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Research Paper

Effects of Xin-Ji-Er-Kang formula on 2K1C-induced hypertension and cardiovascular remodeling in rats

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ABSTRACT

Ethnopharmacological relevance: Xin-Ji-Er-Kang (XJEK), a Chinese herbal formula, is effective against hypertension induced coronary heart disease, viral myocarditis and toxic myocarditis. In this study, the effect of XJEK on cardiovascular system was investigated. To test the hypothesis that Xin-Ji-Er-Kang (XJEK) has an anti-hypertensive effect mediated through attenuation of cardiac remodeling, and amelioration of vascular endothelial dysfunction and oxidative stress.

Materials and methods: Hypertension was induced in Wistar rats by 2 kidney 1 clip (2K1C) treatment. The hypertensive rats were then randomly assigned into four groups and treated as follows: group 1 (Sham-operated [Sh-Op] group received only drinking water), group 2 (induced hypertensive model+no treatment), and group 3 (induced hypertensive+a single daily oral dose of 24 g kg^{-1} XJEK treatment) and group 4 (induced hypertensive+a single oral dose of 15 mg kg^{-1} Fosinopril treatment). The rats in all the defined groups were respectively treated for a period of 4 weeks. Cardiovascular parameter such as systolic blood pressure (SBP) was measured weekly by using tail-cuff apparatus; left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP) and the rate of the rise in left ventricular pressure ($\pm dp/dt$ max) were measured by using a PowerLab 8/30 apparatus (AD Instruments, Australia) at the end of the 8th week; heart weight/body weight (HW/BW) was determined as an index of myocardial hypertrophy (MH). Hematoxylin and eosin (H&E) and Van Gieson (VG) stain were used to assess the cardio-histological changes. Colorimetric analysis was used to assay serum superoxide dismutase (SOD) activity, malondialdehyde (MDA), nitric oxide (NO), and hydroxyproline (Hyp) contents in cardiac tissue. Angiotensin II (Ang II) content in serum was assessed by radioimmunoassay; tetrahydrobiopterin (BH_4) content in cardiac tissue, BNP and endothelial NOS (eNOS) in serum were determined by using ELISA, and the protein expressions of c-Jun NH2-terminal kinase (JNK), P-JNK, p38, P-p38, and NADPH oxidases-2 (Nox-2) were measured by western blot.

Results: XJEK therapy could impair the heart systolic and diastolic function, potently improve the heart weight index, inhibit the elevation of HW/BW ratio, and markedly ameliorate hemodynamic indices and vascular remodeling index. It has blunted the decrease of SOD, NO and the increase in MDA and Ang II serum contents, myocardial cross-section area (CSA), collagen volume fraction (CVF) and perivascular circumferential collagen area (PVCA) compared to the hypertensive model group. It also reduced the serum content of Hyp while increased BH4 levels in cardiac tissue. In addition, the expressions of Nox-2, P-JNK and P-p38MAPK were all suppressed compared to the hypertensive model group. Moreover, treatment with XJEK improved endothelial dysfunction (ED) manifested by promoting eNOS activities and enhancing the NO activity in serum.

Conclusion: The results of the present study show that XJEK attenuates 2K1C-induced hypertension in rats, which confirms our hypothesis that XJEK has an anti-hypertensive and cardiovascular remodeling effect *via* attenuation of cardiac remodeling and improvement of endothelial dysfunction and oxidative stress.

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1. Introduction

Hypertension is the most common risk factor for cardiovascular disease worldwide (Kearney et al., 2005). It has been on the increase in China in recent years, leading to complications, morbidity, and mortality among all age groups. Not only does hypertension lead to abnormal blood dynamics, sugar and fat metabolic disorders, but also it affects target organs such as heart, brain, and kidney (Li et al., 2007). Growing evidence indicates that oxidative stress (OS) plays an important role in cardiac remodeling and hypertension (Chan et al., 2006). Excessive productions of oxidant molecules lead to oxidative damage by overwhelming the anti-oxidant defense systems (Lönn et al., 2012). The oxidant molecules including free radicals and non-free radicals may cause damage to DNA, proteins, and lipids, leading to alterations in cellular functions or cell death (Lönn et al., 2012).

Endothelial dysfunction (ED) is also known to play an important role in the pathogenesis and progression of hypertensive heart disease. It is related to a reduced bioavailability of nitric oxide (NO) and oxidative stress resulting from excessive generation of ROS (Schiffrin and Touyz, 2004). It is clear that ROS can stimulate multiple signaling pathways involved in cell growth, differentiation, apoptosis, and hypertrophy.

Normally tetrahydrobiopterin (BH_4) is a cofactor of nitric oxide synthase (NOS) that is required for nitric oxide (NO) production from endothelial NO synthase (eNOS). A lack of BH_4 leads to eNOS uncoupling and then generation of superoxide (O^{2-}). As we know, eNOS is responsible for the production of NO. Mounting evidence indicates that a major source of ROS in the vessel wall is the family of NADPH oxidases (Griendling et al., 2000; Cave et al., 2006), in which Nox2-containing NADPH oxidase mainly contributes to the production of superoxide and ROS in endothelial cells (Ushio-Fukai et al., 2002; De Silva et al., 2009). ROS generation leads to the activation of MAPKs, c-Jun N-terminal kinases (JNK) and p38 MAPK, which are the most widely studied members of the MAPK family (Balbi et al., 2009). The activation of JNK and p38 MAPK plays a key role in regulating the progression from adaptable compensation hypertrophy. It is quite clear that a large number of biochemical factors are involved in the initiation and progression of endothelial dysfunction and oxidative stress.

Recent reports indicate that there has been an enormous increase in the demand for herbal and medicinal products over the last few years (Langmead and Rampton, 2001). Traditional Chinese Medicine (TCM) has been practiced more than two millennia, representing one of the most long used and tested alternative medicines in the world. TCM has seen vigorous scientific investigations recently because of its general acceptance, and one such TCM is the Xin-Ji-Er-Kang (XJEK). XJEK, a Chinese herbal formula, is composed of fourteen herbs including *Panax ginseng*, C.A.Mey., *Astragalus mongholicus* Bunge, *Ophiopogon japonicus* (Thunb.) Ker Gawl, *Polygonatum odoratum* (Mill.) Druce *etc*. It is effective against hypertension induced coronary heart disease, viral myocarditis and toxic myocarditis (Wang et al., 1998a, 1998b, 2000). The previous reports showed that XJEK exerted protective effects against isoproterenol-induced ventricular remodeling in mice through its actions in reducing the oxidative stress and improving the antioxidant activity of the body (Saw et al., 2012). This study evaluated the effect of XJEK on 2 kidney 1 clip (2K1C) induced hypertension in a rat model, as a means of providing a further scientific basis for future clinical application of XJEK.

2. Materials and methods

2.1. Animals

A total of forty-five healthy male Wister rats with an average weight of 200 ± 10 g were obtained from the Laboratory Animal Center of Nanjing Medical University. All the animals were handled and used by strictly observing the rules and regulations outlined in the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH publication no. 85-23, revised 1996), and approval was obtained from the Animal Care and Use Committee of Anhui Medical University.

Table 1
Recipe of XJEK formulation.

Components	Voucher specimens number	Part used	Rate (%)
Panax ginseng, C.A.Mey.	PCAHMU-20121005	Root	11.71
Polygonatum odoratum (Mill.), Druce	PCAHMU-20121006	Rhizome	7.03
Panax pseudoginseng var. notoginseng (Burkill), G.Hoo and C. L.Tseng	PCAHMU-20121007	Root	3.09
Allium macrostemon, Bunge	PCAHMU-20121008	Ramulus	7.80
Angelica sinensis (Oliv.), Diels	PCAHMU-20121009	Root	7.80
Ophiopogon japonicus (Thunb.), Ker Gawl.	PCAHMU-20121010	Root	7.80
Schisandra chinensis (Turcz.), Baill.	PCAHMU-20121011	Fruit	3.93
Salvia miltiorrhiza f. alba, C.Y. Wu & H.W. Li	PCAHMU-20121012	Root	7.80
Sophora flavescens, Aiton	PCAHMU-20121013	Root	7.80
Glycyrrhiza acanthocarpa (Lindl.), J.M.Black	PCAHMU-20121014	Rhizome	7.80
Astragalus mongholicus, Bunge	PCAHMU-20121015	Root	11.69
Epimedium acuminatum, Franch.	PCAHMU-20121016	Aerial part	7.80
Trichosanthes obtusiloba, C.Y. Wu	PCAHMU-20121017	Seed	7.80
Dryobalanops aromatica, C.F.Gaertn.	PCAHMU-20121018	Resin	0.15

2.2. Chemicals

XJEK, which consists of fourteen different medicinal herbs (Table 1), was obtained from Hefei Company of Traditional Crude Drugs (Hefei, China). Fosinopril was bought from Bristol-Myers Squibb (Shanghai, China).

2.3. Preparation of XJEK

All these herbs were carefully authenticated by Dr. He-ping Huang (AnHui University of Traditional Chinese Medicine, HeFei, China). Voucher specimens (numbers are listed in Table 1) were deposited at the Herbarium of Department of Pharmacology, Basic College of Medicine, Anhui Medical University (Hefei, China), and the preparation of XJEK extract could be found in the Supplement Data.

2.4. HPLC analysis

Water HPLC-Classchao Ultra High Performance Liquid Chromatography method, which is equipped with a TOSOH TSK-GEL ODS-80Tm column (460×150 mm^2, 5 μm, TOSOH, Japan), was performed with Empower3 software system. The mobile phase consisted of 0.05 M sodium dihydrogen phosphate solution (A) and acetonitrile (B) (0–50 min A: 85%, 40%; B: 15%, 60%). Detection wavelength was set at 270 nm. The injection volume was 10 μl and the flow rate was 1 ml/min at 30 °C. The dried aqueous extract, 5 g of XJEK, was accurately weighed, and dissolved in 30 ml of 50% methanol solution, and then an ultrasonicator was used for 30 min at 60 °C (100 w,40 HZ), centrifuged for 15 min at 4000 rpm. The precipitate was mixed with 15 ml of 50% methanol solution, and then we did the above procedure once again. The methanol solution was added into the mixing supernatants. The solutions were filtered through a 0.22 μm membrane filter and applied to HPLC. Fig. 1 shows the HPLC fingerprint of XJEK extracts.