p38 MAPK plays an important role in vascular inflammation and remodeling processes. In this regard, p38 MAPK activation was observed in medial and adventitial lamina of remodeling blood vessels from hypertensive animals. These results are not limited to hypertension and seem to be consistent with sustained activation of p38 MAPK noted in remodeling blood vessels after mechanical injury and hypercholesterolemia (Ju et al., 2002). Chronic treatment with selective p38 MAPK inhibitors limit vascular remodeling in these normotensive vascular injury models suggesting that p38 MAPK plays a broad role in vasculopathies linking cardiovascular disorders such as hypertension and atherosclerosis.

The mechanism(s) of salt sensitivity in the SHR-SP are unknown; however, brief exposure to a high-salt diet has been shown to induce endothelial dysfunction independent of changes in blood pressure (Liu et al., 1999). In addition, high salt has been shown to increase ROS and the activity of ROS-generating enzymes NAD(P)H oxidase and xanthine oxidase (Lenda and Boegehold, 2002a,b). These observations suggest that the initiating events in salt sensitivity serve to reduce bioavailable NO (Hamilton et al., 2001). The role of p38 MAPK in these initiating events has not been evaluated directly but is implicated in the present study by the attenuation of salt-induced hypertension induced by treatment with selective p38 MAPK inhibitors. The present study also suggests a relationship between endothelial dysfunction and end-organ damage such as renal dysfunction and stroke. However, it is unclear whether endothelial protection is the sole mechanism by which p38 MAPK inhibitors ameliorate hypertensive end-organ damage.

In conclusion, salt sensitivity in the SHR-SP is associated with a progressive increase in blood pressure and concomitant endothelial and renal dysfunction, as well as enhanced morbidity and mortality. Treatment with a p38 MAPK inhibitor restored endothelial-dependent relaxation and reduced the mortality rate. Salt-sensitive hypertension was also ameliorated by treatment with a p38 MAPK inhibitor providing the first evidence for p38 MAPK-dependent hypertension.
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