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Effects of Puerarin on Clinical Parameters, Vascular Endothelial Function, and Inflammatory Factors in Patients with Coronary Artery Disease

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Background: The aim of this study was to investigate the effects of puerarin on vascular endothelial function and inflammatory factors in coronary artery disease (CAD) patients with stable angina pectoris (SAP).

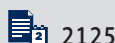
Material/Methods: To evaluate the effects of angina pectoris, the differences of scores of the Seattle angina questionnaire (SAQ), vascular endothelial function [endothelial progenitor cells (EPCs), nitric oxide (NO) and endothelin 1 (ET-1)], and inflammatory factors [tumor necrosis factor α (TNF- α), hypersensitive C-reactive protein (hs-CRP), interleukin-6 (IL-6)] in 2 groups were assessed before and after treatment.

Results: Regarding the curative effect of angina pectoris, the total effective rate of the treatment group was significantly superior to that of the control group (89% vs. 65%, $P < 0.05$). The duration of angina pectoris, the number of abnormal leads, the improvement of the ST segment depression of electrocardiogram, and the scores of SAQ life quality indexes in the treatment group were better than those of the control group ($P < 0.05$). In the 2 groups, EPCs and NO were both elevated, while ET-1 was decreased, and the improvements of the treatment group were superior to those of the control group ($P < 0.05$). After treatment, the average levels of serum TNF- α , hs-CRP and IL-6 in the 2 groups were all decreased, which the treatment group showed a much sharper decrease than in the control group ($P < 0.05$).

Conclusions: Puerarin effectively improves clinical symptoms and vascular endothelial function and reduces the levels of inflammatory factors in patients with CAD.

MeSH Keywords: **Angina, Stable • Coronary Artery Disease • Endothelial Cells**

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29



Background

Coronary artery disease (CAD) refers to a group of diseases that includes stable angina, unstable angina, myocardial infarction, and sudden cardiac death [1]. In 2015, CAD affected 110 million people and resulted in 8.9 million deaths [2,3], and it accounts for 15.9% of all deaths, making it the most common cause of death globally [3]. Risk factors include high blood pressure, smoking, diabetes, lack of exercise, obesity, high blood cholesterol, poor diet, depression, and excessive alcohol intake [4,5].

Vascular endothelium contributes to the development of atherosclerosis via abnormal cell proliferation and apoptosis [6]. Endothelial cells maintain the homeostasis of the vascular system [7]. However, the precise molecular mechanisms which underlie the contributions of the vascular endothelium to CAD remain unclear and are complex.

Puerarin, one of several known isoflavones, is found in a number of plants and herbs, such as the root of pueraria (*Radix puerariae*) [8]. Puerarin was reported to improve arrhythmia and acts as a vasodilator, increasing blood supply of the myocardium, as well as acting as an antioxidant [9]. Puerarin is reported to inhibit the inflammatory response in atherosclerosis [10] and to stimulate serum NO production in rats with myocardial infarction [11]. A recent study found that puerarin protects vascular endothelial cells [12], but the precise molecular mechanisms are unknown. These studies indicate the function of puerarin in inflammation and endothelial function, which might be beneficial for CAD.

The present study investigated the effects of puerarin on vascular endothelial function and inflammatory factors in CAD patients with SAP, and explored the possible molecular mechanisms.

Material and Methods

Patients

Between Jun 2016 and Sep 2017, 120 CAD patients with SAP who were treated in our hospital was enrolled in our study. Patients were randomly separated into 2 different groups: a control group (conventional treatment group, 55 patients, 18 females and 37 males, aged 64.19 ± 12.74 years) and a treatment group (puerarin group, 65 patients, 20 females and 45 males, aged 63.48 ± 11.56 years).

The excluded criteria were: (1) Uncontrolled hypertension (systolic blood pressure (SBP) of more than 140 mmHg and diastolic blood pressure (DBP) of more than 90 mmHg), hyperlipidemia (fasting serum total cholesterol more than 5.72 mmol/L

or fasting triglyceride (TC) more than 1.70 mmol/L, or fasting high density lipoprotein less than 0.91 mmol/L), and diabetes (fasting glucose more than 130 mg/dl); (2) Severe infection, liver and kidney dysfunction, immune diseases, thyroid dysfunction, and severe anemia; and (3) Congenital heart disease, rheumatic heart disease, valvular heart disease, heart failure (left ventricular ejection fraction <45%), and malignant tumors.

Treatments

Patients in the control group were treated with nitrate, β -receptor blockers, calcium antagonists, statins, and aspirin, while patients in the puerarin group were injected once with puerarin (cat. no. H20051499, Rui Yang Pharmaceutical Co.) 0.4 g/day in 250 ml 5% glucose or normal saline in addition of nitrate, β -receptor blockers, calcium antagonists, statins, and aspirin.

At baseline, the characteristics in the control group and puerarin group were assessed. There was no change in medical therapy for any individual. After treatment for 28 consecutive days, the treatment efficacy in the control group and puerarin group were assessed.

Evaluation of EPCs

The assessment of EPCs was carried out by FACSCalibur to test the number of CD34⁺ cells in blood leukocyte membrane.

Assessment of NO

The expression levels of NO in serum was test by NOe reductase method using kits from Guangzhou JSK Biotechnology Co., China.

ELISA

The expression levels of ET-1, TNF- α , hs-CRP, and IL-6 were evaluated by ELISA (Bioscience Technology Co., Shanghai, China) according to the manufacturer's protocol.

SAQ

Quality of life was evaluated using the Seattle angina questionnaire (SAQ), which assesses degree of activity limitation, episodes of angina pectoris, stable state of angina pectoris, degree of disease awareness, and degree of satisfaction. The total possible score was 100, with a higher score representing better patient physical condition.

Statistical analysis

SPSS 19.0 was used for the analysis of data. Mean and standard deviation ($\bar{x} \pm s$) was used to describe the results. The *t*

Table 1. The characteristics of patients in gender, age, blood pressure, heart function, body mass index and blood fat.

Group	n	Age	Gender		Heart function		SBP (mmHg)	DBP (mmHg)	BMI (kg/m ²)	TC (mmol/L)	LDL-C (mmol/L)
			Male	Female	I-II	III-IV					
Control	55	64.19 ±12.74	37	18	38	4	127.55 ±12.68	84.56 ±8.86	22.65 ±4.12	7.38 ±0.48	4.38 ±0.53
Puerarin	65 >0.05	63.48 ±11.56 >0.05	45 >0.05	20	36 >0.05	6	130.18 ±13.30 >0.05	86.12 ±8.11 >0.05	21.32 ±4.36 >0.05	7.72 ±0.40 >0.05	4.91 ±0.58 >0.05

SBP – systolic blood pressure; DBP – diastolic blood pressure; BMI – body mass index; TC – triglyceride; LDL-C – LDL cholesterol.

Table 2. The comparison of efficacy between control group and puerarin group.

Group	n	Aggravated, n (%)	Ineffective, n (%)	Effective, n (%)	P
Control	55	1 (1.82)	18 (32.72)	36 (65.45)	<0.05
Puerarin	65	1 (1.54)	6 (9.23)	58 (89.23)	

test was used for comparisons between groups, single-factor analysis of variance was used for intra-group comparisons, rank sum test was applied for the comparison of ranked data, and the chi-square test was used for the analysis of count data. P<0.05 was considered statistical significance.

Results

Baseline characteristics of patients

The basic characteristics of patients – gender, age, blood pressure, heart function (assessed by New York Heart Association functional classification), body mass index, and blood fat – in the control group and puerarin group were analyzed and are exhibited in Table 1. The chi-square test was used for comparisons between groups. We found no significant differences between groups in number (65 vs. 55), age (63.48±11.56 vs. 64.19±12.74), gender (45 male vs. 37 male; 20 female vs. 18 female), heart function (36 I-II vs. 38 I-II; 6 III-IV vs. 4 III-IV), SBP (mmHg) (130.18±13.30 vs. 127.55±12.68), DBP (mmHg) (86.12±8.11 vs. 84.56±8.86), body mass index (BMI) (kg/m²) (21.32±4.36 vs. 22.65±4.12), TC (mmol/L) (7.72±0.40 vs. 7.38±0.48), and LDL cholesterol (LDL-C) (mmol/L) (4.91±0.58 vs. 4.38±0.53), there was no significant difference (P>0.05) in any characteristic between the control group and puerarin group.

The comparison of efficacy between the 2 groups

As exhibited in Table 2, in the control group 1 (1.82%) patient showed aggravated CAD and 1 (1.54%) patient in the treatment group showed aggravated CAD. In the control group, puerarin was ineffective in 18 (32.72%) patients and effective in 36 (65.45%) patients; while puerarin was ineffective in 6 (9.23%) patients and

effective in 58 (89.23%) patients. The total effective rate of the treatment group was as high as 89.23%, which was significantly better than the 65.45% recorded in the control group (P<0.05). The rank sum test was used for the comparison of ranked data.

The comparison of duration of angina pectoris, number of abnormal leads, and ΣST

As presented in Table 3, puerarin significantly (P<0.05) improved the duration of angina pectoris (min) (4.16±0.80 vs. 7.38±1.44), number of abnormal leads (n) (4.10±1.11 vs. 5.92±1.78), and ΣST (mm) (4.93±1.58 vs. 6.28±1.70) when compared with the control group.

In the control group, there was no significant difference (P>0.05) in duration of angina pectoris (7.38±1.44 vs. 10.23±2.03), number of abnormal leads (5.92±1.78 vs. 6.42±2.01), or ΣST (6.28±1.70 vs. 8.32±1.28) after and before the treatment; however, in the puerarin group, there was a significant difference (P<0.05) in duration of angina pectoris (4.16±0.80 vs. 10.55±1.91), number of abnormal leads (4.10±1.11 vs. 6.34±2.12), and ΣST (4.93±1.58 vs. 8.56±1.38) before vs. after treatment. The rank sum test was used to compare ranked data.

Comparison of Seattle angina questionnaire (SAQ) scores

As presented in Table 4, puerarin significantly (P<0.05) improved the degree of activity limitation (59.05±4.13 vs. 50.13±3.85), episodes of angina pectoris (20.42±2.76 vs. 16.35±2.09), stable state of angina pectoris (17.76±1.98 vs. 12.46±1.76), treatment satisfaction (23.45±2.51 vs. 19.36±2.31), and degree of disease awareness (17.43±1.97 vs. 13.76±2.03) when compared with the control group.

Table 3. The comparison of duration of angina pectoris, number of abnormal leads, and Σ ST.

Group	Duration of angina pectoris (min)	Abnormal leads (n)	Σ ST (mm)
Control			
Before	10.23±2.03	6.42±2.01	8.32±1.28
After	7.38±1.44	5.92±1.78	6.28±1.70
Puerarin			
Before	10.55±1.91	6.34±2.12	8.56±1.38
After	4.16±0.80* [#]	4.10±1.11* [#]	4.93±1.58* [#]

* P<0.05 puerarin vs. control; # P<0.05 after treatment vs. before treatment.

Table 4. The comparison of SAQ.

Group	n	Degree of activity limitation	Episodes of angina pectoris	Stable state of angina pectoris	Treatment satisfaction	Degree of disease awareness
Control						
Before	55	42.35±3.76	10.21±2.54	8.35±0.85	16.21±1.43	10.08±2.14
After	55	50.13±3.85 [#]	16.35±2.09 [#]	12.46±1.76 [#]	19.36±2.31 [#]	13.76±2.03 [#]
Puerarin						
Before	65	41.74±4.07	10.15±2.21	8.43±1.09	16.87±1.86	10.19±2.09
After	65	59.05±4.13* [#]	20.42±2.76* [#]	17.76±1.98* [#]	23.45±2.51* [#]	17.43±1.97* [#]

* P<0.05 puerarin vs. control; # P<0.05 after treatment vs. before treatment. SAQ, seattle angina questionnaire.

Table 5. The comparison of CD34⁺, ET-1 and NO.

Group	CD34 ⁺ (%)	ET-1 (ng/L)	NO (μmol/L)
Control			
Before	1.12±0.50	97.91±6.36	49.58±6.90
After	1.42±0.44	91.52±5.67	50.11±5.93
Puerarin			
Before	1.06±0.43	95.13±8.52	46.86±8.44
After	2.12±0.70* [#]	67.30±9.10* [#]	76.53±10.54* [#]

* P<0.05 puerarin vs. control; # P<0.05 after treatment vs. before treatment. CD34⁺ – CD34⁺ cells in blood leukocyte membrane; ET-1 – endothelin 1; NO – nitric oxide.

In the control group, there were significant differences (P<0.05) in degree of activity limitation (50.13±3.85 vs. 42.35±3.76), episodes of angina pectoris (16.35±2.09 vs. 10.21±2.54), stable state of angina pectoris (12.46±1.76 vs. 8.35±0.85), treatment satisfaction (19.36±2.31 vs. 16.21±1.43), and degree of disease awareness (13.76±2.03 vs. 10.08±2.14) before vs. after treatment. In the puerarin group, there were significant differences (P<0.05) in degree of activity limitation (59.05±4.13 vs. 41.74±4.07), episodes of angina pectoris (20.42±2.76 vs. 10.15±2.21), stable state of angina pectoris (17.76±1.98 vs. 8.43±1.09), treatment satisfaction (23.45±2.51 vs. 16.87±1.86),

and degree of disease awareness (17.43±1.97 vs. 10.19±2.09) before vs. after treatment. The rank sum test was used to compare ranked data.

The comparison of CD34⁺, ET-1, and NO

As presented in Table 5, puerarin significantly (P<0.05) improved CD34⁺ (%) (2.12±0.70 vs. 1.42±0.44), ET-1 (ng/L) (67.30±9.10 vs. 91.52±5.67), and NO (μmol/L) (76.53±10.54 vs. 50.11±5.93) when compared with the control group.

Table 6. The comparison of TNF- α , hs-CRP and IL-6.

Group	TNF- α (ng/L)	hs-CRP (mg/L)	IL-6 (ng/L)
Control			
Before	27.41 \pm 5.50	36.79 \pm 8.45	33.98 \pm 6.90
After	18.62 \pm 5.23	23.18 \pm 1.07	14.49 \pm 5.93
Puerarin			
Before	26.73 \pm 5.40	29.56 \pm 9.68	35.62 \pm 7.44
After	13.12 \pm 3.40* [#]	15.22 \pm 0.45* [#]	12.81 \pm 5.54* [#]

* P<0.05 puerarin vs. control; # P<0.05 after treatment vs. before treatment. TNF- α – tumor necrosis factor α ; hs-CRP – hypersensitive C-reactive protein; IL-6 – interleukin-6.

In the control group, there was no significant difference ($P>0.05$) in CD34⁺ (%) (1.42 \pm 0.44 vs. 1.12 \pm 0.50), ET-1 (ng/L) (91.52 \pm 5.67 vs. 97.91 \pm 6.36), or NO (μ mol/L) (50.11 \pm 5.93 vs. 49.58 \pm 6.90) before vs. after treatment; however, in the puerarin group, there was a significant difference ($P<0.05$) in CD34⁺ (%) (2.12 \pm 0.70 vs. 1.06 \pm 0.43), ET-1 (ng/L) (67.30 \pm 9.10 vs. 95.13 \pm 8.52), and NO (μ mol/L) (76.53 \pm 10.54 vs. 46.86 \pm 8.44) after and before the treatment. The rank sum test was applied for the comparison of ranked data.

Comparison of TNF- α , hs-CRP, and IL-6

As presented in Table 6, puerarin significantly ($P<0.05$) improved TNF- α (ng/L⁻¹) (13.12 \pm 3.40 vs. 18.62 \pm 5.23), hs-CRP (mg/L) (15.22 \pm 0.45 vs. 23.18 \pm 1.07), and IL-6 (ng/L⁻¹) (12.81 \pm 5.54 vs. 14.49 \pm 5.93) when compared with the control group.

In the control group, there was no significant difference ($P>0.05$) in TNF- α (ng/L⁻¹) (18.62 \pm 5.23 vs. 27.41 \pm 5.50), hs-CRP (mg/L) (23.18 \pm 1.07 vs. 36.79 \pm 8.45), or IL-6 (ng/L⁻¹) (14.49 \pm 5.93 vs. 33.98 \pm 6.90) after and before the treatment; however, in the puerarin group, there were significant differences ($P<0.05$) in TNF- α (ng/L⁻¹) (13.12 \pm 3.40 vs. 26.73 \pm 5.40), hs-CRP (mg/L) (15.22 \pm 0.45 vs. 29.56 \pm 9.68), and IL-6 (ng/L⁻¹) (12.81 \pm 5.54 vs. 35.62 \pm 7.44) after and before the treatment. The rank sum test was applied for the comparison of ranked data.

Discussion

It has been verified that CAD can be the result of endothelial dysfunction [13]. Studies demonstrate that the amount and number of colonies of EPCs of CAD patients, which are negatively related with the extent of CAD and degree of stenosis, are significantly downregulated [14–16]. Recent research indicates that the decrease in EPCs in PBMCs of CAD patient is independent of traditional risk factors such as age, total cholesterol, hypertension, and smoking; however, the risk factors for CAD not only decrease the EPCs amount, but also damage

cell adhesion, cell migration, and cell proliferation abilities of EPCs [17]. A recent vascular endothelium research focus has been how to improve the function of EPCs in PBMC of CAD patient, and drugs that target the vascular endothelium are being studied.

ET-1 is a vasoconstrictor secreted by vascular endothelium, and it is also involved in the pathophysiological process of multiple disease [18,19]; for example, ET-1 promotes cell proliferation of vascular smooth muscle and induces platelet aggregation [20]. NO takes part in regulating the vasodilatation of endothelial cells and cardiovascular protection through inhibiting the adhesion and aggregation of platelets and monocytes to endothelium [21].

Inflammatory factors are also involved in acute coronary events; for instance, TNF- α promotes cell proliferation of stromal cells and infiltration of inflammatory cells [22]. hs-CRP, a marker of inflammation, is also a significant risk index and factor for asymptomatic myocardial ischemia and local inflammatory response, which, in addition to TNF- α , is proposed to be independently associated with cardiovascular risk in occlusive atherosclerosis patients [23]. IL-6, an upstream inflammation marker, is correlated with the pathophysiological process of adverse cardiovascular events, myocardial infarction, and heart failure [24].

Puerarin was discovered to inhibit the inflammatory response in atherosclerosis by regulating the activation of NF- κ B signaling pathway [10]. Puerarin is reported to stimulate serum NO production in rats with myocardial infarction [11]. The intermediate effect of puerarin on tissue factors, which are produced by monocyte-derived macrophage of patients with coronary heart disease, was verified [25]. Puerarin showed protective roles in acute ischemic myocardial injury in rats [26]. In addition, puerarin improves cardiac function of myocardial infarction in rats [27] and induces immune hemolytic anemia [28].

Currently, there is no report about how puerarin protects the function of vascular endothelium in CAD patients. Our study adds to the literature showing the effects of puerarin in CAD, as well as the role of puerarin in inflammation and endothelium function. We found that the total effective rate and curative effect of angina pectoris in the treatment group was significantly superior to that of the control group. The duration of angina pectoris, the number of abnormal leads, the improvement of ST segment depression of electrocardiograms, and SAQ life quality scores in the treatment group were better than those of the control group. The levels of EPCs and NO in the 2 groups were all elevated, the level of ET-1 decreased, and the improvements of the treatment group were superior to those of the control group. After treatment, the average levels of serum TNF- α , hs-CRP, and IL-6 in the 2 groups were all decreased, and the treatment group showed a much sharper decrease than in the control group. A previous study reported an approach to identify biomarkers associated with CAD [29].

Our results suggest a correlation between CAD and these markers, which were not commonly used in clinical practice. However, there are some limitations in the present study: (1) We did not assess the clinical applicability of these markers; (2) We did not assess the commonly used markers for CAD patients in clinical practice; and (3) We did not assess the role of puerarin in treatment of CAD using markers of endothelial function and inflammation.

Conclusions

Puerarin effectively improves clinical symptoms and reduces levels of inflammatory factors in patients with CAD, and improves vascular endothelial function.

Conflicts of interest

None.

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