**Antihypertensive effects of fargesin in vitro and in vivo via attenuating oxidative stress and promoting NO release**

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Antihypertensive effects of fargesin in vitro and in vivo via attenuating oxidative stress and promoting NO release

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Abstract: Fargesin is a bioactive neolignan isolated from Magnolia plants, which is widely used in the treatment of managing rhinitis, inflammation, histamine, sinusitis and headache. To provide more biological information about fargesin, we investigated the effects of fargesin on rat aortic rings and 2-kidney, 1-clip (2K1C) hypertensive rats. In vitro, fargesin caused concentration-dependent vasorelaxation in rat isolated aortic rings induced by KCl and NE. The effect was weakened by endothelium denudation and NO synthesis inhibition. In vivo, the evolution of systolic blood pressure was followed by weekly measurements. AngiotensinII (Ang II) and endothelin (ET) levels, nitric oxide (NO) and nitric oxide synthase (NOS), plasma and liver oxidative stress markers were determined at the end of the experimental period. After five weeks treatment of fargesin, we found that fargesin treatment reduced systolic blood pressure (SBP), cardiac hypertrophy, Ang II and ET levels of hypertensive rats. Increased NOS activity and NO level were observed in fargesin treated rats. Normalisation of plasma MDA concentrations and improvement of the antioxidant defences system in plasma and liver accompanied the antihypertensive effect of fargesin. Taken together, these results provided substantial evidences that fargesin has antihypertensive effect in 2K1C hypertensive rats via inhibiting oxidative stress and promoting NO release.

Keywords: fargesin; vasorelaxation; 2K1C hypertensive rats; antioxidant; rat aortic rings
Introduction

Hypertension is the most common risk factor for myocardial infarction, stroke, heart failure, arterial fibrillation, aortic dissection and peripheral arterial diseases (Nurmuhammat et al. 2014). It is frequently related to the activation of the renin-angiotensin system (RAS), as a result of the fall in the renal blood flow and pressure perfusion produced after renal artery stenosis (Textor and Wilcox 2001). In recent years, it has been suggested that oxidative stress is a pathogenetic factor in the development of essential hypertension (Kojda and Harrison 1999). Oxidative stress can be caused by reactive oxygen species (ROS), which is an important mediator of the progression of renal injury in different animal models of hypertension (Datla and Griendling 2010). Under pathological conditions, excessive ROS bioactivity can induce endothelial dysfunction and lipid peroxidation, increase contractility of vascular smooth muscle cells (VSMC) growth and deposition of extracellular matrix proteins, which are important factors in hypertensive vascular and renal damage (Diep et al. 2002). Recently, attention has been focused on herbal and mineral preparations which are traditionally used as potential therapeutic agents in the prevention and management of cardiovascular diseases, however, many new antihypertensive drugs with improved efficacy introduced to the market still possess serious side effects (Arakawa et al. 2006; Herrera-Arellano et al. 2007; Sinitsyna et al. 2006; Tahraoui et al. 2007).

Fargesin is a neolignan isolated from Magnolia plants such as Magnolia biondii and Magnoliae flos (Shen et al. 2008), which are widely used in the treatment of managing rhinitis, inflammatory, histamine, sinusitis and headache in Asian countries such as China and Japan. Most of the recent studies of fargesin have focused on their anti-inflammatory and cardioprotective effects (Kim et al. 2009; Shen et al. 2008; Wang et al. 2015). However, there is no report about whether it has antihypertensive effect until now. Thus, in this study, we investigated this activity of fargesin using rat aortic ring assays and 2-kidney, 1-clip (2K1C) model of hypertension, respectively. The results indicated that fargesin can relax rat aortic ring in vitro and reduce elevated blood pressure of 2K1C hypertensive rats via attenuating oxidative
damage and promoting NO release.

**Materials and method**

**Chemicals and materials**

Fargesin (Far) (control standard, purity $\geq 98\%$ by high-performance liquid chromatography, cat.no.wkq-00603) was obtained from Sichuan Weikeqi biological technology Co., Ltd. (Chengdu, China). Fargesin used in the aorta ring experiments was dissolved in the dimethylsulfoxide (DSMO). The dry powder of fargesin used *in vivo* was prepared into vehicle (0.5% sodium carboxymethyl cellulose). The N$^\text{G}$-nitro-L-arginine methyl ester (L-NAME) and Indometacin (Indo) was purchased from sigma-Aldrich Co. LLC (St. Louis, MO, USA). Captopril (CAP) was purchased from Changzhou Pharmaceutical Factory Co., Ltd. (Changzhou, China). Deionized water was purified using a Milli-Q water purification system (A10, Millipore, Billerica, MA, USA).

**Experimental animals**

The study was conducted in eight-week old male Wistar rats (200±20g). The rats were obtained from the Experimental Animal Center of Shanxi Medical University (Taiyuan, China) and maintained under suitable and standard housing conditions at 25 ± 2 °C, relative humidity of 50 ± 15%, and normal photoperiod (12 h dark and 12 h light) in a standard experimental animal laboratory for 2 weeks prior to the start of the experiments. Animals received human care in compliance with the Chinese guidelines for animal care and protection. All experimental procedures were approved by the Institutional Animal Care and Use Committee.

**Aortic ring preparations**

Male Wistar rats were killed by cervical dislocation and exsanguinated. The thoracic aortas were isolated and immersed immediately into chilled (4°C, pH 7.4) HEPES solution of the following composition
(mmol/L): NaCl 144, KCl 5.8, CaCl₂ 2.5, MgCl₂ 1.2, HEPES 5.0, and D-glucose 11.0. Care was taken not to touch the lumen of the thoracic aortas during dissection to ensure the endothelium intact, unless otherwise stated. The thoracic aortas were quickly removed and cleaned of all adhering tissue, and cut into rings of 3mm length under cold conditions. The rings were suspended on two wire hooks in water-jacketed tissue baths containing 5 ml of HEPES solution kept at 37°C, pH 7.4 and gassed with 100% O₂. The upper hook was connected to a force transducer and changes in isometric force were recorded by Chart 5.4 (PowerLab, AD Instruments Co., Ltd) and saved in computer hard disk. The resting tension was stepwise and slowly adjusted to 2.0 g of basal tension within 90 min during which the solution was renewed every 15 min (Altura et al. 1970). The endothelium was either kept intact or removed by gentle rubbing its lumen with a toothpick.

Protocol for checking the functionality of aortic preparations

At the end of an equilibration period of 2 h, the viability of the vessel segments was checked by KCl (40 mmol/L) and NE (1.0μmol/L). Consecutive KCl and NE responses were obtained in each ring until the contractile response reached a basal tension. Acetylcholine (Ach) (1μmol/L) was added to all preparations to verify endothelium integrity (Furchgott and Zawadzki 1980). The vascular endothelium was considered intact when the aortic rings showed more than 50% of relaxation (Ajaya et al. 2003). The removal of the endothelium was demonstrated by the relaxation induced by Ach on the contraction being less than 10%.

Effects of fargesin on KCl- and NE-induced contractions in endothelium-intact and-denuded rat aortic rings

After the verification of endothelium integrity, the thoracic aortas were contracted by adding KCl (40 mmol/L) and NE (1.0μmol/L). After 30min, a concentration response curve of fargesin was obtained by the cumulative addition of fargesin to the thoracic aortas. The concentration (EC₅₀) of each of these
stimuli to produce 50% of its maximal contraction was calculated from each concentration–response curve of rat aortic rings with both endothelium-intact and -denuded aorta.

**Effects of L-NAME and Indo on fargesin-induced relaxation of the rat aortic rings precontracted with KCl and NE**

Consecutive KCl and NE responses were obtained in each ring until the contractile response reached a basal tension. Then L-NAME (0.1mmol/L) (Rees et al. 1990) and Indo(10µmol/L) (Sigthorsson et al. 2000) were respectively added to all preparations. After the contractile response reached another basal tension, cumulative fargesin was add to the control and experiemntal groups. Relaxations are expressed as the percentage of tension decline on the precontraction.

**Establishment of 2K1C renal hypertensive rat model**

In order to induce hypertension, we used the experience of the Goldblatt 2K1C model. 200±20g male Wistar rats were anesthetized with pentobarbital sodium (50 mg/kg intraperitoneally). The left renal artery was exposed by retroperitoneal flank incision and dissected free of the renal vein and connective tissue. A silver clip with a lumen of 0.20 mm was placed around the artery for partial occlusion; in sham operations, the artery was not clipped.

After 6 weeks, the systolic blood pressure (SBP) was measured using the tail-cuff method in conscious rats. Only hypertensive rats (SBP≥150 mmHg) were used in the experiments. The 2K1C hypertensive rats were divided into six groups (n=8/group), named: SHAM (sham surgery-treated with vehicle), MODEL (2K1C surgery-treated with vehicle), CAP (2K1C surgery-treated with captopril, 25mg/kg/day), Far-H (2K1C surgery-treated with fargesin, 50mg/kg/day), Far-M (2K1C surgery-treated with fargesin, 25mg/kg/day) and Far-L (2K1C surgery-treated with fargesin, 12.5mg/kg/day). In the model and control groups, rats received the indicated preparations or identical quantities of vehicle (0.5% sodium carboxymethyl cellulose as placebo) orally by daily gavage for 5 weeks, respectively. All rats
were weighed and treatment adjusted to bodyweight once a week.

**Blood pressure measurements**

SBP was determined by tail-cuff method (BP-6 non-invasive blood pressure measuring device, Chengdu Taimeng Technology Co., Ltd). The rats were all familiarized to a restrainer at the beginning of the experiment and kept in a chamber at 30°C for 15-20 min to ensure BP measurements. Blood pressure was measured one hour after administration once a week. Each measurement was taken three times and averaged.

**Samples collection and storage**

After the last measurement of blood pressure and weight, animals were anaesthetised with sodium pentobarbital (30 mg/kg, intraperitoneally) and blood was collected from the abdominal aorta. Sample for serum was collected without added anticoagulant. Sample for blood plasma was promptly poured into anticoagulated tubes with pre-added enzyme inhibitor (10%, 30 µl disodium EDTA and 40 µl aprotinin). Plasma and serum were obtained by blood centrifugation at 3000×g for 15 min, aliquoted and frozen. All biological samples were stored at −80°C until analysis.

Anesthetized rats were decapitated and then their livers and hearts were carefully excised, cleaned and weighed. The atria and the right ventricle were then removed and the remaining left ventricle was weighed. The cardiac and left ventricular weight indices were respectively calculated by dividing the heart and left ventricle weight by the body weight.

**Measurement of plasma and serum biochemical markers**

Malondialdehyde (MDA, cat.no.A003-1), nitric oxide (NO, cat.no.A013-2) levels and activities of superoxide dismutase (SOD, cat.no.A001-1), catalase (CAT, cat.no.A007-1) in serum were measured using commercial kits (JianCheng Bioengineering Institute, Nanjing, China) and analyzed with a
spectrophotometer. Plasma concentrations of Angiotensin II (Ang II, cat.no.CK-E30668R) and Endothelin-1(ET-1, cat.no.CK-E30591R) were measured using commercial kits (Shanghai Yuanye biological technology Co., Ltd. (Shanghai, China). The measurement process of MDA, NO, Ang II, ET-1, SOD and CAT was performed according to the manufacturer’s instructions.

**Determination of liver total nitric oxide synthase (NOS) and GSH-related enzymes activities**

Liver samples were deproteinized by homogenisation with ice-cold saline solution (0.9%). Homogenates were centrifuged at 4000×g for 10 min and an aliquot of the supernatant was taken for the measurement of activity of GSH-Px (cat.no.A005) and NOS (cat.no.A014-2) using commercial kits (JianCheng Bioengineering Institute, Nanjing, China).

**Statistical Analysis**

The data were expressed as means ± SD. The relaxation responses were measured as tension reduction in a percentage of the KCl and NE induced precontraction. N represents the number of rats from which the thoracic aortas were isolated. Statistical significance was performed with Student's t-test. Multiple means were compared with One-Way ANOVA. Arrhythmia parameters among different groups were analyzed using the chi-square test. A value of $P < 0.05$ was considered statistically significant. Statistical Product and Service Solutions (SPSS) version 13.0 was used for the analyses.

**Results**

**Relaxation of rat aorta by fargesin**

After the KCl (40 mmol/L) and NE (1.0 µmol/L) -induced contraction reached a sustained/steady-state condition, cumulative application of fargesin (1.0-100 µmol/L) caused a concentration-dependent relaxation and a maximum relaxation of 98.41±1.59 % and 81.68±5.02 % were observed in endothelium-intact preparation.
In endothelium-denuded preparations, fargesin (over the same concentration range) elicited a lesser degree of relaxation in a concentration-dependent manner. The maximum relaxation were 82.88±8.41 % and 62.38±7.30 % for KCl (Fig. 1A) and NE-induced contraction (n=5-6). There was significant difference between the response of the endothelium-intact and endothelium-denuded aorta rings (P<0.05). NE-induced contraction was more resistant to the relaxation of fargesin (Fig. 1B). In aortic rings with (n=8) or without (n=7) vascular endothelium, fargesin had no significant effect on the tissue in a resting state (without adding KCl and NE) (data not show).

Effects of L-NAME and Indo on fargesin-induced relaxation of the rat aortic rings precontracted with KCl and NE

After the KCl (40 mmol/L) and NE (1.0 µmol/L) -induced contraction reached a sustained/steady-state condition, cumulative application of fargesin (1.0-100µmol/L) caused a concentration-dependent relaxation. Preincubation of the rings with L-NAME significantly attenuated fargesin-elicited relaxation in KCl-induced or NE-induced precontraction( P<0.01). The maximum effects of L-NAME were 72.2 ±5.2 and 59.4 ±3.9% for KCl and NE-induced contraction (n=5-6) (Fig. 2). Preincubation of the rings with Indo profoundly attenuated fargesin-elicited relaxation in KCl-induced or NE-induced precontraction ( P<0.05). The maximum effects of Indo were 82.3 ±6.2 and 68.9 ±5.2% for KCl and NE-induced contraction (n=5-6) (Fig. 2)

Effect of fargesin on blood pressure and cardiac indices

Blood pressure kept stable throughout the 6 weeks experimental study period and showed an average SBP of 118 mmHg (Table 1) in the sham group. After clipping the left renal artery, blood pressure increased gradually in the 2K1C model group. SBP had risen to an average of 177 mmHg. SBP was then maintained almost at this level till the end of the experimental period. Treatment with captopril resulted in a reduction in blood pressure though it never reached the sham control values. Treating with fargesin to the hypertensive rats produced a significant reduction of the blood pressure. The reduction of the middle
dose group was more than that of captopril (Table 1).

Values of body weight and cardiac indices are shown in Table 1. During the experimental period, the heart weight, left ventricular and the heart weight/body weight ratio of the untreated hypertensive rats were significantly increased compared to the sham group. The values in the captopril groups were decreased greatly. The heart weight to body weight ratio in the Far groups reduced significantly, but didn’t show dose dependence. Values of the middle dose group had decreased the most and were similarly to that of captopril (Table 1).

Effects of fargesin on the plasma ET-1 and Ang II

The changes in ET-1 and angiotensin-II in each group are shown in figure 3. At the end of the experiment, ET-1 reached a high level in the model group. In the captopril groups, ET was lower than that in the model group \( (P<0.05) \), but didn’t reach the level of the sham group. In the Far groups, ET decreased greatly and similarly to that of captopril. Angiotensin-II was higher in model than in sham rats. Treatment with Far, angiotensin-II decreased even lower than values in the captopril group.

Effects of fargesin on the plasma MDA and NO level, CAT and SOD activities in 2K1C rats

Membrane lipid oxidation is one of the primary events in oxidative damage, which can be assessed by its degradation product MDA. In our study, plasma MDA concentration and NO levels were increased in the model group \( (P<0.05) \). 2K1C hypertensive animals chronically treated with fargesin showed lower plasma MDA and NO concentrations \( (P < 0.05) \), in the range of those shown by animals of control groups (Fig. 4). The values in the Far groups didn’t show dose dependence and the middle dose group for fargesin was not different from that of the captopril group\( (P >0.05) \).

In renal hypertensive rats according to the figure 4, the activities of some endogenous antioxidative enzymes, such as SOD and CAT, were significantly decreased in the untreated hypertensive rats compared to the sham rats \( (P<0.05) \). Captopril opposed these changes for all parameters, again without
return to basal values, which was lower than in the sham control rats. Fargesin increased these induced parameters with the middle dose resulting in numbers that were very close to those of captopril. These data suggested that fargesin reduced oxidative injury by enhancing the endogenous anti-oxidant capacity.

**Effects of fargesin on the liver total NOS and GSH-Px activities**

Total NOS and GSH-Px activities in the liver tissues reduced in the hypertensive model group, compared with those of the corresponding tissues from sham group ($P<0.05$). While treatment with fargesin or captopril effectively ameliorated the activities of NOS and GSH-Px (fig.5, $P<0.05$, vs. model). The NOS and GSH-Px activity in the fargesin and captopril groups did not differ significantly.

**Discussion**

Fargesin is one of the bioactive lignans contained in several TCMs such as *Magnolia biondii Pamp* (Wang et al. 2015). Some reports have suggested that fargesin can stimulate glucose uptake in L6 myotubes, improve lipid and glucose metabolism in 3T3-L1 adipocytes and high-fat diet-induced obese mice (Choi et al. 2013; Lee et al. 2012). In our previous study, we found that fargesin was a potential β1AR blocker candidate by downregulation of cAMP and PKA *in vitro* and has ability to protect myocardium from I/R damage *in vivo* (Wang et al. 2015). But there is no report about its antihypertensive effect. In this study, we evaluated this effect of fargesin *in vitro* and *in vivo*.

In rat aortic ring assays, fargesin showed a significant potential to induce full relaxation, and it relaxed rat aorta pre-contracted with KCl and NE in a concentration-dependent manner on both intact and denuded endothelium. Furthermore, the fargesin showed more vasorelaxant effect on endothelium-intact rat aorta, which suggested that the relaxing effect may be related with the condition of endothelium. Preincubation of the rings with either L-NAME or Indo profoundly attenuated fargesin-elicited relaxation in KCl-induced or NE-induced precontraction. These results were lighted up in elution curves that fargesin might have antihypertensive effects via antioxidatant and promoting NO release. However, very
little information is available regarding the pharmacological effects of fargesin, we need to provide more evidence.

Hypertension is a leading modifiable risk factor for cardiovascular disease. It is now considered to be part of a complex syndrome of changes in the structure and function of the cardiac and vascular systems (Kalra et al. 2010). As a characteristic symptom, it causes an increase in blood pressure and can cause multi-organ and multi-system damage in hypertensive patients (Galzerano et al. 2010). 2K1C hypertension is an experimental model that reproduces in animals’ human renovascular hypertension, a secondary form of hypertension related to the activation of the RAS, in which oxidative stress is clearly involved (Higashi et al. 2002; Lerman et al. 2001; Minuz et al. 2002; Welch et al. 2003). In this study, we tested the effects of fargesin on a rat model of hypertension, on determinants of the hypertension (angiotensin, ET) and its consequence (myocardial hypertrophy).

Renin–angiotensin system (RAS) can adjust water-sodium balance and vasoconstriction and play a crucial role on the development of renal hypertension, the key factor in which is angiotensin II (AngII) and Endothelin (ET) (Zhou et al. 2014). ET is able to greatly contract the coronary and renal arteries, and elevate systemic blood pressure, which makes it the strongest vasopressive substance known so far. Endothelin (ET) and AngII are mutually promotive (Li and Schiffrin 1995; Reinhold et al. 2009; Tran et al. 2009; Willette et al. 1998; Wang et al. 2007; Yoshida et al. 1991). These are proved in our hypertensive rats, where simultaneously the AngII increases with ET, and all changes that are opposed but not fully reversed by the angiotensin converting enzyme inhibitor captopril. On the other hand, sustained high blood pressure is one of the most powerful determinants of cardiac hypertrophy. In our study, the hypertensive rats treated with fargesin showed reduced cardiac and left ventricular indices as compared to the model group, reaching values similar to those of captopril rats. This fact suggests that fargesin possesses antihypertrophic properties. The present results show for the first time that chronic orally treatment of 2K1C hypertensive rats with fargesin reduced SBP and decreased cardiac hypertrophy. But
we found no dose dependence of this effect, which thus is probably already maximal for this drug.

One of the possible mechanisms that have been proposed to explain the antihypertensive effect of the fargesin is promoting the release of NO. NO is one of the major endothelial-derived vasorelaxant substances. Under normal physiological conditions, NO is released from the constitutive enzyme, NO synthase (cNOS), where it plays a role in regulating essential kidney functions such as blood flow and vascular tone. Under pathological conditions, however, activation of the inducible isoenzyme (iNOS) leads to overproduction of NO, which then interacts with ROS producing peroxynitrites and related compounds that are highly damaging to the tissues (Mansour et al. 2011). The level of NO was found to be markedly increased and the activity of NOS was enhanced in the fargesin treated rats. These findings were consistent with our results in rat aortic ring assays.

A further possible mechanism to explain the antihypertensive effect of fargesin may be related to its antioxidant activity. Oxidative stress was caused by reactive oxygen species (ROS), which also was an important mediator of the progression of renal injury in different animal models of hypertension (Datla and Griendling 2010). ROS were generated by normal respiration of cells and regulated by xanthine oxidase (XOD), nicotinamide adenine dinucleotidephosphatase (NADPH) oxidase, glucose oxidase, cyclooxygenase (COX), all of which were found in the hypertension (Mansour et al. 2011; Nishiyama et al. 2004). Under pathological conditions, increased ROS bioactivity lead to endothelial dysfunction, increased contractility, vascular smooth muscle cell (VSMC) growth, lipid peroxidation and increased deposition of extracellular matrix proteins, which were important factors in hypertensive vascular and renal damage (Diep et al. 2002). MDA, a biomarker for oxidative stress, increased markedly in plasma of hypertensive rats. This fact possibly indicated significant damage as a result of ischaemia and subsequent elevation of AngII. At the same time, cells maintain an endogenous anti-oxidative capacity consisting of SOD, CAT, NOS and GSH-Px enzyme systems that defend against possible deleterious effects of ROS and protect against various forms of oxidative cardiovascular injuries (Han et al. 2008; Ryter et al. 2007).
SOD scavenges superoxide radical by accelerating its conversion to hydrogen peroxide (H_2O_2) and CAT acts in the decomposition of H_2O_2 to water and oxygen. The biochemical role of GSH-Px is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. Our present findings showed that the activity of SOD, CAT, NOS and GSH-Px were markedly reduced in the hypertensive rats and that treatment with fargesin was associated with arise in the activity of these antioxidant enzymes, possibly in an attempt to limit tissue damage induced by the increased production of free radicals in the ischemic kidneys. Furthermore, it is likely that fargesin can reduce SBP (at least in part) by attenuating oxidative damage.

In conclusion, the present study provided proof of concept that fargesin can relax rat aortic ring in vitro and reduce elevated blood pressure of 2K1C hypertensive rats via attenuating oxidative damage and promoting NO release. But these effects of fargesin didn’t show dose-dependence, the middle dose had better effect than the low dose and the high dose. The results may be related to the target spot or receptor that fargesin acts on. So we need further study on the precise mechanisms of fargesin. Although the precise mechanisms underlying the effects remain to be clarified, this finding suggests that fargesin may be a promising agent for treatment of hypertension.

Acknowledgment

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Conflict of interest

The authors declare that they have no competing interests.

References


Table 1 SBP and cardiovascular parameters in renovascular hypertensive rats after 5 weeks of daily oral treatment with captopril, low-dose fargesin (Far-L), medium-dose fargesin (Far-M) and high-dose fargesin (Far-H), compared with untreated controls (model) and sham-operated rats (sham)

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<th>Groups</th>
<th>MODEL</th>
<th>SHAM</th>
<th>CAP</th>
<th>Far-L</th>
<th>Far-M</th>
<th>Far-H</th>
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<td>SBP(mmHg)</td>
<td>177±4*</td>
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<td>156±11*</td>
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<td>BW(g)</td>
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<td>LHW(mg)</td>
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<td>655±19*</td>
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<td>HW/BW</td>
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<td>LVAWTh(cm)</td>
<td>0.39±0.03*</td>
<td>0.30±0.04*</td>
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<td>0.33±0.01*</td>
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Abbreviations: BW, body weight; HW, heart weight; HW/BW, heart weight to body weight ratio; LHW, left heart weight; LVAWTh, left ventricular anterior wall thickness; SBP, systolic blood pressure.

* Values are mean ± s.e.m.

* P < 0.05 vs. model; † P < 0.05 vs. sham.
**Fig.1.** Fargesin-induced endothelium-dependent and independent relaxation in the rat isolated thoracic aorta. Concentration-dependent vasodilation by cumulative administration of fargesin with KCL (60 mmol/L) and NE (10 µmol/L) as contractor in both endothelium intact and denuded thoracic aorta rings. Data are expressed as mean ± SD, n=5-6. *P<0.05, **P<0.01 compared to endothelium-dependent.

**Fig.2.** Effects of cyclooxygenase inhibitor indomethacin (Indo, 10µmol/L), nitric oxide synthase inhibitor N^G^ -nitro-L-arginine methyl ester (L-NAME, 0.1 mol/L) on fargesin-induced relaxation of the rat isolated thoracic aorta precontracted with KCl (40 mmol/L) and NE (1.0 µmol/L). One of these inhibitors was added 20min before fargesin was cumulatively added into the organ chambers. Relaxations (mean ± SD, n= 6-8) are expressed as the percentage of tension decline on the precontraction.

*P<0.05, ** P<0.01 compared to control (fargesin alone)

**Fig.3.** Effect of captopril and Far at low, medium and high doses (Far-L, Far-M, Far-H) on plasma ET-1 and Ang II in two-kidney, one-clip hypertensive rats, compared with sham-operated rats and untreated control (model) rats.

*P <0.05 vs model ; 5P <0.05 vs. sham.

**Fig.4.** Effect of captopril and Far at low, medium and high doses (Far-L, Far-M, Far-H) on plasma MDA and NO levels, activities of SOD and CAT in two-kidney, one-clip hypertensive rats, compared with sham-operated rats and untreated control (model) rats.

*P <0.05 vs . model; 5P <0.05 vs. sham.

**Fig.5.** Effect of captopril and Far at low, medium and high doses (Far-L,Far-M, Far-H) on activities of liver NOS,GSH-Px in two-kidney, one-clip hypertensive rats, compared with sham-operated rats and untreated control (model) rats.

*P <0.05 vs. model; 5P <0.05 vs. sham.
Fig. 1. Fargesin-induced endothelium-dependent and independent relaxation in the rat isolated thoracic aorta. Concentration-dependent vasodilation by cumulative administration of fargesin with KCL (60 mmol/L) and NE (10 μmol/L) as contractor in both endothelium intact and denuded thoracic aorta rings. Data are expressed as mean ± SD, n=5-6.

*p<0.05, **p<0.01 compared to endothelium-dependent.

150x78mm (300 x 300 DPI)
Fig. 2. Effects of cyclooxygenase inhibitor indomethacin (Indo, 10\(\mu\)mol/L), nitric oxide synthase inhibitor NG-nitro-L-arginine methyl ester (L-NAME, 0.1 mol/L) on fargesin-induced relaxation of the rat isolated thoracic aorta precontracted with KCl (40 mmol/L) and NE (1.0 \(\mu\)mol/L). One of these inhibitors was added 20 min before fargesin was cumulatively added into the organ chambers. Relaxations (mean ± SD, \(n = 6-8\)) are expressed as the percentage of tension decline on the precontraction.

*\(p<0.05\), **\(p<0.01\) compared to control (fargesin alone)

150x71mm (300 x 300 DPI)
Fig. 3. Effect of captopril and Far at low, medium and high doses (Far-L, Far-M, Far-H) on plasma ET-1 and Ang II in two-kidney, one-clip hypertensive rats, compared with sham-operated rats and untreated control (model) rats. *P < 0.05 vs model.; #P < 0.05 vs. sham.
Effect of captopril and Far at low, medium and high doses (Far-L, Far-M, Far-H) on plasma MDA and NO levels, activities of SOD and CAT in two-kidney, one-clip hypertensive rats, Fig.4 compared with sham-operated rats and untreated control (model) rats.

*P < 0.05 vs. model; #P < 0.05 vs. sham.
Fig. 5. Effect of captopril and Far at low, medium and high doses (Far-L, Far-M, Far-H) on activities of liver NOS and GSH-Px in two-kidney, one-clip hypertensive rats, compared with sham-operated rats and untreated control (model) rats. *P < 0.05 vs. model; #P < 0.05 vs. sham.

150x62mm (300 x 300 DPI)