Experimental study of blood pressure and its impact on spontaneous hypertension in rats with Xin Mai Jia

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ARTICLE INFO

Keywords: Xin Mai Jia
Primary hypertension
Spontaneously hypertensive rats
Renin-angiotensin-aldosterone system
Angiotensin II type 1 receptor
Vascular endothelial function

ABSTRACT

Aim: The aim of this study was to evaluate the antihypertensive effect of Xin Mai Jia (XMJ) and to explore the mechanism of its hypotensive effect.

Methods: A total of 50 spontaneously hypertensive rats (SHR) were randomised into five groups. A total of 30 Wistar-Kyoto rats were randomised into three groups, comprising the control group. All of the rats were administered medicine through a gastrogavage once a day for 8 weeks. The tail-cuff method was applied to their monitor blood pressure. After 8 weeks of treatment, serum NO, SOD activity, MDA level, ET, ALD, AngII, and CGRP in the serum were detected in all of the rats. Pathological changes in the aorta were observed via haematoxylin-eosin (HE) and immunohistochemical staining. Vasodilation function was assessed by measuring acetylcholine-induced vessel relaxation in the rats’ organ chambers. The function of the mesenteric arteries was measured using DMT wire myography. Human aortic smooth muscle cells (HASMCs) and human umbilical vein endothelial cells (HUVECs) injury models were induced by hydrogen peroxide (H2O2). HASMCs and HUVECs were injured by H2O2 and then exposed to various drugs. HASMC and HUVEC migration was evaluated using the cell scratch test. The expression of the AT1 receptors (AT1R) in the HASMCs was detected via immunofluorescence (IFC) assay.

Results: After 8 weeks of treatment, XMJ reduced the systolic blood pressure of the SHR. XMJ significantly reduced the serum RE, AngII, ALD, and ET-1 levels and increased the content of CGRP and NO in the SHR, upregulated the SOD content, and downregulated MDA level of the SHR. XMJ improved pathological damage of the aorta to varying degrees, decreased the expression of AT1R in the SHR aortic vessels, and improved the mesenteric microvascular relaxation of the SHR. Cell experiments confirmed that XMJ inhibited the migration of the HUVECs and HASMCs induced by H2O2 and the expression of AT1R in the HASMCs.

Conclusion: XMJ had satisfactory hypotensive action on the SHR in this study. Its mechanism may be associated with inhibiting RAAS activity and improving RAAS function, inhibiting hypertensive-induced vascular diastolic dysfunction, and improving vascular endothelial function.

1. Introduction

Primary hypertension (PH) is a disease with high blood pressure as the main clinical manifestation and unclear aetiology. It is a major risk factor for stroke, coronary heart disease, and other high-risk diseases. It accounts for 90% of the incidence of hypertension, and the incidence is increasing year by year [1,2]. Therefore, effectively controlling blood pressure remains a global public health problem.
Substantial research has shown that the renin-angiotensin-aldosterone system (RAAS) plays an important role in the occurrence and development of hypertension and is an important mechanism for regulating blood pressure. Improper activation of the RAAS can lead to hypertensive heart disease and stroke [3]. Angiotensin II (AngII) is a type of main active peptide and one of the strongest vasoconstrictive active substances. It has a significant effect on blood pressure by binding to a specific AngII receptor. AngII effects such as oxidative stress, vasoconstriction, aldosterone secretion, and vasopressin release are mediated by AT1 receptors (AT1R) [4].

Traditional Chinese medicine has a long-held and unique theory for the diagnosis and treatment of primary hypertension, and there are many different prescriptions for hypertension. Xin Mai Jia (XMJ) is a Chinese medicinal prescription that is available in capsule form. The formula contains functional red koi rice, kudzu flavonoid, soybean isoflavone, bamboo leaf flavones, and resveratrol (Patent No. ZL 2010 10536001.x). A previous study showed that XMJ can alleviate cardiovascular and cerebrovascular diseases and decrease blood lipid levels. XMJ has clear anti-inflammatory and anti-oxidant effects. XMJ can alleviate the symptoms of AS and reduce or eliminate complications after a few months. Moreover, XMJ can reduce blood lipids, normalise blood pressure, and improve sleep quality [5,6]. However, the effects and mechanisms of XMJ on primary hypertension have not been clarified. In the present study, XMJ was used to treat SHR, after which we determined the changes in blood pressure, AngII, endothelin (ET), aldosterone (ALD), renin-1 (RE-1), and calctomin gene-related peptide (CGRP) levels to explore the possible mechanisms.

2. Materials and methods

2.1. Composition and preparation of XMJ

XMJ crude drugs were purchased from Beijing Tong Ren Tang Co., Ltd. (Beijing, China). The preparation of XMJ was adopted from the published methods [7,8]. The formula contained 10–35% functional red koi rice powder, 1–10% kudzu flavonoid powder, 1–8% soybean isoflavone powder, 1–8% bamboo leaf flavone powder, 1–8% resveratrol powder, 1–6% hawthorn powder, 0.1-0.2% powdered hippocampus body, 0.008-0.04% astaxanthin powder, 0.1-0.3% menthol powder, and 20–50% resistant starch. These were ground into a superfine powder with a diameter of 10 μm or less using a microniser and prepared as capsules.

2.2. Animals and experimental designs

Fifty healthy male spontaneous hypertensive rats (SHR) (10 weeks old, SCXX (Jing) 2016-0011) and 30 healthy male Wistar-Kyoto (WKY) rats (10 weeks old, SCXX (Jing) 2016-0011) were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. They were housed in a temperature-controlled (21 ± 1 °C) and humidity-controlled (40-60%) environment with a 12h light/dark cycle and provided free access to clean water and laboratory chow. The animal use protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Henan Xinxiang Medical University (Xinxiang, China). All of the experiments were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

After one week of adaptive feeding, the SHR with systolic blood pressure (SBP) more than 170 mmHg and the WKYs with SBP less than 120 mmHg were used in this investigation. The WKYs were randomly divided into 3 groups: a WKY group (WG); a WKY + XMJ group (WXG) (3.24 g/kg/day, oral), and a SHR + XMJ group (SXG) (3.24 g/kg/day, oral), an SHR + XMJ + telmisartan II group (STAG) (8.32 mg/kg/day + 0.15 mg/kg/day, oral), an SHR + XMJ + telmisartan + angiotensin II group (SXTAG) (8.32 mg/kg/day + 0.15 mg/kg/day, oral).}

2.3. Measurement of blood pressure

SBP was determined in conscious rats using the tail-cuff method [9,10]. The rats were placed in a 37°C thermostatic fixator for approximately 10 min. The rat tails were occluded with the appropriate size tube-shaped tail cuff connected to the tail cuff device. The SBP and HR of the rats were measured via an XH200 thermostatic non-invasive blood pressure meter and an MD3000 biological signal collection and processing system (Huabei Zhenghua Biological Instrument Equipment Co., Ltd., Anhui, China). Each result was averaged from 3 repeats at the beginning of the study, 1 week, and every 2 weeks subsequently until euthanasia. The rats were habituated to this procedure at least 1 week prior to the experiments.

2.4. Collection and examination of blood samples

At the end of the experiments, all of rats fasted for 12 h, were weighted and anaesthetised with 10% chloral hydrate and then 5 ml of blood samples were collected from the neck aorta. Serum NO, SOD activity, MDA, ET, ALD, AngII, RE, and CGRP levels were examined using commercial Elisa kits (Jiancheng Biological Company; Nanjing, China).

2.5. Measurement of endothelium-dependent vasorelaxation (EDR)

The techniques used in this study were adopted from the published methods [11,12]. After the animals were anaesthetised, blood samples were collected. The common carotid artery was isolated by removing the adhering perivascular tissue carefully, cut into rings (3–4 mm in length), and then mounted in an organ chamber perfused with modified Krebs buffer. The contractile response was evoked by treatment with phenylephrine (PE 1 μM). At the plateau of contractions, accumulative acetylcholine (Ach) was added to elicit EDR as described previously to observe the effect of different drugs on vascular tension.

2.6. Pathomorphological observation

After the animals were anaesthetised, blood samples were collected. The thoracic aorta was obtained and embedded in paraffin. The thoracic aorta was processed in 5% CO2, 95% O2 mixture at 4°C until dissection. The mesenteric small arteries were isolated from the adipose or connective tissue under a dissecting microscope. After passage through two stainless steel wires (40 μm in diameter), the dissected mesenteric small arteries were mounted as ring-shaped preparations on a quadruple wire to measure the microvascular function.
2.8. Fundus photography

Ophthalmoscopy observations were performed using fundus photography in the rats anaesthetised with pentobarbital sodium (30 mg/kg, I.P.). The optic fundi were viewed and photographed using a fundus camera (Optomed, VET2). The fundus photography was based on the optic disc and the posterior polar region of the retina. The arteriolar changes were visualised directly by examining the ocular fundus.

2.9. Cell culture

Cell cultures were performed as described previously [7,15]. Human umbilical vein endothelial cells (HUVECs) from the Chinese Academy of Sciences were grown in DMEM supplemented with 10% FBS in a 37°C humidified atmosphere of 5% CO2 and 95% air. In all of the experiments, the cells were between passages 3 and 8. The ability of XMJ to change the migration of the HUVECs was evaluated using a scratch-wound assay. Under the suspension logarithmic phase, the vaccinated endothelial cells were seeded in 12-well plates at a density of 2.5 × 105 cells/well and treated with a medium without serum for 24 h to reach 80–90% confluency. The cultured cells were obtained for the subsequent tests.

Human aortic smooth muscle cells (HASMCs) were obtained from HASMC cell lines purchased from American Type Culture Collection (Manassas, VA, USA). The cells were grown in basal medium (Clonetics, Inc. Walkersville, MD, USA) supplemented with 2% foetal bovine serum, penicillin (100 U/ml), and streptomycin (10 mg/ml) in a 37°C incubator containing 5% CO2. The cells were used between passages 3 and 8. Cultured cells were obtained for the subsequent tests.

The experimental groups were as follows: (1) a normal group (NG), (2) a normal + XMJ group (NXG), (3) a normal + angiotensin II group (NAG), (4) a model group (MG), (5) a model + XMJ group (MXG), (6) a model + XMJ + angiotensin II group (MXAG), (7) a model + telmisartan group (MTG), and (8) a model + XMJ + telmisartan + angiotensin II group (MXTAG).

2.10. Cell scratch-wound assay

The ability of XMJ to change the migration of HUVECs and HASMCs was evaluated using a scratch-wound assay. The scratch-wound test was performed as described previously [16]. The cell layers were then scraped using a 200 μL pipett tip and the cell debris was rinsed with PBS. All of the cells were handled in 200 μmol/L H2O2 for 1 h except for the normal group (NG), the normal + XMJ group (NXG), and the normal + angiotensin II group (NAG). The cells were then treated with different drugs for 48 h, respectively. Images were taken immediately via bright field microscopy after the addition of media for 0 h and 48 h. The wound closure rate was calculated as follows: wound closure rate (%) = (scratched area before treatment - scratched area at 48 h after treatment)/scratched area before treatment × 100%.

2.11. Immunofluorescence (IFC) analysis

As described previously [17], the 48-well plate-cultured HASMCs were fixed at 15 min in 4% paraformaldehyde and then washed three times for 10 min. They were cultured in PBS (overnight at 4°C) and incubated with rabbit anti-rat AT1 receptor (1:200) antibodies (Abcam, Cambridge, MA, UK). After washing three times for 10 min, the cells were incubated in PBS for 1 h at room temperature with secondary antibodies (goat anti-rabbit Alexa Fluor 594 (1:200)). The nuclei were counterstained with 4-0-6-diamidino-2-phenylindole (DAPI; Invitrogen, Carlsbad, CA, USA). The immunofluorescent staining was analysed using a laser-scanning confocal microscope (SLM 510, Carl Zeiss Meditec, Inc., Jena, Germany).

3. Results

3.1. Effect of XMJ on the rats’ blood pressure

We first performed in vivo experiments to investigate whether XMJ has an effect on blood pressure in rats. As shown in Fig. 1, before administration, the SBP of the SHR was significantly higher than that of the WKYs (P < 0.05). After administration, the SBP of the SXG decreased by approximately 12 mmHg in the first week, continued to decrease significantly in the second week, and then maintained at approximately 155 mmHg. Compared with the SG, the SBP of the SXG was significantly lower than that of the SG from the first week of administration (P < 0.05). In addition, the SBP of the WXG was slightly lower than that of the WG, and the SBP of WAG increased significantly. The SBP of the SXG, STG, and SXTAG was all lower than that of SG, and there were significant differences between the two groups (P < 0.05). The results showed that XMJ significantly reduced the arterial SBP in the SHR.

3.2. Effects of XMJ on the rats’ serum NO, ET-1, and CGRP levels

NO and ET-1 are important endothelium-dependent vasodilator factors and vasoconstrictor factors [18]. They play key roles in the regulation of cardiovascular activity. CGRP is an important peptide...
transmitter of capsaicin-sensitive sensory nerves [19]. It is one of the known vasodilators and interacts with other vasoactive substances to regulate peripheral vascular resistance. As shown in Table 1, the content of ET-1 in the serum of the SG was significantly higher than that of the WG, and the content of NO was significantly lower than that of the WG, indicating abnormal ET metabolism in the SHR vascular endothelium, and the synthesis and utilisation of NO were also impaired. XMJ significantly inhibited ET-1 (P < 0.05) and increased the content of NO and CGRP (P < 0.05).

3.3. Effects of XMJ on the rats’ serum AngII, ALD, and RE levels

The RAAS plays an important role in the occurrence and development of hypertension and is key mechanism of regulating blood pressure. Overactivation of the RAAS is one of the main reasons for hypertension [20,21]. The RAAS is mainly composed of RE, ACE, and angiotensin receptors. RE acting on angiotensin originally generated AngI under the action of angiotensin-converting enzyme AngII. AngII is an important substance in the RAAS and its role in AT1R. It causes contraction of the blood vessels, stimulates the secretion of aldosterone, increases water sodium retention, stimulates the sympathetic nervous activity, and elevates the blood pressure. As shown in Table 2, the levels of RE, AngII, and ALD in SG were significantly higher than those in WG (P < 0.05), Compared with SG, XMJ significantly reduced the serum RE in the SHR, AngII, and ALD levels (P < 0.05).

3.4. Effects of XMJ on the rats’ oxidative stress

The decrease in the antioxidant system is one of the main complications of hypertension [22]. SOD is one of the important antioxidant enzymes in vivo and can effectively scavenge superoxide anions. MDA is a product of lipid peroxidation. Its content directly reflects the rate and intensity of lipid peroxidation. We examined whether XMJ affected the oxidative stress levels in the rats and found that SOD activity in the SHR serum was significantly reduced as shown in Fig. 2B and the MDA levels were significantly increased as shown in Fig. 2A. XMJ and telmisartan reduced the abnormal MDA and SOD activity in the SHR.

3.5. Effect of XMJ on vascular endothelial function in the SHR

PH patients have endothelium-dependent vasodilation dysfunction, and vascular endothelial dysfunction is common in PH. Our previous research found that XMJ significantly increased the endothelium-dependent relaxation response of the AS rats [5], Then we tested whether

| Table 1 |
| Comparison of the levels of NO, ET-1, and CGRP in the rats’ serum. |
| Groups | N | NO (μmol/L) | ET (ng/L) | CGRP (X value) |
| WKY group (WG) | 8 | 79.91 ± 5.41 | 44.39 ± 7.99 | 200.32 ± 10.75 |
| WKY + XMJ group (WXG) | 9 | 81.26 ± 6.58 | 45.69 ± 8.94 | 195.67 ± 12.52 |
| WKY + angiotensin II group (WAG) | 9 | 50.10 ± 3.73 | 66.73 ± 2.48 | 178.92 ± 5.31 |
| SHR group (SG) | 8 | 45.31 ± 3.26 | 78.27 ± 2.53 | 160.69 ± 5.92 |
| SHR + XMJ group (SXG) | 10 | 71.61 ± 2.10 | 59.27 ± 2.69 | 197.82 ± 4.73 |
| SHR + angiotensin II group (SXAG) | 8 | 57.36 ± 3.91 | 61.02 ± 2.72 | 171.49 ± 5.90 |
| SHR + telmisartan group (STG) | 8 | 75.95 ± 4.26 | 55.24 ± 3.52 | 211.18 ± 7.00 |
| SHR + XMJ + angiotensin II group (SXTAG) | 9 | 57.14 ± 2.18 | 58.92 ± 3.52 | 220.40 ± 4.16 |

NO, nitric oxide; ET-1, endothelin-1; CGRP, calcitonin gene-related peptide. The data were expressed as mean ± SEM.

* P < 0.05 vs SHR.

| Table 2 |
| Comparison of the levels of ALD, Ang II, and RE in the rats’ serum. |
| Groups | N | ALD (ng/L) | Ang II (ng/L) | RE (X value) |
| WKY group (WG) | 8 | 177.62 ± 8.14 | 98.56 ± 5.43 | 28.33 ± 2.26 |
| WKY + XMJ group (WXG) | 9 | 169.82 ± 9.74 | 96.55 ± 5.86 | 26.97 ± 5.22 |
| WKY + angiotensin II group (WAG) | 9 | 172.10 ± 9.26 | 93.13 ± 5.41 | 52.85 ± 2.89 |
| SHR group (SG) | 8 | 203.22 ± 17.22 | 171.33 ± 8.26 | 63.08 ± 4.92 |
| SHR + XMJ group (SXG) | 10 | 184.17 ± 6.84 | 117.47 ± 6.25 | 41.53 ± 2.68 |
| SHR + angiotensin II group (SXAG) | 8 | 189.65 ± 6.32 | 96.26 ± 6.60 | 43.42 ± 2.62 |
| SHR + telmisartan group (STG) | 8 | 176.39 ± 8.81 | 103.26 ± 7.66 | 34.61 ± 2.46 |
| SHR + XMJ + telmisartan + angiotensin II group (SXTAG) | 9 | 174.60 ± 10.42 | 111.23 ± 6.96 | 38.53 ± 2.67 |

ALD, aldosterone; Ang II, angiotensin II; RE, renin. The data were expressed as mean ± SEM.

* P < 0.05 vs SHR.
XMJ had an effect on the SHR vascular endothelial function. As shown in Table 3, the Ach Emax of the SG rats was 44.54 ± 9.55%. XMJ significantly increased the Ach Emax value of the SHR, which was different from the SG (P < 0.05). The Ach EC50 of the SG rats was 2.65 ± 0.43 μmol/L, while the Ach EC50 of the SXG rats was significantly reduced (P < 0.05). XMJ improved the EDR function of the SHR and protected the endothelial cells of the SHR.

3.6. Pathomorphological changes in the rats’ aortas

A naked-eye morphological observation revealed (Fig. 3A) that, compared with WG, the SG group had obvious endothelial injury and decreased vascular elasticity. Telmisartan decreased the SHR vascular endothelial injury and increased vascular flexibility. XMJ also alleviated the SHR vascular endothelial injury, enhanced vascular endothelial continuity, and increased vascular elasticity. As indicated in Fig. 3B, under a light microscope, the aortic walls of the SHR were obviously thickened, the intimal surface was not smooth, the endothelial continuity was poor, the endothelial cells were not orderly arranged, and the morphology was irregular. The smooth muscle cells were disordered. The endothelium of the XMJ-treated SHR was relatively smooth and the endothelial cells were arranged neatly. The membrane was slightly thickened compared with WG. The smooth muscle cells were arranged neatly, and their degree was significantly reduced compared with SG. This indicated that XMJ significantly improved the morphology of the SHR aortas.

3.7. Effect of XMJ on AT1R levels in rats’ aortas

AT1R is the main receptor of Ang II, and its mediated vasoconstriction, promoting the VSMCs proliferation effect in the development of hypertension, plays an important role [23]. As indicated in Fig. 4, the immunohistochemical results of AT1R showed that the positive staining was brown, mainly distributed in the membrane or cytoplasm of the aortic vascular endothelial cells and smooth muscle cells. AT1R was weakly positive in the WG. High positive expression of AT1R was found in the SG and WAG, with a large amount of brown in the cytoplasm and interstitium; In the SXG and STG, some scattered brown-yellow stains were found in the aortic membrane, cytoplasm, and interstitium, showing a weak positive reaction. The expression of AT1R was

Table 3
Effects of XMJ on endothelium-dependent vasodilatation in the rats’ aortas.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Ach Emax (%)</th>
<th>Ach EC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY group (WG)</td>
<td>8</td>
<td>93.43 ± 11.32*</td>
<td>0.27 ± 0.07*</td>
</tr>
<tr>
<td>WKY + XMJ group (WXG)</td>
<td>9</td>
<td>95.65 ± 12.14*</td>
<td>0.28 ± 0.08*</td>
</tr>
<tr>
<td>WKY + angiotensin II group (WAG)</td>
<td>9</td>
<td>49.84 ± 6.52</td>
<td>1.36 ± 0.29</td>
</tr>
<tr>
<td>SHR group (SG)</td>
<td>8</td>
<td>44.54 ± 9.55</td>
<td>2.65 ± 0.43</td>
</tr>
<tr>
<td>SHR + XMJ group (SXG)</td>
<td>10</td>
<td>56.32 ± 7.66*</td>
<td>1.63 ± 0.33*</td>
</tr>
<tr>
<td>SHR + XMJ + angiotensin II group (SXAG)</td>
<td>8</td>
<td>43.68 ± 8.44</td>
<td>2.72 ± 0.51</td>
</tr>
<tr>
<td>SHR + telmisartan group (STG)</td>
<td>8</td>
<td>89.36 ± 9.87*</td>
<td>0.35 ± 0.09*</td>
</tr>
<tr>
<td>SHR + XMJ + telmisartan + angiotensin II group (SXTAG)</td>
<td>9</td>
<td>58.92 ± 6.88*</td>
<td>1.72 ± 0.32*</td>
</tr>
</tbody>
</table>

The data were expressed as mean ± SEM.

* P < 0.05 vs SHR.
significantly lower than that in the SG.

3.8. Effect of XMJ on the rats’ microvasculature

The mesenteric artery is often used to evaluate the function of the microvessels. We used DMT to evaluate the effect of XMJ on the SHR mesenteric microvascular function. Ach can induce endothelium-dependent microvascular relaxation. As indicated in Fig. 5A, the microvascular diastolic rate induced by 10 mol Ach in the SG was significantly lower than in the WG. XMJ significantly improved the endothelium-dependent microvascular diastolic function of the SHR [24]. The retinal vascular diameter is also an important index to evaluate microvascular remodelling. We further confirmed the effect of XMJ on the SHR microvasculature via fundus photogrammetry. As indicated in Fig. 5B, the diameter of the retinal artery in the SHR was slightly narrow. Treatment with XMJ partially reversed this phenomenon. These data indicate that XMJ improved the microvascular dysfunction induced by hypertension.

Fig. 4. Effect of XMJ on the AT1R levels in the rats’ aortas (magnification, x400). a, WKY group. b, WKY + XMJ group. c, WKY + angiotensin II group. d, SHR group. e, SHR + XMJ group. f, SHR + XMJ + angiotensin II group. g, SHR + telmisartan group. h, SHR + XMJ + telmisartan + angiotensin II group. N = 8–10 rats in each group.

Fig. 5. Effect of XMJ on the rats’ microvasculature. (A) Effect of XMJ on the mesenteric microvessel endothelial diastolic function of the SHR measurement using DMT wire myography. Relative relaxation of the small mesenteric arteries from the WKY and SHR groups in response to acetylcholine (Ach). (B) The diameter of the retinal artery was determined via ophthalmoscopy. The representative pictures are shown. a, WKY group. b, WKY + XMJ group. c, WKY + angiotensin II group. d, SHR group. e, SHR + XMJ group. f, SHR + XMJ + angiotensin II group. g, SHR + telmisartan group. h, SHR + XMJ + telmisartan + angiotensin II group. The data were expressed as mean ± SEM. N = 8–10 rats in each group. *P < 0.05 vs SHR.
Effect of XMJ on migration of HUVECs and HASMCs

Endothelial cell migration is crucial to the repair of injured endothelium [25]. We studied the effect of XMJ on H₂O₂-induced HUVECs migration, conducted a scratch test of the endothelial cells, and observed the migration ability of the HUVECs. As indicated in Fig. 6A and B, XMJ inhibited cell migration in the scratch HUVECs and increased the ability of the HUVECs to repair scratch injury. As the main

Fig. 6. Effect of XMJ on H₂O₂-induced HUVEC and HASMC migration. (A) The HUVECs were scratched and the wound healing was determined 48 h after the scratch test. (B) Quantitative analyses of endothelial HUVEC migration. (C) The HASMCs were scratched and the wound healing was determined 48 h after the scratch test. (D) Quantitative analyses of endothelial HASMC migration. a, Normal group. b, Normal + XMJ group. c, Normal + angiotensin II group. d, Model group. e, Model + XMJ group. f, Model + XMJ + angiotensin II group. g, Model + telmisartan group. h, Model + XMJ + telmisartan + angiotensin II group. The data were expressed as mean ± SEM. *P < 0.05 vs the model group.
Fig. 7. Immunofluorescence analysis was used to detect the levels of AT1R in the HUVECs. (A) and (B) Immunofluorescence staining of the antibodies to AT1R (red) in all of the groups' HUVECs. DAPI (blue) was used to stain the nucleus (magnification, x400). (C) Quantitative analyses of AT1R protein levels. The data were expressed as mean ± SEM. *P < 0.05 vs the model group (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).
component of the vascular wall, HASMCs are closely related to the occurrence of hypertension. Therefore, we also performed HASMCs scratch experiments. As indicated in Fig. 6C and D, the scratch width of the model group was significantly reduced, the cell migration ability was enhanced, and the scratch width was significantly increased after XMJ treatment. XMJ inhibited the migration of HASMCs.

3.10. The effect of XMJ on the expression of AT1R in HASMCs

Most of the functions of AngII are mediated by AT1R. The combination of AngII and AT1R stimulates the hypertrophy and proliferation of the HASMCs, resulting in thickening of the vascular wall and narrowing of the lumen. The expression of AT1R in the HASMCs was detected by IFC assay. AT1R protein was mainly expressed on the cell membrane of the HASMCs. As indicated in Fig. 7, AT1R protein was only slightly expressed in the normal group, and the intensity of the red fluorescence was very weak; the number of cells in the model group was significantly higher than in the normal group. The distribution of AT1R increased, and the intensity of the red fluorescence was the strongest. The fluorescent expression of the AT1R protein in the XMJ group was significantly decreased, indicating that XMJ could inhibit the distribution of AT1R in the HASMCs.

4. Discussion

4.1. This study’s major discovery

PH is a type of cardiovascular disease with unknown causes and is related to many factors. It is mainly caused by elevated blood pressure, which seriously threatens human health [26]. Traditional Chinese medicine and its formulas have received substantial attention for the treatment of hypertension, which has a broad prospect of clinical application and research value. In this study, we mainly discussed the hypertension effect of XMJ on SHR and related mechanisms. SHR have the characteristics of a 100% spontaneous hypertensive rate and a pathological change similar to human hypertensive cardiovascular disease. SHR have been widely used in experimental studies on the pathogenesis of hypertension and the efficacy of antihypertensive drugs [27]. In this study, the SHR were used as an animal model and the WKYs were set as a control strain to evaluate the effectiveness of XMJ. In the experiment, the rats were given XMJ, telmisartan, XMJ, and angiotensin II, respectively, for 8 weeks. The SBP in the XMJ group was significantly lower than that in the model group, indicating that XMJ reduced the SHR blood pressure.

4.2. How does XMJ reduce blood pressure?

The RAAS, which has important regulatory effects on blood pressure and cardiovascular remodelling, has become the focus of hypertension research in the past few decades [28]. AngII is one of the most important components of the RAAS and is known as the strongest of the vascular active substances. It participates in the vasomodulation, proliferation, and migration of the smooth muscle cells, acts on AT1R, decreases NO content, and increases ET content. AngII can also strongly stimulate the adrenocortical zona glomerulosa cells to promote the synthesis and release of ALD. ALD can promote the renal tubules to reabsorb Na+, so that the amount of extracellular fluid increases, leading to water retention. CGRP is a type of vascular factor with strong physiological activity. An increased content of CGRP can relax the renal artery, reduce peripheral resistance, and decrease blood pressure. The results showed that XMJ significantly reduced the serum RE, AngII, and ALD levels and increased the content of CGRP in the SHR. The hypotensive effect of XMJ might be related to inhibiting RAAS activity and improving RAAS function.

4.3. How does XMJ protect blood vessels?

This study found that in the chronic course of hypertension, the internal and external diameter of the middle arteries decreased, the ratio of the vessel wall thickness to the lumen diameter increased, while the cross-sectional area of the vessel wall remained unchanged, which was called “vascular remodelling” [29]. At present, substantial evidence has proved that vascular remodelling is an important feature of hypertensive patients. It mainly manifests as vascular wall thickening, vascular endothelial destruction, smooth muscle cell migration and proliferation, extracellular matrix deposition, and elastic protein fracture, among other factors [30]. In this study, the SHR aorta manifested multiple thickening, fibrosis, increased smooth muscle cell layer, disordered arrangement, thickening of the wall, etc. After treatment with XMJ, the SHR intima was improved, and pathological damage to the aorta also improved to varying degrees. The results of the immunohistochemical experiments indicated that the expression of AT1R was strongly positive in the aortic vessels of the SHR. XMJ significantly decreased the expression of AT1R in the SHR aortic vessels. Vascular smooth muscle cells (VSMCs) and vascular endothelial cells (VECs) are the main components of the vascular wall, and their proliferation and migration are the central link of vascular remodelling [31]. The cell scratch test is an experimental method to establish a cell trauma model, measure the movement characteristics of the cells on the extracellular matrix, and observe the influence of some factors on wound healing in vitro. The scratch test can be used to observe the migration ability of cells in vitro. This study showed that XMJ inhibited the migration of the HUVECs and HASMCs induced by H2O2. An immunofluorescence assay showed that XMJ inhibited the expression of AT1R in the HASMCs. Therefore, XMJ could have a significant protective effect on the aortic injury in the SHR. Hypertension can lead to atherosclerosis, decreased arterial compliance, decreased elasticity, and impaired diastolic and contractile functions [32]. At the same time, it can also lead to endogenous remodelling of the microvessels and is often found in animal models of hypertension and in patients with hypertension in whom microvascular diastolic function is also impaired [33]. We further observed the effect of XMJ on the vasomotion of the SHR microvessels. The results showed that XMJ improved the mesenteric microvascular relaxation of the SHR.

4.4. Effect of XMJ on vasoconstriction

The dysfunction caused by vascular endothelial cell (VEC) injury is closely related to hypertension diseases. The vascular endothelium is a large endocrine organ, and NO and ET-1 are a pair of vasoconstrictor factors released by the VEC. ET-1 is a potent vasoconstrictor that causes constriction of the vascular smooth muscles, proliferation of the vascular endothelial cells, thickening of the vascular wall, and narrowing of the lumen, resulting in increased blood pressure and vascular remodelling [34]. NO is a strong vasodilator that can dilate the blood vessels, regulate blood pressure, inhibit endothelial cell proliferation and migration, and participate in vascular remodelling, which is an important sign of normal vascular endothelial function. The results of this experiment showed that XMJ increased NO and decreased ET-1, suggesting that XMJ could improve the vascular pathological changes induced by hypertension by repairing the vascular endothelium and inhibiting endothelial dysfunction.

4.5. How does XMJ reduce oxidative stress?

Hypertension damages the vascular endothelium, which produces large amounts of reactive oxygen species (ROS), which, in turn, act on hypertension. Excessive ROS attacks unsaturated fatty acids on the cell membrane and induces lipid peroxidation, thus increasing the level of MDA. The MDA content directly reflects the rate and intensity of lipid peroxidation. SOD is one of the most important antioxidant enzymes in vivo. It can effectively scavenge superoxide anions and maintain the
balance of free radicals in vivo. In this study, XMJ upregulated the SOD content and downregulated the MDA level of the SHR. This indicates that XMJ has an antioxidant effect, suggesting that this is one of the mechanisms of vascular endothelial protection.

5. Summary

The present study supports that Xin Mai Jia possesses hypotensive action on SHR, which is associated with inhibiting RAAS activity and improving RAAS function, inhibiting hypertensive-induced vascular diastolic dysfunction, and improving vascular endothelial function. This study provides a theoretical basis for XMJ for the prevention and treatment of hypertension and arterial injury protection and lays a foundation for further research.

Competing interest

The authors declare no competing financial interests.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (U1804197U81874312, 81571696, U1704175 and U1704168), the National Natural Science Foundation of China (19420051000, 17IRTSTHN022, 162300410216 and 182300410332), the Research Project of Xinxiang Medical University (XYBSKYZZ201626, 2016PN-KFKT-02, XYBSKYZZ505319, and 2017CXY-2-6). This work was supported by Vascular Remodelling Intervention and Molecular Targeted Therapy Drug Development Innovation Team and the Cardiovascular Remodelling Intervention and Molecular Targeting Drug Research and Development Key Laboratory.

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