Original Article

Cardioprotective Effect of a Combination of Rho-Kinase Inhibitor and P38 MAPK Inhibitor on Cardiovascular Remodeling and Oxidative Stress in Dahl Rats

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Aim: Rho-kinase plays a critical role in various cellular functions. p38 mitogen-activated protein kinase (p38 MAPK) plays a central role in the inflammatory cytokine response to immune challenge. We evaluated the effects of a combination of fasudil, a Rho-kinase inhibitor, and FR167653, a p38 MAPK inhibitor, on cardiovascular remodeling, inflammation, and oxidative stress in Dahl salt-sensitive (DS) rats.

Methods: DS and Dahl salt-resistant (DR) rats were fed a high-salt diet at 6 weeks of age. Vehicle, fasudil (100 mg/kg per day), FR167653 (2 mg/kg per day), and a combination of fasudil and FR167653 were administered to 6-week-old DS rats for 5 weeks.

Results: At the age of 11 weeks, in the left ventricle, DS rats were characterized by increased myocardial fibrosis, phosphorylation of p38 MAPK, and myosin phosphatase targeting subunit (MYPT-1), and NAD(P)H oxidase p22phox, p47phox, gp91phox, tumor necrosis factor-α and interleukin-1β expression compared with DR rats. Fasudil improved cardiovascular remodeling, inflammation, NAD(P)H oxidase subunits, and phosphorylation of p38 MAPK and MYPT-1. FR167653 also similarly ameliorated these indices but not MYPT-1 phosphorylation. Compared with either agent alone, a combination of fasudil and FR167653 was more effective for the improvement of myocardial damage, inflammation and oxidative stress.

Conclusion: These findings suggest that the Rho-kinase and p38 MAPK pathways may play a pivotal role in ventricular hypertrophy; thus, we obtained the first evidence that a combination of Rho-kinase inhibitor and p38 MAPK inhibitor may provide a potential therapeutic target in hypertension with cardiovascular remodeling.


Key words; Hypertrophy, Oxidative stress, p38 MAPK, Rho-kinase

Introduction

Left ventricular hypertrophy (LVH) can develop as a consequence of hypertension or cardiovascular disease, and constitutes by itself a risk factor for the development of arrhythmias, diastolic dysfunction and progression to congestive heart failure. It appears that local release of neuroendocrine ligands such as angiotensin II (Ang II) can modulate myocyte hypertrophy and function as well as extracellular matrix reorganization. Via its various G protein-coupled receptors subtypes, Ang II can activate mitogen-activated protein kinase (MAPK) signaling pathways. Intracellular MAPK signaling cascades probably play a critical role in the pathogenesis of cardiac and vascular disease. Among the members of this family, the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 MAPK are known to be in-
involved in this process. p38 MAPK is markedly activated in cardiovascular cells by a variety of cellular stresses including Ang II stimulation. Some studies have revealed that activation of the p38 MAPK signaling pathway is involved in cardiovascular diseases. Moreover, p38 MAPK plays a central role in the inflammatory cytokine response to immune challenge and the development of sickness behavior. This understanding may present the possibility of using p38 MAPK inhibitors to reduce chemotherapy-induced inflammatory cytokine production and consequently treatment-related fatigue.

Rho-kinase, a target protein of small GTP-binding protein Rho, plays crucial roles in various cellular functions and mediates cellular events, such as changes in cell morphology, cell motility, focal adhesions, and cytokines. The possibility that Rho is involved in vascular proliferation and migration is suggested by the involvement of Rho in the growth of nonvascular cells in response to heterotrimeric G protein receptor stimulation and in the migration of endothelial cells in response to mechanical strain or tyrosine kinase growth factors. Indeed, the Rho-kinase pathway is involved in DNA synthesis and migration in vascular smooth muscle cells of rat aorta. Moreover, studies have demonstrated that cardiomyocyte hypertrophy and myofibrillar assembly are blocked by inhibitory mutants of Rho-kinase, which suggests a role for this Rho effector in cellular growth responses. Therefore, inhibition of the Rho-kinase pathway may be useful in the treatment of arteriosclerotic cardiovascular diseases. Previously, we have reported that upregulation of Rho-kinase plays a critical role in the pathogenesis of Ang II-induced hypertensive rats, and Dahl salt-sensitive hypertensive (DS) rats with end-stage severe heart failure.

NAD(P)H oxidase proteins are major sources of reactive oxygen species (ROS). Upon stimulation, the cytosolic complex migrates and assembles with the membrane subunits to form an active oxidase capable of producing superoxide anion, and NAD(P)H oxidase plays a critical role in intracellular redox signaling and has been implicated in various cardiovascular diseases. Specifically, the interaction of superoxide anion with nitric oxide reduces nitric oxide bioavailability and produces toxic peroxynitrite, resulting in endothelial dysfunction, which is a hallmark of hypertension, heart failure, and atherosclerosis. Recent studies demonstrated that p38 MAPK activates NAD(P)H oxidase by enhancing the phosphorylation and assembly of NAD(P)H oxidase subunits, and the activation of NAD(P)H oxidase is suppressed by p38 MAPK inhibitors. In addition, Rho-kinase also upregulates NAD(P)H oxidase and augments Ang II-induced ROS production; however, their effects are limited, and combination therapy involving Rho-kinase inhibitor and p38 MAPK inhibitor in DS rats remains unknown. The aim of our present work was to investigate for the first time the effects of a combination of fasudil, a Rho-kinase inhibitor, and FR167653, a p38 MAPK inhibitor, on cardiovascular remodeling, inflammation, and ROS production in the left ventricle (LV) of DS rats.

**Methods**

All procedures were in accordance with our institutional guidelines for animal research and with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Animal Models and Experimental Designs**

After weaning, male inbred DS and Dahl salt-resistant (DR) rats (Eisai Co Ltd, Tokyo, Japan) were fed a 0.3% NaCl (low-salt) diet until the age of 6 weeks; thereafter, they were fed a diet containing 8% NaCl (high-salt) until the stage of concentric LVH at 11 weeks (DSVH). The rats were weighed, and their systolic blood pressure (SBP) and heart rate (beats/min) was measured by the tail-cuff method before feeding with the 8% NaCl diet and at 1-week intervals thereafter. Rats were acclimatized in a rodent restrainer for half an hour before recording. The same investigator monitored and the animal in order to detect artifacts such as animal distress or movement. An average of three recordings was made for each rat. At 6 weeks of age, the DS rats were randomly divided into four groups: vehicle treated (DSVH-V), fasudil treated (100 mg/kg per day; DSVH-RK), FR167653 treated (2 mg/kg per day; DSVH-FR) or a combination of fasudil and FR167653 treated (DSVH-RK+FR) for 5 weeks. Age-matched male DR rats fed the same diet served as a control group (DR-C). FR167653 was administered with an ALZET Osmotic Pump. Fasudil was administered in drinking water. After oral administration, fasudil is metabolized to hydroxyfasudil, a major active metabolite of fasudil that specifically inhibits Rho-kinase. Recent studies have demonstrated that the levels of hydroxyfasudil in plasma after 4 weeks of oral treatment with fasudil (30 to 100 mg/kg per day) are within the specific therapeutic ranges of Rho-kinase inhibitor. Fasudil has been shown by kinase assay to selectively inhibit Rho-kinase activity; thus, fasudil is a relatively selective inhibitor of Rho-kinase.
Sample Processing
After all of the final measurements had been obtained, each rat was killed by an injection of a pentobarbital overdose, and the heart was excised, separated immediately from fat and fibrous tissue, cleaned carefully, blotted dry, and weighed on a precision balance. After the great vessel, atria, and right ventricular free wall had been removed (in that order), the LV mass (including the intraventricular septum remaining with the LV free wall) was measured. Wet weights of the ventricles were normalized with respect to the body weight and expressed as the ventricular mass index (mg/g). Tissue samples were placed into plastic tubes, immediately frozen in liquid nitrogen, and stored at −80°C.

Western Blot Analysis
NAD(P)H oxidase p22phox, p47phox, gp91phox, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), monocyte chemoattractant protein-1 (MCP-1), procollagen type I and III, and fibronectin protein expressions were measured as described previously18-20. LV was homogenized (25% wt/vol) in 10 mmol/l HEPES buffer, pH 7.4, containing 320 mmol/l sucrose, 1 mmol/l EDTA, 1 mmol/l DTT, 10 μg/ml leupeptin, and 2 μg/ml aprotinin at 0°C to 4°C with a polytron homogenizer. Protein concentrations were determined with bovine serum albumin as a standard protein. Equal amounts of protein were loaded in each lane of SDS-PAGE using 13% gels. The proteins transferred to the sheets were detected using the ECL immunoblotting detection system (Amersham Life Science Inc.).

Detection of Superoxide Anion in the LV
Histologic detection of superoxide anion in the LV was performed using dihydroethidium (DHE) as described previously18, 19, 21, 23.

Histologic Examination and Evaluation of Cardiovascular Remodeling
Histologic examination was performed as described in detail previously10, 11, 18-23. The wall-to-lumen ratio (the area of the vessel wall divided by the area of the total blood vessel lumen) was determined. The area of fibrosis immediately surrounding the blood vessels was calculated, and perivascular fibrosis was determined as the ratio of the area of fibrosis surrounding the vessel wall to the total area of the vessel. To assess the area of myocardial interstitial fibrosis, the area of pathological collagen deposition was measured in the microscopic field of each Masson’s trichrome-stained section. The ratio of the total area of fibrosis within the left ventricular myocardium to the total area of the left ventricular myocardium in each heart was calculated and used for analysis.

Statistical Analysis
All values are expressed as the mean ± SEM. Mean values were compared among the 5 groups by ANOVA and the Bonferroni post-hoc test for multiple comparisons. P<0.05 was considered significant.

Results
Physiological Profiles
The physiological profiles of the five groups at the age of 11 weeks are shown in Table 1. Body weight (BW) was significantly lower in DS rats than in DR rats, and was not changed by the administration of fasudil, FR167653, and combination therapy. In contrast, DS rats had higher LVW/BW than DR rats. Fasudil or FR167653 monotherapy significantly decreased LVW/BW compared with non-treated DS rats, and the combination of fasudil and FR167653 further reduced LVW/BW. DS rats had markedly higher SBP using the tail-cuff method than DR rats. None of long-term fasudil, FR167653, or combination therapy affected SBP. There were no significant differences in heart rate among the 5 groups (Table 1).

Effect of Fasudil and FR167653 on Cardiovascular Remodeling
We tested the effect of fasudil, FR167653, and combination therapy on cardiovascular remodeling, such as the wall-to-lumen ratio, perivascular fibrosis, and area of interstitial fibrosis of the LV in Fig. 1A to 1J, and Table 1 (n = 10 tissue sections per group). The wall-to-lumen ratio was significantly increased in DS-VH-V rats compared with DR-C. Long-term fasudil and FR167653 monotherapy caused significant ame-
lioration of the wall-to-lumen ratio, and the combination of fasudil and FR167653 therapy further reduced this ratio. Moreover, perivascular and interstitial fibrosis was significantly greater in DSVH-V rats than in DR-C rats. Long-term fasudil and FR167653 monotreatment also caused significant improvement in perivascular and interstitial fibrosis, and the combination of fasudil and FR167653 therapy further reduced the rate (Table 1, Fig. 1A-J).

**Effect of Fasudil and FR167653 on MYPT-1 and p38 MAPK Phosphorylation**

Next, we tested the phosphorylation of p38 MAPK and MYPT-1, a target of Rho-kinase, in the LV of DS rats by fasudil, FR167653, and combination therapy. Fig. 2 shows a representative Western blot of equal amounts of protein from the LV in the five groups. Phospho-specific (activated) p38 MAPK and MYPT-1 levels were significantly greater in the DSVH-V compared with DR-C. Fasudil or FR167653 monotherapy significantly decreased the increase in LV p38 MAPK activity, and the combination of fasudil and FR167653 further reduced p38 MAPK activity. In addition, fasudil monotherapy significantly inhibited MYPT-1 activity, but not FR167653 alone, and the combination of fasudil and FR167653 therapy reduced MYPT-1 activity to the same level as fasudil monotherapy. In contrast, no significant differences were found in total p38 MAPK and total MYPT-1 expression among the five groups (Fig. 2).

**Effect of Fasudil and FR167653 on NAD(P)H Oxidase Expression and Superoxide Production**

Next, we tested the effects of fasudil, FR167653, and combination therapy on NAD(P)H oxidase expression and superoxide production.
p22phox, p47phox, gp91phox protein expression and superoxide anion production. NAD(P)H oxidase p22phox, p47phox, gp91phox levels in the LV were significantly higher in DS rats than in DR rats. Long-term fasudil and FR167653 monotherapy significantly decreased the NAD(P)H oxidase subunit expression, and the combination of fasudil and FR167653 therapy further reduced these expressions (Fig. 3). Moreover, Fig. 4 show superoxide anion production by DHE in each group after 5 weeks of fasudil and FR167653 alone and combination therapy. Superoxide anion production was higher in DS rats than in DR rats. Long-term fasudil and FR167653 monotherapy in DS rats significantly reduced superoxide anion production, and the combination of fasudil and FR167653 therapy further reduced this production (Fig. 4A-E).
Effect of Fasudil and FR167653 on Inflammation

Next, chronic cardiac inflammation plays a pivotal role in aggravating hypertensive cardiac remodeling; therefore, we tested the effects of fasudil, FR167653, and their combination on TNF-α and IL-1β, and MCP-1, a chemokine for monocytes/macrophages, protein levels in the LV of DS rats. The expression levels of TNF-α, IL-1β, and MCP-1 in the LV were significantly higher in DS rats than in DR rats. Long-term fasudil and FR167653 monotherapy in DS rats significantly reduced TNF-α, IL-1β, and MCP-1 expression, and the combination of fasudil and FR167653 therapy further reduced these expressions (Fig. 5).

Effect of Fasudil and FR167653 on Fibrosis Factor

Next, we tested the effects of fasudil, FR167653, and their combination on fibrosis factor such as procollagen type I and III and fibronectin protein levels. The expression levels of procollagen type I and III and fibronectin in the LV were significantly higher in DS rats than in DR rats. Long-term fasudil and FR167653 monotherapy in DS rats significantly reduced procollagen type I and III and fibronectin expression, and the combination of fasudil and FR167653 therapy further reduced these expressions (Fig. 6).

Discussion

In the present study, dietary salt intake Dahl rats with LVH had significantly induced p38 MAPK and MYPT-1 activation and increased NAD(P)H oxidase subunit protein expression, resulting in cardiovascular remodeling and cardiac inflammation. Treatment of DS rats with fasudil and FR167653 alone suppressed p38 MAPK and MYPT-1 activation and ROS production, and attenuated cardiovascular remodeling and inflammation, and the combination of fasudil and FR167653 therapy further reduced these parameters. These observations provide the first in vivo evidence

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**Fig. 5.** Effects of a combination of fasudil and FR167653 on TNF-α, IL-1β, and MCP-1 protein expression

Values are the means ± SEM. *p < 0.05 vs DR-C;  †p < 0.05 vs DSVH-V; ‡p < 0.05 vs DSVH-RK; §p < 0.05 vs DSVH-FR.

**Fig. 6.** Effects of a combination of fasudil and FR167653 on procollagen type I and III, and fibronectin protein expression

Values are the means ± SEM. *p < 0.05 vs DR-C;  †p < 0.05 vs DSVH-V; ‡p < 0.05 vs DSVH-RK; §p < 0.05 vs DSVH-FR.
that a combination of fasudil and FR167653 may improve cardiovascular remodeling and inflammation associated with the oxidative stress pathway in dietary salt intake Dahl rats with LVH. Thus, Rho-kinase and p38 MAPK pathways may play a critical role in LVH, and the combination of Rho-kinase and p38 MAPK inhibitors may provide a potential therapeutic target in hypertension with LVH.

In the heart, the principal isoforms of stress-regulated MAPKs are p38α, p38β, JNK-1, and JNK-2. Downstream targets of p38 MAPKs and JNKs are largely transcription factors, including c-Jun, the p38 activating transcriptional factor (ATF)-2, ATF-6, Elk-1, p53, and nuclear factor to activated T cells 4 (NFAT-4). Overexpression of activated MAPK kinases for p38 MAPK in neonatal cardiomyocytes resulted in increased cell size, enhanced sarcomeric organization, and induction of ANF. p38 MAPK inhibition by the expression of dominant-negative p38 MAPKs or pharmacologic inhibitors blunted agonist-stimulated hypertrophy of cultured neonatal cardiomyocytes. A synthesized low-molecular-weight pyrazolotriazine derivative, FR-167653, is a potent suppressor of TNF-α and IL-1β production via specific inhibition of p38 MAPK activity. Administration of FR167653 has been shown to exert beneficial effects in animal models of disseminated intravascular coagulation, pulmonary ischemia-reperfusion injury, and glomerulonephritis. In the present study, treatment with a p38 MAPK inhibitor, FR167653, resulted in regression of cardiac hypertrophy and fibrosis and improvement in cardiac inflammation. Matsuoka et al. demonstrated that p38 MAPK is involved in bleomycin-induced pulmonary fibrosis, and its inhibitor, FR167653, may be a feasible therapeutic agent. In addition, FR167653 inhibited p38 MAPK activation and ameliorated fibronectin expression and apoptosis in diabetic glomeruli and in mesangial cells cultured under high-glucose conditions. Moreover, a number of studies demonstrated that inhibition of p38 MAPK protected against organ damage. Doucet et al. showed that FR167653 improved renal damage in association with the reduction of inflammation and interstitial fibrosis in a non-heart-beating donor model. Furuichi et al. reported that FR167653 also had marked anti-inflammatory effects after ischemia-reperfusion injury, as measured by lymphocyte and monocyte/macrophage infiltration. Yin et al. described that treatments with p38 MAPK inhibitor suppressed myocardial fibrosis and LV remodeling, and attenuated the expressions of p-p38-MAPK, TNF-α, α-SMA and type I collagen as compared with rats with myocardial ischemia. Bao et al. indicated that Ang II-induced hypertension, organ damage, and ROS production are possibly mediated by p38 MAPK and inhibition of p38 MAPK may offer a therapeutic approach for cardiovascular disease. In addition, their group also investigated that chronic treatment with p38 MAPK inhibitor significantly reduces target-organ damage in salt-sensitive stroke-prone spontaneous hypertensive rats, and is associated with the preservation of endothelial function. Some studies demonstrated that p38 MAPK inhibitor significantly improves cardiac function and LV remodeling in mice and rats with heart failure resulting from myocardial infarction. Tojo et al. showed that the inhibition of p38 MAPK by FR167653 reduced renal IL-1β and TNF-α production and ameliorated renal damage in hypertensive rats via suppression of NAD(P)H oxidase and enhanced nitric oxide bioavailability. These results are consistent with the idea that p38 MAPK is necessary and sufficient in the hypertrophy response, and suggest that p38 MAPK inhibitor FR167653 could be a potential target for preventing cardiovascular remodeling and inflammation. On the other hand, it has been reported that targeted inhibition of p38 MAPK promoted hypertrophic cardiomyopathy, including the increase of the LVW/BW ratio and interstitial cell fibrosis; however, the discrepancy of p38 MAPK inhibitor between our present and similar results in many previous studies and this opposite finding is unclear. This will be evaluated in a future study.

Rho-kinase, the first Rho effector to be described, is a serine/threonine kinase that is important in fundamental processes of cell migration, cell proliferation and cell survival. Knowledge of the involvement of Rho-kinase in the cardiovascular system has mostly been derived from studies utilizing pharmacological inhibitors. Fukui et al. demonstrated that cardiac Rho-kinase activity was strongly associated with myocardial stiffness of LV in a rat model of diastolic heart failure, and long-term inhibition of Rho-kinase with fasudil ameliorated diastolic heart failure. In addition, Migashi et al. reported that Rho-kinase is substantially involved in Ang II-induced cardiovascular hypertrophy in vivo and that the mechanisms include enhanced oxidative stress associated with upregulation of endothelial NAD(P)H oxidase and the resultant endothelial dysfunction. Fasudil was shown to decrease ischemia–reperfusion injury, infarct size and myocardial fibrosis in response to experimental myocardial infarction and in a rat model of chronic hypertension-induced congestive heart failure. Moreover, Rho-kinase is the best-known downstream effector of Rho A. RhoA-GTP activates Rho-kinase, which in...
turn phosphorylates a non-catalytic subunit of MYPT-1 to inhibit myosin phosphatase activity. In the present study, the results indicated a significant upregulation of MYPT-1 phosphorylation in the LV, and fasudil significantly inhibited MYPT-1 phosphorylation in DS rats. These findings suggest that dietary salt intake Dahl rats with LVH led to Rho-kinase-dependent phosphorylation of MYPT-1, which represents a selective marker of Rho-kinase activation in the LV. Thus, the Rho-kinase pathway may play a crucial role in the pathogenesis of LVH and could be an important therapeutic target of hypertension.

Oxidative stress is elevated systemically and in the myocardium of patients with myocardial hypertrophy and heart failure. ROS have multiple effects on cell function depending on the amount and subcellular location of ROS generated. The cause of increased ROS in this setting is not known, but may relate to increased production of ROS due to increased metabolic activity, stimulated production by mechanical strain, neurohumoral activation, inflammatory cytokines, and decreased antioxidant activity. Controlled intracellular ROS production from NADPH oxidase has been shown to be necessary for normal cellular development and function, whereas excessive ROS generation is implicated in myocardial hypertrophy and heart failure. The possibility that p38 MAPK mediates the effects of oxidative stress on myocyte growth is supported by in vitro and in vivo overexpression studies using molecular constructs of specific kinases or activators, and examining the effect on myocyte growth. Wang et al. demonstrated that in neonatal rat ventricular myocytes, overexpression of constitutively active p38 MAPK causes a hypertrophic response. The present study showed that a p38 MAPK inhibitor, FR167653, inhibited the production of ROS, which is associated with the suppression of NAD(P)H oxidase subunit expression. Dhingra et al. demonstrated that exposure of cells to SB-203580, a specific p38 MAPK inhibitor, prevented TNF-α-induced increases in oxidative stress and apoptosis, which further implies that p38 MAPK is a downstream target of TNF-α-induced oxidative stress in cardiac myocytes that activates the apoptotic signaling cascade. In addition, Sedeek et al. demonstrated high glucose stimulated phosphorylation of p38 MAPK and increased expression of transforming growth factor-β1 (TGF-β1) and fibronectin, indicating the importance of Nox4-based NADPH oxidase in renal ROS production and redox signaling. To further investigate the relationship between glucose-stimulated redox-sensitive p38 MAPK and profibrotic signaling, cells stimulated by high glucose were preexposed to SB-203580, which inhibited glucose-stimulated expression of TGF-β1 and fibronectin. These findings define a pathway in proximal tubule cells whereby a prodiabetic milieu promotes fibrosis through Nox4-ROS-p38MAP kinase-TGF-β signaling. These findings suggest that elevated ROS activated p38 MAPK and contributed significantly to cardiac inflammation and cardiovascular remodeling. Furthermore, we demonstrated that fasudil inhibited NAD(P)H oxidase p22phox, p47phox, and gp91phox expression and superoxide anion production in the LV. Thus, fasudil may reduce the production of ROS by suppressing NAD(P)H oxidase expression. It has demonstrated that long-term concomitant treatment with fasudil markedly suppressed Ang II-induced upregulation of NAD(P)H oxidase p22phox, gp91phox nox1, and nox4 mRNA expression. The inhibitory effect of fasudil indicates that Rho-kinase is substantially involved in Ang II-induced upregulation of NAD(P)H oxidase. Production of superoxide anions in Ang II-infused rats was normalized by apocynin, a selective NAD(P)H oxidase inhibitor, indicating increased activity of NAD(P)H oxidase in Ang II-infused animals. Moreover, hypertension caused oxidative stress in the blood vessels and produced superoxide by NADPH oxidase in endothelial and vascular smooth muscle cells, therefore, the effects of Rho-kinase inhibitor fasudil on NAD(P)H oxidase expression and ROS production may play an important role in cardioprotection.

As mentioned above, we and many investigators have already demonstrated similar findings using Rho-kinase or p38 MAPK inhibitor monotherapy; however, in the present study, we provide the first evidence that a combination of fasudil and FR167653 improved cardiovascular remodeling in dietary salt intake DS rats with LVH more than monotherapy. No reference has been made in previous reports, and no other detailed study has reported the combination of Rho-kinase and p38 MAPK inhibitor treatment. The combination of Rho-kinase and p38 MAPK inhibitor may provide a novel therapeutic strategy for the treatment of hypertension with cardiovascular remodeling.

In conclusion, dietary salt intake Dahl rats with LVH had significantly induced p38 MAPK and MYPT-1 phosphorylation, increased oxidative stress, resulting in cardiovascular remodeling. Treatment with fasudil and FR167653 alone suppressed p38 MAPK and MYPT-1 activation and ROS production, and attenuated cardiovascular remodeling, and the combination of fasudil and FR167653 therapy further reduced these parameters. These findings suggest that a combination of fasudil and FR167653 may improve cardio-
vascular remodeling and inflammation associated with the oxidative stress pathway in DS rats with LVH. Thus, we have obtained the first evidence that Rho-kinase and p38 MAPK pathways may play a pivotal role in LVH, and the combination of Rho-kinase and p38 MAPK inhibitors may provide a potential therapeutic target for hypertension with LVH.

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**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

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