

Original Article

Hypertensive vascular remodeling was inhibited by Xuezhikang through the regulation of Fibulin-3 and MMPs in spontaneously hypertensive rats

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Abstract: Fibulin-3, an extracellular glycoprotein, has been suggested as having functions in vessels. In hypertension, extracellular matrix, matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) play important roles in cardiovascular remodeling. However, the role of Fibulin-3 as an extracellular glycoprotein in hypertensive vascular remodeling remains unclear. Our study was to determine whether Fibulin-3 and TIMPs/MMPs would affect vascular structure during hypertension and the treatment of Xuezhikang. Thirty spontaneously hypertensive rats (SHRs) aged 8 weeks were randomized to three groups: SHRs control group (SHRs group, n=10), group treated with low dose Xuezhikang (XZK-L, 20 mg/kg/d, n=10) and group treated with high dose Xuezhikang (XZK-H, 200 mg/kg/d, n=10), the normal group was comprised of ten Wistar-Kyoto (WKY) rats of the same age. We showed that serum nitric oxide (NO) in control group was significantly lower than WKY group ($P<0.05$). Concomitantly, serum oxidized low-density lipoprotein (ox-LDL) was higher than WKY group ($P<0.05$). The treatment of high dose Xuezhikang significantly decreased ox-LDL, left ventricular mass index (LVMI) and Wall-to-lumen area ratio (W/L) of thoracic aorta ($P<0.05$), while serum NO was significantly increasing ($P<0.05$). Moreover, the expressions of Fibulin-3 and MMP-2, 9 at both protein and mRNA levels were significantly higher in thoracic aorta of SHRs group compared to WKY group by immunohistochemistry and western blotting ($P<0.05$). However, the levels of Fibulin-3 and MMP-2, 9 were significantly decreased in XZK-H group compared to control group ($P<0.05$). The level of TIMP-3 had no significance difference between SHRs and WKY groups ($P>0.05$). So the levels of Fibulin-3 and MMP-2, 9 in SHRs could be inhibited by Xuezhikang. Furthermore, a strong correlation in transcript expression was established between Fibulin-3, and MMP-2 ($r=0.81$, $P<0.05$) and MMP-9 ($r=0.92$, $P<0.05$) through immunohistochemistry. In summary, the overexpression of Fibulin-3 and MMP-2, 9 levels were associated with hypertension and vascular remodeling and inhibited by Xuezhikang. Fibulin-3 is a candidate in the pathogenesis of cardiovascular remodeling in hypertension.

Keywords: Fibulin-3, hypertension, matrix metalloproteinase, spontaneously hypertensive rat, vascular remodeling

Introduction

The fibulins are the family of seven extracellular matrix proteins characterized by tandem arrays of epidermal growth factor (EGF)-like domains and a C-terminal fibulin-type module [1]. They are widely distributed in various tissues including vasculature and elastic tissues [2]. The seven-member family can be further classified into two subgroups. Fibulin-3 is a member of the second subgroup, which is similar to fibulin-4

and fibulin-5. They are small in size (50-60 kDa) and highly homologous to one another in modular structure. Fibulin-3 consists of a modified cbEGF domain at the N terminus followed by five tandem cbEGF modules and the fibulin-type C-terminal region. It has been reported that fibulin-4 and fibulin-5 played important roles in the pathogenesis of aortic diseases and the development of atherosclerosis [3, 4]. However, the role of Fibulin-3 in the hypertensive vascular remodeling remains unclear.

Remodeling of ECM is an important role of a number of physiological and pathological processes in the vessel wall [5]. The vascular extracellular matrix not only provides mechanical strength to the normal vessel wall but is also involved in cellular activities that are important for the development of atherosclerosis and restenosis [6]. Matrix metalloproteinases (MMPs) are believed to play a significant role in the degradation of extracellular matrix components [7]. The activity of MMPs is strictly regulated to maintain tissue homeostasis at a minimum of three steps: transcription, activation and inhibition. The tissue inhibitor of metalloproteinase 3 (TIMP-3), which binds to MMPs with a 1:1 molar stoichiometry, is an endogenous inhibitor of MMPs and regulate the matrix remodeling collaborated with MMPs. Higuchi M et al has reported that TIMP-3 plays essential roles in L-arginine methyl ester (L-NAME)-induced elevation of blood pressure and cardiac vascular remodeling [8]. At the same time, TIMP-3 is a binding partner of Fibulin-3 [9]. Then Fibulin-3 would potentially reduce extracellular matrix(ECM) proteolysis and remodeling, because Fibulin-3 could influence the expression of MMPs and TIMPs in endothelial cell [10].

Xuezhikang, the extract of red yeast rice, has been widely used as a Chinese traditional medicine for the therapy of patients with cardiovascular diseases. It contains natural Lovastatin and its homologues, as well as unsaturated fatty acids, flavonoids, plant sterols and other biologically active substances [11]. Previous clinical studies showed that Xuezhikang had a marked modulating effect on C-reactive protein (CRP) [12] and MMP-9 concentrations [13]. Statins have beneficial effects on endothelial NOS (eNOS) production, nitric oxide (NO) and antioxidant defence mechanisms in the vasculature [14]. However, no attention has been directed to the potential effects on hypertensive vascular remodeling.

We speculate that Fibulin-3, TIMP-3 and MMPs would play a important role in hypertensive vascular remodeling. Concomitantly, it would be involved in the mechanism of Xuezhikang treatment in hypertention. Then we performed this study.

Materials and methods

Animals and blood pressure measurement

8-week-old Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHRs) weighing 120-140 g were used. All rats were purchased from Beijing Vital River Experimental Animal Technology Co., Ltd. The SHR rats were randomly divided into SHR control group ($n=10$) and Xuezhikang (XZK)-treated groups. Treatment dosages were 20 and 200 mg/kg/d for the low-dose group (XZK-L group, $n=10$) and the high-dose group (XZK-H group, $n=10$), respectively. Xuezhikang was infused to stomach of rats every morning for 8 weeks, whereas the control group received normal saline. The normal group (WKY rats, $n=10$) received no addition. Blood pressure was serially determined in conscious, trained mice using a noninvasive tail-cuff device by a researcher blinded to the groups. All the rats were killed after 8 weeks of treatment. Tissue samples of aorta as well as peripheral blood from individual animals were collected. The experimental protocols were approved by the Guangdong Pharmaceutical University Animal Care and Use Committee and were performed in accordance with the Sun Yat-Sen University Guidelines for the Care and Use of Laboratory Animals.

Histological analysis

Rats were sacrificed with an overdose of pentobarbital after 8 weeks of administration. The thoracic aorta were removed and fixed in 4% paraformaldehyde (pH 7.4) or liquid nitrogen for protein isolation and western blotting. Fixed tissues were sectioned and stained with hematoxylin and eosin for the evaluation of vascular wall thickness or immunohistochemistry. The extents of medial thickening and of perivascular fibrosis of arteries were evaluated by the ratio of medial thickness to internal diameter and by the ratio of perivascular fibrosis area to total vascular area, respectively. The quantitative analysis on the thicknesses and areas were calculated by using Image-Pro Plus6.0.

Measurements of nitric oxide (NO) and oxidized low-density lipoprotein (ox-LDL)

Plasma was collected from all experimental rats after 8 weeks of administration. Plasma

Table 1. Primers used for reverse transcriptase polymerase chain reaction analysis

Target mRNA	Primer sequences	Product size
Fibulin-3	forward 5'-ACATGCCACTGTCTTCCTGG-3'	153 bp
	reverse 5'- GTTTGCTGCCAGCTGAAACC-3'	
MMP-9	forward 5'-CCCCTACTGCTGGTCCTTCTG-3'	127 bp
	reverse 5'-CCGACCGTCCTTGAAGAAATGC-3'	
MMP-2	forward 5'- CCATCGAGACCATGCGGAAG-3'	107bp
	reverse 5'-CCTGTATGTGATCTGGTTCTTG-3'	
GAPDH	forward 5'-ACAGCCGCATCTTCTTGTC-3'	162 bp
	reverse 5'-TTGAGGTCAATGAAGGGGTCG-3'	

concentrations of Ox-LDL were evaluated by using a standard commercial enzyme-linked immunoassay (Oxidized LDL ELISA, Beijing Xin Fangcheng Biotechnology Co., Ltd, China). Serum NO levels were measured using a Glucose Oxidase commercial kit (Sigma, St. Louis, MO, USA).

Immunohistochemistry

The thoracic aortas from experimental rats were dissected and deparaffinized by immersion in xylene, following a series of alcohol treatments. The sections were rinsed in PBS three times for 15 min each. Endogenous peroxidase activity was quenched by immersing the slides in 0.3% hydrogen peroxide in methanol for 15 min. The sections were rinsed in PBS three times for 10 min each and were blocked with 5% normal goat serum for 30 min. Primary antibodies were incubated for overnight. Sections were washed and incubated with secondary antibodies for 60 min. The slides were counterstained with hematoxylin (Sigma, St. Louis, MO, USA). The result was considered to be positive when brown precipitate developed in the cytoplasm. For quantification, pictures were captured and pixels were counted using Image Pro Plus Version 6.0 software (MediaCybernetics Inc., Bethesda, MD). The following primary antibodies were used: Fibulin-3 antibody (AP9095a, ABGENT, San Diego, CA), MMP2 antibody (A-AJ1497b, ABGENT, San Diego, CA), MMP-9 antibody (A-AJ1503b, ABGENT, San Diego, CA), TIMP-3 antibody (A-AO1052a, ABGENT, San Diego, CA). The secondary antibody in immunohistochemistry was from Dako.

Protein isolation and western blotting

Protein was extracted from the aorta by a protein extraction reagent. Aortas were dissected clear (3 pooled aortas per experiment) ground in liquid nitrogen. Samples were lysed on ice for 1 h with lysis buffer (1×PBS, 1% NP40, 0.1% SDS, 5 mM EDTA, 0.5% Sodium Deoxycholate, and 1 mM Sodium Orthoyanadate) containing 1% protease inhibitor PMSF (KeyGEN BioTECH, China), and clarified by centrifugation at 14000 g for 10 min at 4°C. The equal amounts of proteins were loaded into each lane of a 10% SDS-PAGE gel for protein separation in every experiment.

The protein bands were transferred to Polyvinylidene Difluoride membrane (Millipore, USA) which was pretreated with methanol. The membranes were incubated with primary antibodies overnight at 4°C and washed for three times before incubating secondary antibody and DAB staining. The following primary antibodies were used: Fibulin-3 antibody (AP9095a, ABGENT, San Diego, CA), MMP-2 antibody (A-AJ1497b, ABGENT, San Diego, CA), MMP-9 antibody (A-AJ1503b, ABGENT, San Diego, CA), TIMP-3 antibody (A-AO1052a, ABGENT, San Diego, CA).

Total RNA extraction and reverse transcriptase-polymerase chain reaction (RT-PCR)

A 3 mm-long segment of cryopreserved aorta was prepared for RNA extraction. One milliliter of Trizol reagent (Invitrogen Company, USA) was added and total RNA was extracted using chloroform and isopropyl alcohol. RNA A260/A280 was tested by an UV spectrophotometer to detect its purity. The A260/A280 of all the samples was greater than 1.7. Total RNA was extracted from aorta by using Trizol reagent (Invitrogen). 1 µg of total RNA from the aorta was used for the synthesis of the first-strand cDNA with a Revert Aid first-strand cDNA synthesis kit (Fermentas, Madison, WI) in a total volume of 20 µl. For quantitative PCR, SYBR Green supermix was used, and standard curves for each primer set were generated to confirm that only one amplicon was generated at the same efficiency as the reference gene GAPDH. The sequences of primers used for amplifying rat Fibulin-3, MMP-9, MMP-2 and internal refer-

Table 2. The datas about body weight, systolic blood pressure, heart rate, nitric oxide and oxidized low-density lipoprotein in four groups of experimental rats

group	n	body weight (g)	LVMI (%)	SBP (mmHg)	HR (beats/min)	NO (IU/ml)	oxLDL (pg/ml)
WKY	10	265.50±15.15	0.20±0.03	122±8	289±11	121.59±9.93	154.14±18.22
SHRs	10	270.75±14.15	0.30±0.02**	224±14*	311±11	81.18±6.94*	221.37±17.52*
XZK-L	10	259.25±16.02	0.28±0.02	214±11*	310±8	96.53±11.65 [#]	189.44± 24.74 [#]
XZK-H	10	261.81±16.85	0.26±0.03*	196±12*	302±10	105.78±10.43 [#]	174.02±32.35 [#]
F Value		1.774	4.45	9.014	1.523	36.903	18.06
P		0.175	0.011	0.000	0.236	<0.001	0.000

Values are means ± SE. XZK=Xuezhikang; LVMI=left ventricular mass index; HR=heart rate; NO=nitric oxide; ox-LDL=oxidized low-density lipoprotein. Compare to WKY group, * $P<0.05$, ** $P<0.01$; Compare to control group, [#] $P<0.05$.

ence β -actin were as stated in **Table 1**. PCR was performed by using the following thermal profile: 10 minutes at 95°C; denature at 95°C for 15 seconds, annealing for 25 seconds at 62°C, elongation at 60°C for 1 minutes, for 45 cycles; extension for 10 minutes at 72°C, and finally paused at 4°C. Relative expression was calculated by using the comparative Ct method ($2^{-\Delta\Delta Ct}$). $2^{-\Delta\Delta Ct} >3$ or $<1/3$ was deemed statistically significant.

Statistical analysis

Data are shown as the mean ± standard deviation. Differences between groups were analyzed using one-way ANOVA, while differences within two groups were assessed by unpaired t-tests. For statistical evaluation of correlations between parameters we used a univariate Spearman correlation analysis. Statistical analysis was performed using SPSS for Windows version 17.0. Probability values of $P<0.05$ were considered as statistically significant.

Results

General findings

Thirty spontaneously hypertensive rats (SHRs) were randomized to three groups: SHRs control group (SHRs group, $n=10$), group treated with low dose Xuezhikang (XZK-L, 20 mg/kg/d, $n=10$) and group treated with high dose Xuezhikang (XZK-H, 200 mg/kg/d, $n=10$), the normal group was comprised of ten Wistar-Kyoto (WKY) rats of the same age. The body weight, systolic blood pressure (SBP), heart rate (HR), nitric oxide (NO) and oxidized low-density lipoprotein (ox-LDL) were detected in experimental rats. The data are shown in **Table 2**. SBP and HR of SHRs were significantly higher

than those of WKY. For the WKY group, SBP did not differ significantly during the 8 weeks of monitoring. Blood pressure was significantly increased after 8 weeks in SHRs group and XZK-treated group. However, it was slightly higher in the SHRs group than XZK-treated group ($P>0.05$). There were no differences in final body weight and heart rate in rats from all groups. LVMI, an indicator of cardiac hypertrophy, was increased in SHRs group (mean=0.30±0.02) compared with WKY group (mean=0.20±0.03; $P<0.01$), while LVMI was decreased in XZK-H group (mean=0.26±0.03; $P<0.05$). The level of nitric oxide (NO) in SHRs group (mean=81.18±6.94) was significantly lower than WKY group (mean=121.59±9.93; $P<0.05$), while those in XZK-treated groups (mean=101.16±10.98) were significantly higher than SHRs group (mean=81.18±6.94; $P<0.05$). The level of NO in XZK-H group (mean=105.78±10.43) was near to that of WKY group (mean=121.59±9.93; $P>0.05$). The level of ox-LDL in SHRs group (mean=221.37±17.52) was significantly higher than WKY group (154.14±18.22; $P<0.05$). The treatment of Xuezhikang significantly decreased the level of ox-LDL in XZK-treated groups (mean=181.73±26.52) comparing to SHRs group (mean=221.37±17.52; $P<0.05$).

Histological analysis of aortic structure

To investigate the effect of hypertensive vascular remodeling, we investigated vascular wall thickness and Wall-to-lumen area ratio (W/L) of thoracic aorta of SHRs (16 week old), WKY and XZK-treated groups. Thoracic aortas were evaluated in sections stained with hematoxylin and eosin (**Figure 1**). Aortas of SHRs showed wall thickening and hypertrophic vascular smooth

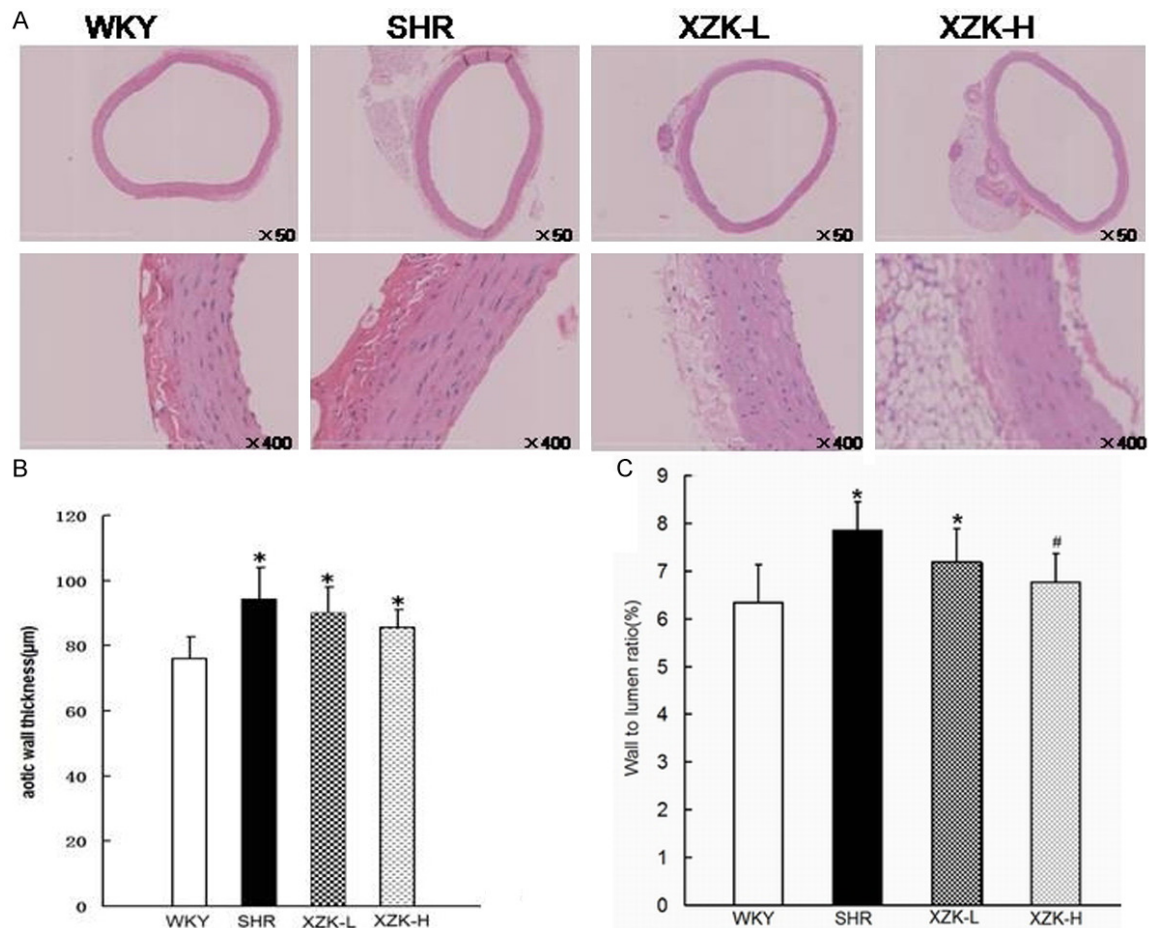
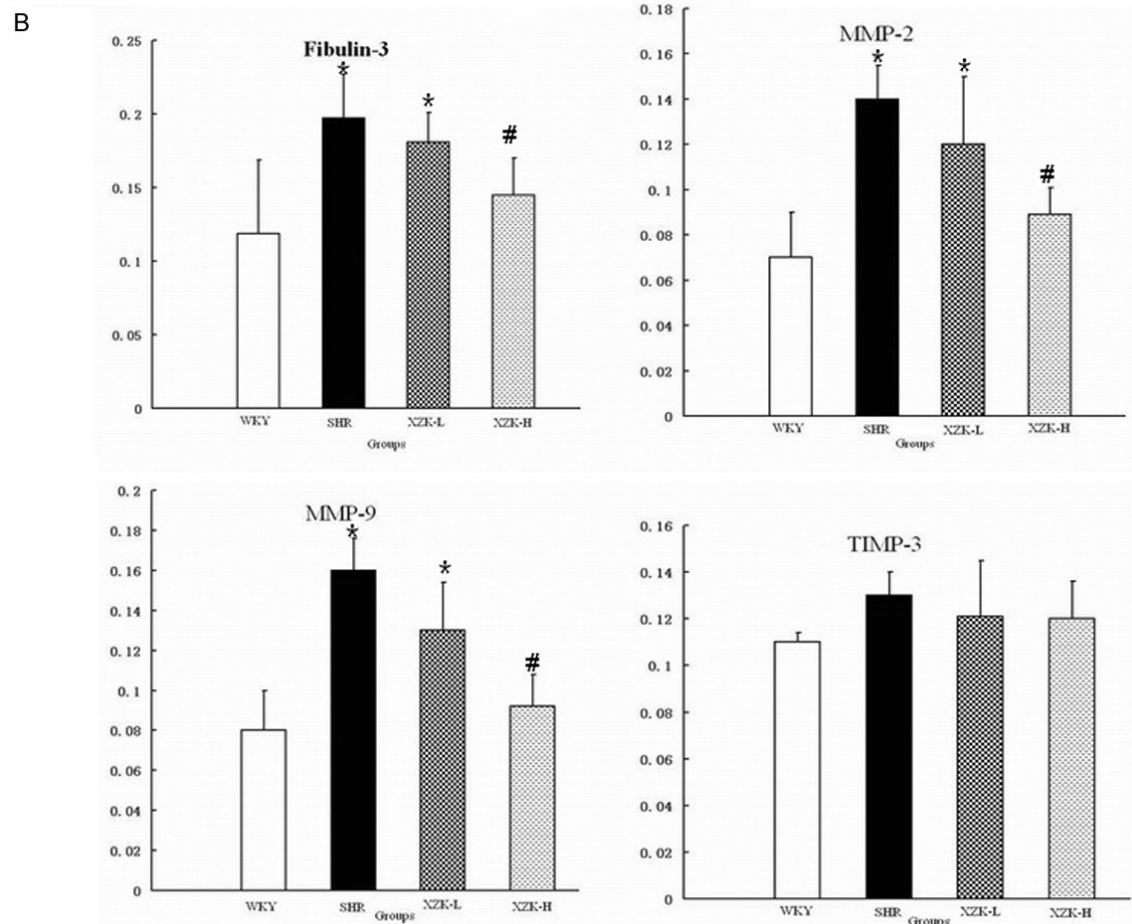
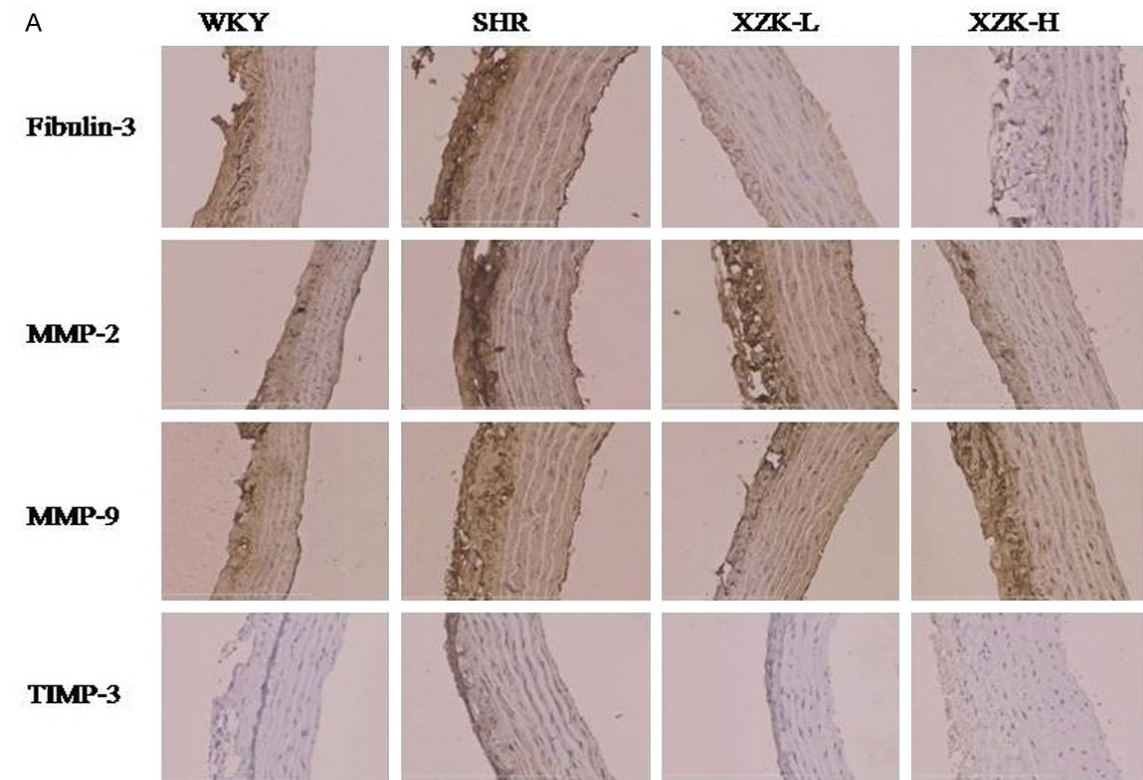


Figure 1. Histological Analysis of thoracic aorta. A. Representative images of thoracic aorta (H&E stain). B. The quantitative analysis on the thickness of thoracic aortic wall. The thickness of vascular wall of SHRs was significantly higher than that of WKY group. C. The quantitative analysis on wall-to-lumen ratio of thoracic aorta. Wall-to-lumen area ratio of aorta in SHRs group was significantly higher than that of WKY group. At the same time, wall-to-lumen area ratio of aorta was significantly decreased in the XZK-H group compared to SHRs group. Compare to WKY group, * $P < 0.05$; Compare to SHRs group, # $P < 0.05$.

muscle cells, which were foamed obviously. The thickness of vascular wall of SHRs (mean = 94.12 ± 9.84) was significantly higher than that of WKY group (mean = 78.65 ± 6.82 ; $P < 0.05$). The thickness of vascular wall of XZK-treated (mean = 87.92 ± 6.64) were less thicker in aortic wall than those of SHR group (mean = 94.12 ± 9.84), but there was no significance between XZK-treated group and SHRs group ($P > 0.05$) (**Figure 1B**). Wall-to-lumen area ratio of aorta in SHRs group (mean = 2.21 ± 0.26) was significantly higher than that of WKY group (mean = 1.21 ± 0.24 ; $P < 0.05$), it was significantly decreased in the XZK-H group (mean = 1.64 ± 0.25) compared to SHRs group (mean = 2.21 ± 0.26 ; $P < 0.05$) (**Figure 1C**).

The expression of Fibulin-3, MMP-2, MMP-9 and TIMP-3 in rat aortic tissue

The protein expression level of Fibulin-3, MMP-2, MMP-9 and TIMP-3 were detected in all groups by immunohistochemistry. Quantitation of the protein content of Fibulin-3, MMP-2 and MMP-9 were assessed by computerized planimetry in the aortic media in immunohistochemically stained slides by using Image-Pro Plus 6.0. In SHRs group, the levels of Fibulin-3, MMP-2 and MMP-9 were significantly increased in the thoracic aorta compared to WKY group ($P < 0.05$). The levels of Fibulin-3 and MMP-2, 9 were significantly decreased in XZK-H group compared to SHRs group ($P < 0.05$; **Figure 2A**). However, the level of TIMP-3 had no signifi-



Fibulin-3 and MMPs were inhibited by Xuezhikang

Figure 2. The protein expression level of Fibulin-3, MMP-2, MMP-9 and TIMP-3 were detected in all groups by immunohistochemistry. A. Fibulin-3, MMP-2 and MMP-9 In SHRs group were significantly increased in the thoracic aorta compared to WKY group. Fibulin-3 and MMP-2, 9 were significantly decreased in XZK-H group compared to SHRs group. TIMP-3 had no significance in the four groups. Positive staining is shown as brown in cells cytoplasm ($\times 400$). B. Quantitative analysis of Fibulin-3, MMP-2, MMP-9 and TIMP-3 expression in different experimental groups was performed. The data are expressed in terms of the fold of the WKY control group as mean \pm SD. Compare to WKY group, * $P < 0.05$; Compare to SHRs group, # $P < 0.05$.

Table 3. The expressions of proteins in rat aortic tissue by immunohistochemistry and quantitation by using Image-Pro Plus 6.0

group	n	Fibulin-3	MMP-2	MMP-9	TIMP-3
WKY	10	0.12 \pm 0.05	0.07 \pm 0.02	0.08 \pm 0.02	0.06 \pm 0.01
SHRs	10	0.19 \pm 0.03*	0.14 \pm 0.03*	0.16 \pm 0.04*	0.07 \pm 0.02
XZK-L	10	0.18 \pm 0.02*	0.12 \pm 0.04*	0.13 \pm 0.02*	0.06 \pm 0.01
XZK-H	10	0.15 \pm 0.03#	0.09 \pm 0.03#	0.09 \pm 0.02#	0.06 \pm 0.02
F Value		13.05	14.91	15.11	1.61
P		0.000	0.000	0.000	0.206

Values are means \pm SE. XZK=Xuezhikang; Compare to WKY group, * $P < 0.05$; Compare to SHRs group, # $P < 0.05$.

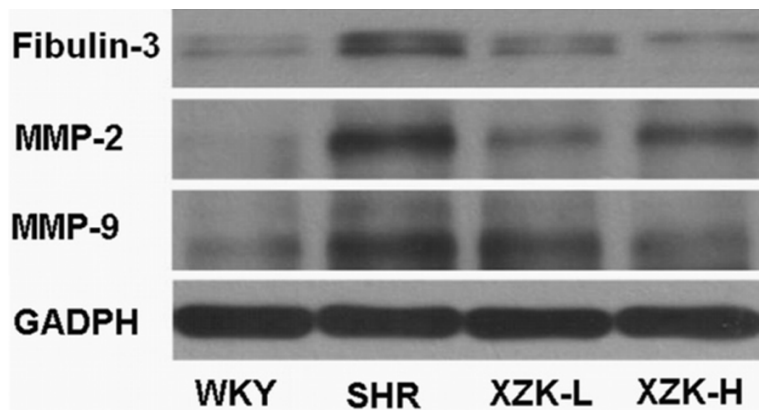


Figure 3. The protein expression level of Fibulin-3, MMP-2, MMP-9 and TIMP-3 of thoracic aorta were detected in all groups by western blotting. The levels of Fibulin-3, MMP-2 and MMP-9 in SHRs group were significantly higher than those in WKY group. Protein expression levels were normalized to GADPH.

cance in the four groups ($P > 0.05$). The data are shown in **Figure 2B** and **Table 3**.

Western blot analysis was also performed to measure the expressions of Fibulin-3, MMP-2, MMP-9 and TIMP-3 in thoracic aorta (**Figure 3**). The expression of Fibulin-3, MMP-2 and MMP-9 were also increased in SHRs group compared to WKY group. The expression of Fibulin-3, MMP-2 and MMP-9 were decreased in XZK-treatment groups. The level of TIMP-3 had no marked change in the four groups.

The transcription levels of Fibulin-3, MMP-2 and MMP-9 mRNA of thoracic aorta were analyzed by real time PCR. The levels of Fibulin-3,

MMP-2 and MMP-9 in SHRs group were significantly higher than those in WKY group ($P < 0.01$; **Figure 4A**). The expressions of Fibulin-3, MMP-2 and MMP-9 mRNA in the XZK-H group were significantly decreased compared to the SHRs group ($P < 0.05$; **Figure 4A**). A positive correlation in transcript expression was established between Fibulin-3 and both MMP-2 and MMP-9 (MMP-2, $r = 0.81$, $P < 0.05$; MMP-9, $r = 0.92$, $P < 0.05$; **Figure 4B**).

Discussion

In this study, we used spontaneously hypertensive rats to investigate whether Fibulin-3 and TIMP-3/MMPs were involved in hypertension and vascular remodeling. Vascular remodeling defined as any enduring change in the size and/or composition of an adult blood vessel following adaptation and repair. Hypertensive vascular remodeling was underlied the pathogene-

sis of major cardiovascular diseases, such as atherosclerosis and restenosis. In our study, remodeling of the aorta encompassed structural changes which were the increase in the wall thickness and wall-to-lumen ratio. However, these alterations might lead to impairment in the ability of vessels to autoregulate and dilate, which were showed an increase in the lumen diameter. Vascular remodeling can be caused by smooth muscle cell hypertrophy or hyperplasia and deposition of extracellular matrix (ECM) elements [15]. Our studies demonstrated the contribution of an increased number of intimal SMCs and proliferation and hypertrophy of medial SMCs. Fibulin-3 and MMP-2, 9

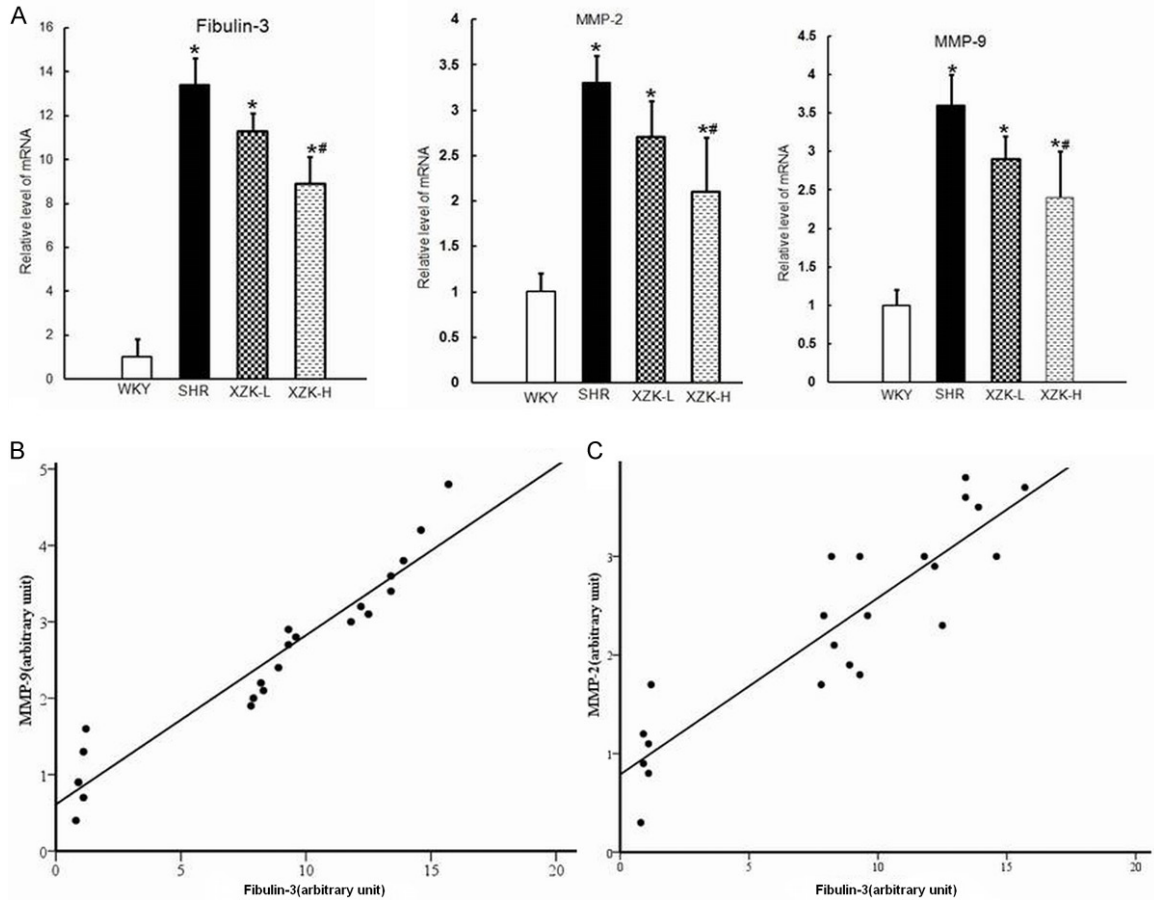


Figure 4. The transcription levels of Fibulin-3, MMP-2 and MMP-9 mRNA of thoracic aorta were analyzed by Real-time PCR. A. Real-time PCR examination of Fibulin-3, MMP-2 and MMP-9 in SHRs, XZK-H, XZK-L and WKY groups. The mRNA expression levels are presented as the increasing fold compared with WKY group or SHRs group and were normalized to GAPDH. Bars represent the SEM. Compare to WKY group, * $P < 0.05$; Compare to SHRs group, # $P < 0.05$. B. The scatter diagram showed the strong correlation between Fibulin-3 and MMP-9 on mRNA level. C. The scatter diagram showed the strong correlation between Fibulin-3 and MMP-2 on mRNA level.

expressed significantly in the media of aortic wall in SHRs than those in WKY group. The correlations between Fibulin-3 and MMP-2, -9 were significant. It indicated that Fibulin-3 and MMP-2, -9 played the important role in hypertensive vascular remodeling. It is generally considered that MMP-2 and MMP-9 were involved in smooth muscle cell migration [16, 17]. However, the effects of MMP-2 and MMP-9 are functionally different. A deficiency in MMP-2(-/-) reduces cell migration and inhibits neointimal formation after ligation of the mouse carotid artery, but it does not affect vessel diameter. However, the overexpression of MMP-9 affect vessel diameter in addition to influencing cell-matrix attachment and matrix reorganization [18, 19].

Interestingly, the changes of TIMP-3 were not apparent in aortic wall of WKY rats and SHRs, indicating that TIMP-3 is not involved in the early stage of hypertensive vascular remodeling. Moreover, a marked decrease in Fibulin-3 and MMP-2, -9 was observed in high dose Xuezhikang-treated SHR group. Xuezhikang has the effect on inhibiting the expression of Fibulin-3 and MMP-2, -9, which is beneficial to the hypertension. The mechanism about the upregulation of Fibulin-3 and MMP-2, 9 in hypertension remains to be elucidated. One possible explanation is that overexpression of Fibulin-3 in hypertension induces extracellular matrix components surrounding peripheral vessels, increasing vascular resistance and elevating the levels of MMPs. Another possibility is

that the action of Fibulin-3 and MMPs were used together in the vascular extracellular matrix of hypertension. It has been reported that fibulins is consistently associated with MMP-2, 9 expression in cancers [20] and arterial stiffening [21]. Thus, we suggested that the changes of the expression of Fibulin-3 and MMPs might induce the vascular structural change of the aortic wall, which led to vascular remodeling in hypertension.

Xuezhikang contains a family of naturally occurring statins. Statins have some other beneficial functions besides lipid regulation. Previous studies showed that Xuezhikang can increase the eNOS expression in aortic endothelia associating with the decrease of plasma lipids [22]. In our study, Xuezhikang also increased the level of serum NO and decreased of plasma ox-LDL. Many observations supported the idea of the MMPs inhibiting effects of statins on matrix contents and inflammation. Statins inhibit MMPs secretion (MMP-1, -2, -3, -9) from rabbit, human SMCs and macrophages [23]. Moreover, statins reduce MMP expression in hyperlipidemic rabbits [24], and increase plaque stability [25].

Clinical and experimental researchs have shown that statins inhibit renin-angiotensin system activation and improve vascular remodeling [26, 27]. In our study, Xuezhikang treatment could inhibit vascular wall thickness and Wall-to-lumen area ratio (W/L) of thoracic aorta in hypertetion. Moreover, Fibulin-3 and MMP-2, 9 were significantly decreased after Xuezhikang treatment. This indicates that Xuezhikang improves hypertensive vascular remodeling alike other statins and plays an important role in preventing restenosis. The downregulation of Fibulin-3 and MMP-2, 9 were the important roles of pathogenesis about Xuezhikang treatment in hypertension.

In this study, we showed for the first time that fibuin-3 plays essential roles in hypertensive vascular remodeling. Xuezhikang improves hypertensive vascular remodeling by inhibiting the levels of fibuin-3 and MMP-2, 9. Fibulin-3 is a candidate in the pathogenesis of cardiovascular remodeling in hypertension.

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Disclosure of conflict of interest

None.

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