Phytomedicine 42 (2017) 18–24

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.com/locate/phymed

Original Article

Protective role and mechanism of snakegourd peel against myocardial infarction in rats

Guanlin Yang[a,1], Dongyu Min[b,1], Junwei Yan[c], Ming Yang[d], Guijun Lin[e,*]

[a] Liaoning University of Traditional Chinese Medicine, Shenyang 110032, China
[b] The Affiliated Hospital of Liaoning University of Traditional Chinese Medicine, Shenyang 110032, China
[c] The Affiliated Hospital of Tsingtao University of Vascular Surgery, Tsingtao 266070, China
[d] The Affiliated Hospital of Tsingtao University of Intensive Care Unit, Tsingtao 266070, China
[e] Department of Anatomy, Harbin Medical University-Daqing, Daqing, Heilongjiang 163319, China

ARTICLE INFO

Keywords:
Myocardial infarction
Snakegourd peel
Apoptosis
p-JNK
p-p38MAPK

ABSTRACT

Background: Injection of snakegourd peel (SP), an herb used in traditional Chinese medicine, is used to treat coronary artery disease and stable angina in China. However, its therapeutic role and mechanism of action for the treatment of myocardial infarction (MI) is not fully understood.

Purpose: The present study was designed to investigate the effect of SP on MI-induced cardiac injury and elucidate its underlying molecular mechanisms.

Methods: To create an *in vivo* model of MI, we ligated the left coronary artery of Wistar rats. For our *in vitro* model of MI, we treated primary neonatal rat ventricular myocytes with hypoxia. Myocardial infarct size was measured by triphenyltetrazolium chloride (TTC) staining. Intracellular calcium concentration (Ca^{2+}) was measured by confocal microscopy, and cardiomyocyte apoptosis was assessed by TUNEL assay. Western blot was applied to determine protein levels.

Results: Three days post-MI, SP significantly improved MI-induced impairment of cardiac function, as indicated by increased left ventricular systolic pressure (LVSP), maximum rate of left ventricular pressure rise and fall (± dp/dt max), and decreased left ventricular end-diastolic pressure (LVEDP). In addition, SP treatment markedly reduced the infarct size and serum lactate dehydrogenase (LDH) activity; inhibited cardiomyocyte apoptosis and Caspase-3 activation both *in vivo* and *in vitro*; and decreased intracellular calcium overload, Cav1.2, phosphorylated JNK (p-JNK), and p38 MAPK (p-p38 MAPK) levels in ischemic myocardium.

Conclusion: SP alleviated cardiac ischemic injury and inhibited cardiomyocyte apoptosis by attenuating intracellular calcium overload, suppressing Caspase-3 activation, and downregulating protein expression of p-JNK and p-p38MAPK. These results suggest that SP may serve as a potential novel therapeutic drug for MI.

Introduction

Myocardial infarction (MI) is one of the leading causes of sudden cardiac death all over the world. It is accompanied by inflammation, cardiomyocyte apoptosis, cardiac fibrosis, and can lead to left ventricular dilatation and eventual heart failure (Wartenberg, 2012; Bogomolov et al., 2013). Cardiomyocyte apoptosis occurs in both the border zone of the infarct and the remote zone of the non-infarcted myocardium (Piro et al., 2000; Saraste et al., 1997). This apoptosis exacerbates post-MI remodeling and contributes to the development of heart failure (Palojoki et al., 2001; Wencker et al., 2003). Thus, inhibition of cardiomyocyte apoptosis at an early stage of MI is critical for the restoration of the injured heart and amelioration of cardiac function (Eltzschig and Eckle, 2011).

Previous studies have shown that increasing the release of calcium from the sarcoplasmic reticulum (SR) and L-type Ca^{2+} current (ICa_L) leads to intracellular calcium overload, which promotes cardiomyocyte apoptosis(Zhao et al., 2008; Chen et al., 2010). The MAPK signaling cascade, including extracellular signal-regulated kinase (ERK), c-Jun NH2-terminal kinase (JNK), and p38 mitogen activated protein kinase

Abbreviations: MI, myocardial infarction; SP, Snakegourd Peel injection; TTC, triphenyltetrazolium chloride; ± dp/dt max, maximum rate of left ventricular pressure rise and fall; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; LDH, lactate dehydrogenase; JNK, c-Jun NH2-terminal kinase; P38 MAPK, p38 mitogen activated protein kinase

* Correspondening author.
E-mail address: linguijun@hmudq.edu.cn (G. Lin).
[1] Guanlin Yang and Dongyu Min contributed equally to this study.

https://doi.org/10.1016/j.phymed.2018.03.014
Received 11 November 2016; Received in revised form 6 November 2017; Accepted 7 March 2018

Table 1
Effects of SP on hemodynamics in MI rat hearts.

Group	Dose (ml/kg/day)	LVSP (mmHg)	LVEDP (mmHg)	+dp/dtmax (mmHg/ms)	−dp/dtmax (mmHg/ms)
Sham	–	136.66 ± 10.52	2.28 ± 0.73	5.80 ± 0.41	−5.17 ± 0.68
MI	–	96.46 ± 19.79**	6.23 ± 1.80**	3.85 ± 0.31**	−3.10 ± 0.87**
SP	1.6	137.25 ± 9.87	2.31 ± 0.33	5.50 ± 0.61	−5.22 ± 0.45
MI + SP	0.4	98.37 ± 17.28	5.79 ± 2.30	3.95 ± 0.35	−3.23 ± 0.96
	0.8	118.65 ± 10.18$^{\#}$	3.83 ± 1.32$^{\#}$	5.05 ± 0.54$^{\#\#}$	−4.92 ± 0.99$^{\#\#}$
	1.6	119.73 ± 11.56$^{\#}$	3.73 ± 1.54$^{\#}$	5.13 ± 0.67$^{\#\#}$	−5.01 ± 1.01$^{\#\#}$
MI+ Tanshinone IIA	2.5	120.24 ± 9.44$^{\#}$	3.56 ± 1.38$^{\#}$	5.30 ± 0.77$^{\#\#}$	−5.15 ± 1.16$^{\#\#}$

Data shown were mean ± SD averaged from 8 rats for each group. **$p < 0.01$ vs. sham group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ vs. MI group.

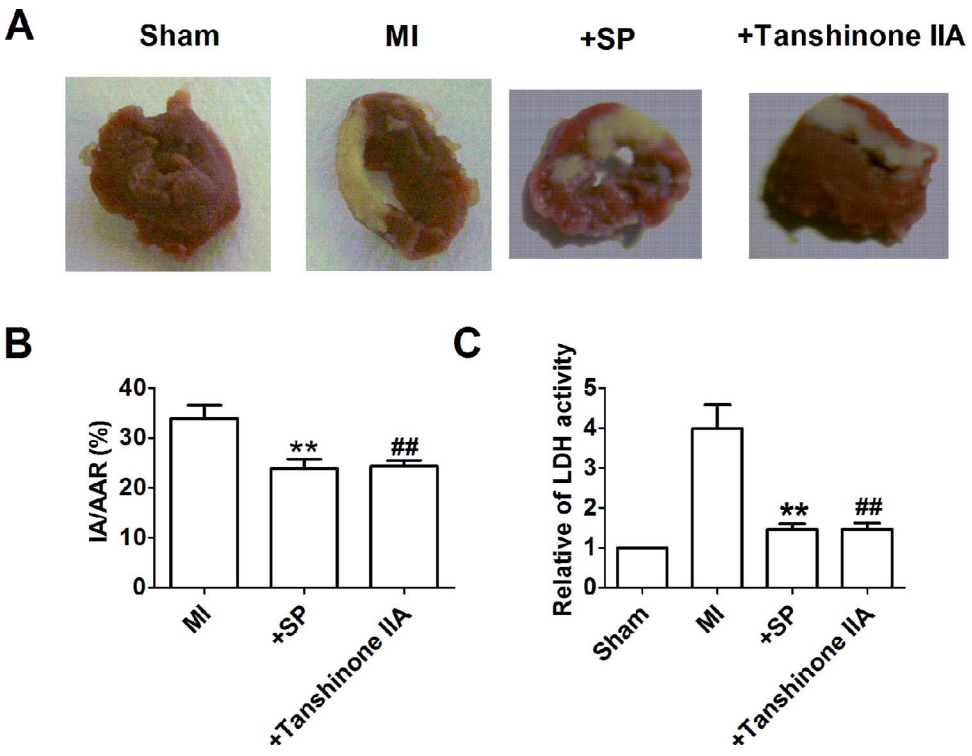

Fig. 1. SP decreases serum LDH activity and reduces infarct size in MI. Rats were treated with SP for 2 weeks, and then the related hemodynamic parameters were examined in rats 3 days post-MI. (A) Representative images showing infarct areas in cross section slices. (B) Statistical analysis of IA/AAR ratio. AAR, area at risk; IA, infarct area. (C) Serum LDH activity. **$p < 0.05$ vs. sham group, $^{\#\#}p < 0.01$ vs. MI group. $n = 8$ rats in each group.

(p-38 MAPK), plays a vital role in cardiomyocyte apoptosis(Xie et al., 2009). Increases in intracellular calcium (Ca^{2+}) can induce phosphorylation of JNK and p38-MAPK (Tfelt-Hansen et al., 2003; Kim and Sharma, 2004a). Moreover, the presence of a JNK agonist or upregulation of phosho-p38MAPK promote cardiomyocyte apoptosis, while their inhibition prevents cardiomyocyte apoptosis (Muslin, 2008; Cao et al., 2011). Caspase-3 is a member of the caspase family that plays a role in the execution phase of the apoptotic cascade (Boulares et al., 1999). Thus, decreasing intracellular calcium, blocking the MAPK signaling cascade, and inhibiting Caspase-3 activation may provide avenues for therapeutic intervention in the case of MI injury.

Snakegourd peel is the dried ripe peel of Chinese herb Trichosanthes kirilowii Maxim or Trichosanthes rosthornii Harms (Fam.Cucurbitaceae). A number of studies have revealed that snakegourd root provides therapeutic benefit for Type 2 diabetes mellitus (Xie et al., 2011). In addition, a previous study suggests that pretreatment with Wufu Jingfang (a compound containing snakegourd fruit) might protect the heart from ischemia reperfusion (I/R) injury via decreasing myocardial cell apoptosis (Li et al., 2013). In addition, SP has been used to treat coronary artery disease and stable angina in China for many years. Based on this evidence demonstrating multiple beneficial effects of the snakegourd plant, the present study was designed to investigate whether SP can protect against MI-induced cardiac injury by preventing cardiomyocyte apoptosis and, if so, to elucidate the underlying molecular mechanisms.

Materials and methods

Rat model of MI and hemodynamic parameters

Male Wistar rats (200–250 g) were maintained with food and water at standard room (temperature 21 ± 2 °C; humidity 60 ± 5%). The *in vivo* MI model was induced by occluding the left coronary artery as described previously (Yang et al., 2007). To investigate the effect of SP on the ischemic heart, SP was administered via intraperitoneal injection for 14 days consecutively before MI was established. The rats were randomly divided into the following groups: sham, MI, SP (1.6 ml/kg/day), and MI + SP (0.4 ml/kg/day, 0.8 ml/kg/day and 1.6 ml/kg/day), MI+ Tanshinone IIA (2 ml/kg/day). SP (4 g/ml) and Tanshinone IIA (10 mg/2 ml) injection were purchased from the Shang Hai First biochemical & pharmaceutical CO.,LTD. Rats in the sham group were administered an equal volume of saline. Three days after MI, hemodynamic parameters were recorded by a heparin-filled pressure transducer (from the right carotid artery into the left ventricle) interfaced with BL-420F biological function experiment system (Chengdu Tai Meng, China). Hemodynamic parameters included left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), and maximum rate of left ventricular pressure rise and fall (+dp/dtmax and −dp/dtmax). After the measurements were completed, rats were sacrificed, and the hearts were immediately dissected and frozen at −80 °C for subsequent experiments.