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Icariside II attenuates myocardial fibrosis by inhibiting nuclear factor-κB and the TGF-β1/Smad2 signalling pathway in spontaneously hypertensive rats

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ABSTRACT

Studies have demonstrated that icariin plays important roles in preventing hypertension and improving myocardial hypertrophy, inflammatory and infiltration. Icariside (ICS II) is the main metabolite of icariin, which has anti-inflammatory and anti-oxidant activities and protects against ischaemic brain injury. Whether ICS II improves myocardial fibrosis in spontaneously hypertensive rats (SHRs) and the related mechanism remain unknown. Some studies have suggested that TGF-β and the nuclear factor κB signalling pathway play a key role in the progression of myocardial fibrosis. Therefore, in the current study, we aimed to evaluate the effects of ICS II on induced myocardial fibrosis in SHRs and explore the mechanism underlying this activity. The SHRs were treated with ICS II (4, 8, and 16 mg/kg) via daily gavage for 12 weeks. Left ventricular function was detected using the Vevo2100 system, and the collagen area was measured by Masson staining. The results indicated that ICS II markedly improved left ventricular function and decreased the left ventricular myocardial collagen area compared with the SHR group. To further investigate the mechanism underlying this activity, we measured the protein expression of interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α), transforming growth factor-β1 (TGF-β1), Smad2, inhibitory κB (IκB), and nuclear factor κB (NF-κB) p65 by Western blot. The results showed that ICS II inhibited NF-κB p65 expression and the TGF-β1/Smad2 signalling pathways. In conclusion, the present results suggest that ICS II suppresses myocardial fibrosis in SHRs, and this effect might be at least partially mediated through suppression of NF-kB signalling and the TGF-β1/Smad2 signalling pathway.

1. Introduction

It is currently believed that myocardial fibrosis is a common pathological change in heart disease that can be, to a certain extent, caused by a variety of pathological factors. Additionally, myocardial fibrosis is considered a key process during which heart functions change from the compensatory stage to the decompensation stage and a key factor in the prognosis of cardiovascular diseases. Myocardial fibrosis is also one of the main pathological features during ventricular remodelling. Improving myocardial fibrosis can effectively improve cardiac function and reduce the risk of cardiovascular disease [1]. Myocardial fibrosis might therefore contribute to the progression of cardiovascular diseases [2]. Hypertension is one of the most common diseases, a serious threat to human health, and a major risk factor for stroke, myocardial infarction, vascular disease, and chronic kidney disease. It might cause heart failure in 50–60% of affected patients, increase the risk of ischaemic stroke by 50%, and increase the risk of haemorrhagic stroke [3]. Hypertension can lead to a sustained increase in heart pressure overload, resulting in excessive proliferation and accumulation of collagen fibres in the interstitium and perivascular vessels, which can itself lead to myocardial fibrosis [4,5].

Some studies have demonstrated that excessive proliferation of cardiac fibroblasts, increased levels of collagen I and III, and an imbalance between these two factors are all major causes of myocardial fibrosis [6]. Some studies have also suggested that TGF-β plays a key role in the progression of myocardial fibrosis [7]. Prostacyclin, a prostacyclin analogue, inhibits myocardial fibrosis by inhibiting the TGF-β/Smad signal pathway [8]. Therefore, inhibiting the TGF-β/Smad signalling pathway might represent a beneficial therapeutic strategy for myocardial fibrosis. Recent studies have demonstrated that chronic inflammation can either directly or indirectly lead to myocardial fibrosis, necrosis and apoptosis [9]. Monocytes and macrophages produce and secrete pro-inflammatory mediators (e.g., cytokines, including interleukin (IL)-1β, tumour necrosis factor (TNF)-α and IL-6, and pro-

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fibrotic growth factors, such as TGF-β). These factors have direct effects on cardiac fibroblasts and induce the expression and release of a variety of mediators during the process of fibrosis to induce the development of myocardial fibrosis [10]. Therefore, the inhibition of inflammation is another approach for the treatment of myocardial fibrosis.

Herba Epimedii is a time-honoured traditional Chinese herbal medicine, and icariin is one of the main active components of Herba Epimedii. Some studies have reported that icariin plays important roles in preventing congestive heart failure and hypertension, regulating the immune system, protecting the cardiovascular system, and improving myocardial hypertrophy, inflammatory infiltration and cardiac dysplasia [11,12]. Icariin inhibits the production of IL-1β-induced extracellular matrix-degrading enzymes in the nucleus pulposus, reduces the generation of extracellular matrix, suppresses IL-1β-induced activation of NF-κB-related signalling pathways and plays a significant anti-inflammatory role [13]. When taken orally, intestinal bacteria can decompose icariin, resulting in the production of a variety of metabolites. Studies have demonstrated that icariside II (ICS II) is the main metabolite of icariin. In addition, ICS II exhibits anti-inflammatory and antioxidant activities and prevents against ischaemic brain injury and other functions [14,15]. However, the role of ICS II in myocardial fibrosis remains unknown.

Hence, in this study, we sought to determine the effects of ICS II on myocardial fibrosis using spontaneously hypertensive rats (SHRs). We additionally explored the mechanism underlying these effects.

2. Materials and methods

2.1. Animals and groups

The study objects included 28 male, 13-week-old, specific pathogen-free (SPF) SHRs and seven male, age-matched, SPF Wistar Kyoto (WKY) rats, which were used as the blank control group. All of the animals were purchased from Vital River Experimental Animal Technology Co., Ltd. (Beijing, China; certificate number SCXK2012-001). The 28 SHRs were randomly divided into the following groups: a model group (SHR) and ICS II low (4 mg/kg, ICS II-L), middle (8 mg/kg, ICS II-M) and high (16 mg/kg, ICS II-H) dose groups. All animals were fed in an SPF-grade animal room for one week. The rats in the ICS II-L, ICS II-M and ICS II-H groups were intragastrically administered ICS II (Nanjing Zelang Medical Technology Co., Ltd., ICS II purity was more than 98% as assayed by HPLC) for 12 weeks, whereas the control WKY rats and SHRs were intragastrically administered an equal volume of double-distilled water.

2.2. Measurements of blood pressure

Blood pressure was measured in the caudal arteries of conscious and tranquil rats using a Kent Scientific CODA instrument (Kent Scientific Corporation, CT, USA). The measurements were obtained thrice, and an average was obtained.

2.3. Left ventricular function measurements

After the rats were subjected to 12 weeks of intragastric feeding, they were anaesthetized via intraperitoneal injection of 2% pentobarbital (2 mL/kg). The hair of the rats was removed, and left ventricular function was measured using a Vevo2100 system (VisualSonics Corporation, Toronto, Canada).

2.4. Left ventricular index calculation

Heart tissues were removed from rats in each group. After dissecting residual roots of the arteries and veins, the free atrium, and the right ventricular connective tissue the left ventricles were weighed. We then calculated the left ventricular index as follows: left ventricular index = left ventricular mass/body weight × 100.

2.5. Masson staining of myocardium

All rats were anaesthetized using an intraperitoneal injection of 2% pentobarbital (2 mL/kg). The left ventricular myocardium was immersed in 4% formaldehyde solution for 48 h. After the tissues were dehydrated, paraffin-embedded and sectioned, Masson staining (Solarbio, Beijing, China) was used to detect the occurrence of interstitial fibrosis in the cardiac tissues. The tissues were then analysed under an optical microscope (BX-43, OLYMPUS, Tokyo, Japan).

2.6. Measurement of the collagen area

Five fields were randomly selected from each section. The images were captured to determine the area containing collagen (Image-Pro-Plus 6.0). The mean percentage of the area covered by collagen was calculated as follows: collagen area/myocardial area ×100%.

2.7. Determination of IL-6 and TNF-α levels in rat serum samples

The IL-6 and TNF-α levels in rat serum were detected through enzyme-linked immunosorbent assays (ELISAs) according to the manufacturer's instructions (Cheng Lin, Beijing, China).

2.8. Quantitative real-time RT-PCR analysis

Total RNA was extracted from the left ventricle using TRIzol reagent (TaKaRa Biotechnology, Dalian, China) and purified. The RNA yields and purity were assessed using a micro spectrophotometer (NanoDrop 8000, Thermo Fisher, USA). Total RNA (1 μg/μL) was transcribed using a reverse transcription reaction system kit (TaKaRa Biotechnology, Dalian, China). RT-PCR reactions were performed in 15-μL reactions that consisted of 7.5 μL of iQ SYBR Green Supermix, 0.5 μL of PCR forward primer, 0.5 μL of PCR reverse primer, 3 μL of cDNA and 4 μL of DEPC water. The TGF-β1, Smad2, IL-1β, TNF-α, NF-κBp65 and IκB-α mRNA levels were normalized using β-actin mRNA for normalization. The mRNA expression levels in the WKY group were set to 100. The primer sequences for each gene were synthesized by Sangon Gene Company and are presented in Table 1.

Table 1
Primer pairs used in real-time PCR.

Gene	GenBank accession No.	Forward primer (5′–3′)	Reverse primer (5′–3′)
TGF-β1	NM_021578.2	CATTGCTGTCCCGTGCAGA	AGGTAACGCCAGGAATTGTTGCTA
Smad2	NM_019191.1	TTACAGATCCATCGAACTCGGAGA	CACTTAGGCACTCGGCAAACAC
IL-1β	NM_031512.2	CCCTGAACTCAACTGTGAAATAGCA	CCCAAGTCAAGGGCTTGGAA
TNF-α	NM_012675.3	TCAGTTCCATGGCCCAGAC	GTTGTCTTTGAGATCCATGCCATT
NF-κBp65	NM_001276711.1	GACCTGGAGCAAGCCATTAG	CACTGTCACCTGGAAGCAGA
IκB-α	NM_030867.2	GCAGTGACAGCGACAGTGACAA	TGGCTCCTGCGACTGTGAAC
β-actin	NM_031144.2	GGAGATTACTGCCCTGGCTCCTA	GACTCATCGTACTCCTGCTTGCTG