Original Article

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Paeoniae Radix Rubra Induces the Relaxation of Porcine Coronary Artery

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Objectives: Angina pectoris is generally caused by spasms or blockage of coronary arteries that supply blood to the myocardium. Common clinical symptoms of angina pectoris include chest pain, discomfort, and pressure. Few medications used in traditional Chinese medicine (TCM) are used clinically to treat angina pectoris. In addition, the effect of *Paeoniae radix rubra* (PRR) on the coronary arteries is unclear.

Methods: PRR and its active ingredients, including paeonol, gallic acid, methyl gallate, ethyl gallate, and 4-hydroxybenzoic acid, were used to investigate their effects on porcine coronary arteries.

Results: PRR induced coronary vasodilation after U46619-induced contraction in porcine coronary arteries in a dose-dependent manner. However, paeonol, gallic acid, methyl gallate, ethyl gallate, and 4-hydroxybenzoic acid did not induce contraction of porcine coronary arteries.

Conclusions: PRR can induce porcine coronary relaxation, which supports the development of PRR in TCM for treating angina pectoris.

Key words: *Paeoniae radix rubra*, coronary artery, relaxation, angina pectoris

Introduction

Angina pectoris is classified as a vasospastic disease (Prinzmetal angina), which is caused by transient vasoconstriction of coronary arteries, and an atherosclerotic disease, which is caused by stenosis of coronary arteries due to atherosclerotic plaques.[1] The common symptoms of angina pectoris are chest pain and discomfort, palpitation, dyspnea, nausea, and sweating. Chest pain or discomfort may include pressure, burning, fullness, and squeezing in the chest. In women, chest discomfort may present in the epigastric area, neck, jaw, shoulders, or back.[2,3] The risk factors of angina pectoris include increased age, male sex, hypertension, hyperlipidemia, diabetes mellitus, smoking, obesity, and family history of coronary heart disease.[4] An electrocardiogram and a cardiac enzyme blood test help diagnose angina pectoris. The common medications for angina pectoris include nitrates, morphine, beta-blockers, antiplatelet drugs, and anticoagulant drugs.[5] Although some studies reported certain Chinese herbal

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medications for treating angina pectoris, their effects are still unsatisfactory.[6] Therefore, it is still important to explore more effective and safer Chinese herbal medications for treating angina pectoris.

Paeoniae radix rubra (PRR) is the dried root of two peony species, *Paeonia lactiflora* Pallas and *Paeonia veitchii* Lynch. According to the Chinese Pharmacopoeia, peony root is the root of *Paeonia lactiflora* that contains not less than 2.0% paeoniflorin.[7] This plant contains many active plant components, such as paeoniflorin, albiflorin, pentagalloylglucose, (+)-catechin, gallic acid, methyl gallate, benzoic acid, and paeonol.[8] PRR has been applied for thousands of years in traditional Chinese medicine (TCM) to treat various diseases for years.[9] Recent studies showed that PRR has anti-inflammatory, immunomodulatory, cytotoxic, antitumor, antibacterial, antifungal, antioxidant, pro-oxidant, and glycemic activities. PRR is also reported to be used to treat viral diseases.[10] In addition, a study showed that a high-dose PRR-containing formula could improve hepatitis B-induced hyperbilirubinemia.[11] Furthermore, PRR may help recover from a stroke.[12]

To the best of our knowledge, no research has been conducted on the effect of PRR on vascular motility. This study aimed to investigate the effects of PRR on porcine coronary arteries. Additionally, PRR is expected to be developed in TCM for treating angina pectoris.

Materials and Methods

Materials

The specimens were taken from pigs with a net weight of approximately 110 kg. The pigs were stunned and exsanguinated in a formal slaughterhouse overseen by the Council of Agriculture, Executive Yuan, Republic of China (Taiwan). Because no live animals were used in this study and the coronary arteries used in this study were obtained from regional slaughterhouses, this study was exempt from review by the Institutional Animal Care and Use Committee of E-Da Hospital. After obtaining the specimens, they were stored in ice-cold oxygenated Krebs-Henseleit solution and were transferred back to the laboratory for use as soon as possible, with a transit time of approximately 30 minutes. Paeonol, gallic acid, methyl gallate, ethyl gallate, and 4-hydroxybenzoic acid were procured from the Cayman Chemical Company (MI, USA). The Krebs-Henseleit buffer solution comprises the following: 4.7 mM potassium chloride (KCl), 1.2 mM monosodium phosphate, 118 mM sodium chloride, 1.8 mM calcium chloride, 25 mM sodium bicarbonate, and 14 mM glucose, which was maintained at a constant pH of 7.4.

Preparation of PRR

The concentrated preparation of PRR in this study was procured from Kaiser Pharmaceutical Co., Ltd. (Tainan, Taiwan). We took 30 mg of TCM concentrated PRR powder and dissolved it in 1 mL of 10% dimethyl sulfoxide for later use.

Measurement of PRR-induced relaxation of the porcine coronary artery after U46619-induced contraction

The anatomy of porcine and human coronary arteries is similar; therefore, porcine coronary arteries are often used to study diseases related to human coronary arteries.[13] We dissected the epicardium of the porcine heart to expose the coronary arteries, then dissected the coronary arteries, which were further dissected with surgical scissors into small arterial tubules approximately 1 cm long and 0.3 cm wide. In addition, these segmental arterial tubules were fixed in a 7 mL organ bath containing 5 mL Krebs-Henseleit buffer solution using triangular clips and silk. Krebs-Henseleit buffer solution in the organ bath was continuously infused with 95% oxygen and 5% carbon dioxide and maintained at 37°C.

The other end of the silk was connected to an isometric transducer (FORT10g; Grass Technologies, Rhode Island, USA). The isometric transducer was then connected to an amplifier (Gould Instrument Systems, OH, USA), and the signal was recorded by a computerized recording system (BIOPAC Systems, CA, USA).[14] In this study, the vascular tone was regulated at 1.0 g. After equilibration for another 30 minutes, Krebs-Henseleit solution comprising 60 mM KCl was used to induce contraction of the arterial tubules in the organ bath, and Krebs-Henseleit solution comprising 60 mM KCl was washed thrice with Krebs-Henseleit buffer solution. After an additional equilibration period of 30 minutes, 100 nM U46619 was added to the organ bath to induce contraction of the arterial tubules. To observe the effect of PRR relaxation on the porcine coronary artery, PRR solutions at concentrations of 3 g/L, 9 g/L, and 15 g/L were added to an organ bath.

Measurement of PRR ingredient-induced relaxation of porcine coronary artery after U46619-induced contraction

Active ingredients of PRR, including paeonol, gallic acid, methyl gallate, ethyl gallate, and 4-hydroxybenzoic acid, were used in this study. The experimental procedure is described in the previous paragraph. After 30 minutes of equilibration, Krebs-Henseleit solution comprising 60 mM KCl was added to the organ bath to induce contraction of the arterial tubules, following which Krebs-Henseleit solution comprising 60 mM KCl was washed thrice with Krebs-Henseleit buffer solution. After another 30-min equilibration, 100 nM U46619 was added to the organ bath to induce contraction of the arterial tubules. After the coronary arteries reached the maximum contraction induced by 100 nM U46619, 10 μM, 30 μM, and 100 μM of the active components of PRR were added separately to test the effect of these active components of PRR on the tension of the coronary artery. In this study, the effect of the active components of PRR on the coronary artery was determined based on the maximum coronary contraction induced by 100 nM U46619 (100%).

Data analysis

Data are presented as the mean ± standard error of the mean. The results were statistically analyzed using Student's t-test or one-way analysis of variance and Tukey's post-hoc test. In all cases, differences were considered significant at $p < 0.05$. All analyses were performed using SPSS statistical software version 24 (IBM Corp., NY, USA).

Results

High performance liquid chromatography (HPLC) analysis of PRR

Figure 1 shows the HPLC of the concentrated preparations of PRR provided by Kaiser Pharmaceutical Co., Ltd. Paeoniflorin was used as a standard compound, and its retention time was 9.7 minutes.

Measurement of PRR-induced relaxation of the porcine coronary artery after

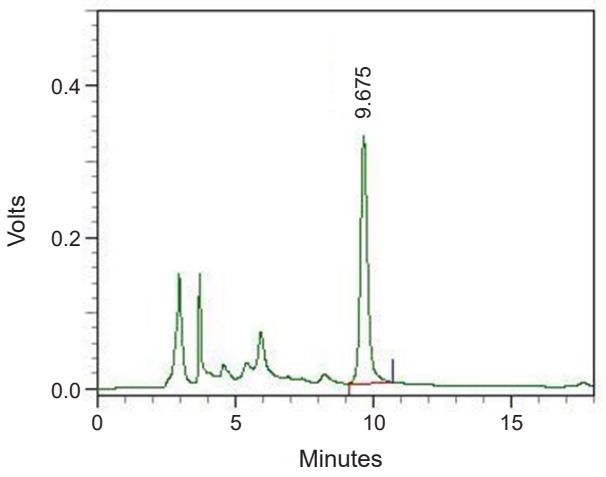

Fig. 1 High performance liquid chromatography of concentrated Paeoniae radix rubra powder. The retention time of paeoniflorin as a standard reference is 9.7 min.

U46619-induced contraction

Figure 2A shows the typical curves of 3 g/L, 9 g/L, and 15 g/L PRR-induced relaxation of the coronary tubules. Relaxation of the coronary tubules was induced by applying different concentrations of PRR. As shown in Figure 2B, PRRs of 3 g/L, 9 g/L, and 15 g/L elicited significant relaxation responses of 18.4 ± 2.4%, 24.3 ± 1.0%, and 38.7 ± 3.4%, respectively in coronary tubules in response to the contraction induced by 100 nM U46619, (n = 4, 5, 5, respectively). There were significant differences between 3 and 9 g/L, 3 and 15 g/L, and 9 and 15 g/L of PRR-induced relaxation of the coronary tubules (all $p < 0.05$).

Measurement of PRR ingredient-induced relaxation of the porcine coronary artery after U46619-induced contraction

As shown in Table 1, the active ingredients of PRR, including paeonol, at cumulative concentrations of 10 μM, 30 μM, and 100 μM, as well as gallic acid, methyl gallate, ethyl gallate, and 4-hydroxybenzoic acid, at a concentration of 100 μM, did not induce significant relaxation responses in the coronary tubules corresponding to 100 nM U46619-induced contraction. These relaxations induced by the active ingredients of PRR were not significantly different compared with the corresponding solvent in the coronary tubules (all $p > 0.05$, $n \geq 2$ in each group).

Discussion

In this study, PRR induced relaxation of porcine coronary arteries following U46619-induced contractions in a dose-dependent manner. In addition, the onset of PRR-induced porcine coronary vasodilation was rapid, and the rapid vasodilation induced by PRR is clinically helpful in patients with acute coronary syndrome. However, the active components of PRR in this study were unable to induce relaxation of the porcine coronary arteries following U46619-induced contraction.

To the best of our knowledge, there is currently no research on the use of PRR in TCM for treating angina pectoris. Some studies have reported that herbal medications are helpful for

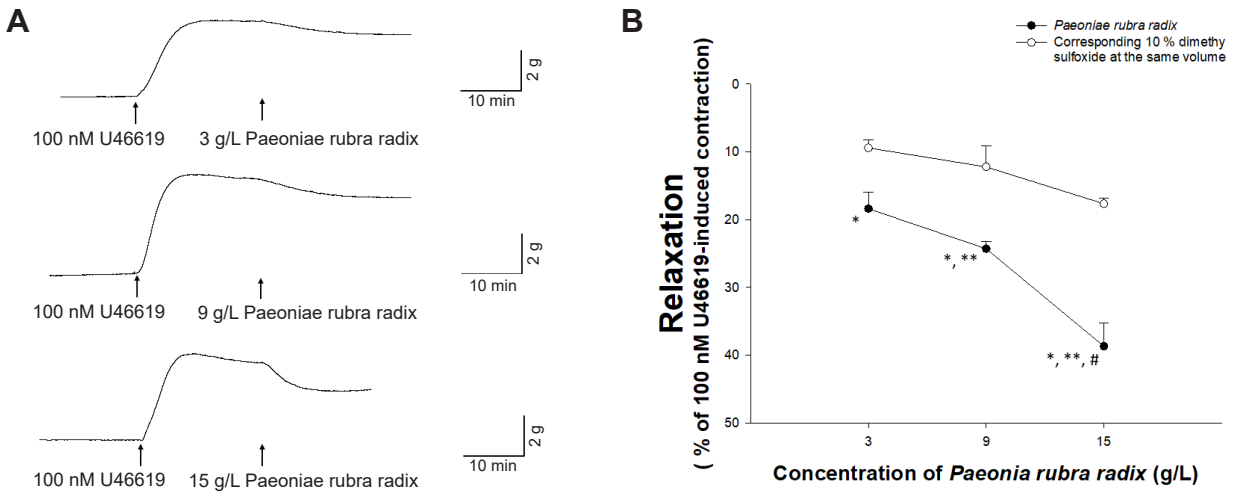

*Fig. 2 Paeoniae radix rubra (PRR) or dimethyl sulfoxide (DMSO, vehicle)-induced relaxation of porcine coronary arteries. (A) Representative tracings of 3 g/L, 9 g/L, and 15 g/L PRR-induced relaxation of coronary tubules. (B) PRR induced significant relaxation of the coronary tubules in a dose-dependent manner. These values are expressed as the percentage of contraction induced by 100 nM U46619. These results are from at least four experiments. Vertical bars represent standard error of the mean. * indicates a significant difference ($p < 0.05$) compared with the corresponding DMSO (vehicle) at the same volume. ** indicates significant difference ($p < 0.05$) compared with the relaxation induced by 3 g/L PRR. # indicates a significant difference ($p < 0.05$) compared with the relaxation induced by 9 g/L PRR.*

Table 1. The effects of the active ingredients of Paeoniae radix rubra-induced relaxation of porcine coronary arteries compared with the corresponding solvents (n ≥ 2).

	Ingredient or vehicle	Concentration of ingredients			*p* value
		10 μM	30 μM	100 μM	
Relaxation of coronary artery induced by ingredients or corresponding vehicles (% U46619 1 μM)	Paeonol	3.8 ± 0.9%	7.9 ± 1.8%	9.6 ± 2%	all > 0.05
	The same volume of vehicle of Paeonol	1.8 ± 0.3%	4.4 ± 0.6%	5.0 ± 0.7%	
	Gallic acid			3.5 ± 1.5%	> 0.05
	The same volume of vehicle of gallic acid			2.9 ± 0.4%	
	Methyl gallate			5.3 ± 2.0%	> 0.05
	The same volume of vehicle of methyl gallate			2.9 ± 0.4%	
	Ethyl gallate			4.6 ± 0.6%	> 0.05
	The same volume of vehicle of ethyl gallate			2.9 ± 0.4%	
	4-hydroxybenzoic acid			4.2 ± 1.1%	> 0.05
	The same volume of vehicle of 4-hydroxybenzoic acid			2.9 ± 0.4%	

angina pectoris. *Salvia miltiorrhiza* (Danshen) induces coronary vasodilatation and helps treat angina pectoris.[15,16] A systematic review of randomized controlled trials of > 3000 individuals showed that *Ginkgo biloba* has antihypertensive effects and decreases mortality of cardiovascular disease and angina pectoris.[17] In addition, *Panax notoginseng* (Burk.) F. H. Chen, *Panax ginseng* C. A. Mey., cardioprotection, and *Aralia chinensis* L. have been used for managing coronary heart disease, angina pectoris, and other cardiovascular diseases.[18] Furthermore, *Crataegus oxyacantha, Commiphora wightii, Allium sativum*, and *Terminalia arjuna* have been used for managing cardiovascular diseases.[19] In addition, Rhodiola formulation may have a positive effect on the management of ischemic heart disease.[20] In this study, the results might provide a potential effect of PRR for treating angina pectoris by rapidly promoting relaxation of coronary arteries.

There are few studies on the effect of PRR on smooth muscles. A study has shown that the ethanol extract of PRR induced relaxation of vessels in rats by activating calcium (Ca^{2+})-activated and adenosine triphosphate (ATP)-sensitive potassium channels and inhibiting L-type Ca^{2+} channels, as well as via endothelium-dependent and protein kinase B- and store-operated Ca^{2+} entry-endothelial nitric oxide synthase-cyclic guanosine monophosphate (cGMP)-mediated pathways.[21] PRR has a significant preventive effect on restenosis after carotid balloon injury in rabbits with atherosclerosis induced by a high-fat diet.[22]

Common active components of PPR include paeoniflorin, albiflorin, pentagalloylglucose, (+)-catechin, gallic acid, methyl gallate, benzoic acid, and paeonol.[8] Among them, paeoniflorin attenuates myocardial fibrosis in rats with isoproterenol-induced chronic heart failure by inhibiting the P38 mitogen-activated protein kinase pathway.[23] Catechin attenuates coronary heart disease in a rat model by inhibiting interleukin-6 and tumor necrosis factor-alpha.[24] Gallic acid inhibits cardiac hypertrophic remodeling and heart failure through an autophagy-dependent mechanism.[25] Methyl gallate decreases doxorubicin-induced cardiotoxicity in rats by suppressing oxidative stress.[26] Paeonol is a phenolic compound and gallic acid is a trihydroxybenzoic acid originally extracted from PRR. In this study, paeonol, gallic acid, methyl gallate, ethyl gallate, and 4-hydroxybenzoic acid were

studied; however, they did not cause significant relaxation of the coronary smooth muscle compared with the corresponding solvents. It may be considered to test more PRR active components other than these compounds in the future to determine which compound causes relaxation of porcine coronary arteries.

In general, the relaxation mechanism of porcine coronary arteries is related to nitric oxide, protein kinase A, protein kinase G, cyclic adenosine monophosphate, cGMP, potassium channels, Ca^{2+}-ATPase pumps, and L-type Ca^{2+} channels. Therefore, further research should be performed to determine the exact mechanism of PRR-induced relaxation of porcine coronary arteries so that PRR can be more precisely applied clinically for treating angina pectoris.

Conclusions

Concentrated PRR preparations induced immediate relaxation of porcine coronary arteries in a dose-dependent manner. Therefore, the study findings suggest that PRR may provide a promising therapeutic approach in TCM for treating angina pectoris.

Author Contributions

Study Design, CCT and SNY; Data Collection, MCY and CCT; Statistical Analysis, YTS, LCC and CCT; Data Interpretation, YTS, LCC, PLW and CCT; Manuscript Preparation, SNY and CCT; Literature Search, YTS, MCY, LCC, PLW and CCT; Funding Acquisition, CCT and SNY. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

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