

Puerarin pre-conditioning on the expression levels of CK-MB, cTnI and inflammatory factors in patients undergoing cardiac valve replacement

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Abstract. Effect of puerarin preconditioning on the expression levels of nuclear factor κ B (NF- κ B), interleukin 6 (IL-6), interleukin 8 (IL-8), troponin I (cTnI), and creatine kinase isoenzyme MB (CK-MB) in the neutrophils of patients undergoing cardiac valve replacement under cardiopulmonary bypass (CPB) was evaluated. We enrolled 50 patients scheduled for cardiac valve replacement and assigned them randomly divided into either a puerarin or a control group. Puerarin was dissolved in 10 ml normal saline before CPB, and administered by intravenous infusion to patients in the puerarin group. The control group was administered an equivalent amount of saline. We used flow cytometry to determine the expression levels of NF- κ B, IL-6 and IL-8 in neutrophils and an auto chemistry analyzer to determine the serum levels of cTnI and CK-MB before anesthesia induction (T0), 30 min after aortic declamping (T1), 4 h after aortic declamping (T2), and 8 h after aortic declamping (T3). We found the mean serum cTnI and CK-MB levels of the puerarin group tended to decrease with time. The positive rates of NF- κ B, IL-6 and IL-8 at different time-points were lower in patients of the puerarin group than in those of the control group (and the differences at T3 were statistically significant). The clinical manifestations of patients in the puerarin group after operation were better than those in the control group ($P < 0.05$). We found that the expression levels of NF- κ B, IL-6 and IL-8 were positively correlated with the levels of CK-MB and cTnI ($P < 0.05$). Puerarin preconditioning can reduce the NF- κ B activation and the overexpression of IL-6 and IL-8 in neutrophils, and it inhibits the release of myocardial enzyme cTnI and

CK-MB reflecting myocardial cell protection. Puerarin seems to improve safety and efficacy of valvular replacement operations.

Introduction

Ischemia-reperfusion injury refers to the phenomenon aggravating tissue injury with blood perfusion recovery after an ischemic event, usually affecting myocardial tissues after heart surgery, percutaneous transluminal angioplasty or thrombolytic therapy. Myocardial ischemia-reperfusion injury is related to the overexpression of certain cytokines and adhesion molecules in local tissues (1). The expression of the inflammatory factor nuclear factor κ B (NF- κ B) is increased at the beginning of myocardial ischemia reperfusion (2), together with interleukin 6 (IL-6) and IL-8 (3,4). For a safe valvular replacement operation, it is critical to protect the myocardium and to carry out the open-heart surgery under cardiopulmonary bypass (CPB). Clinically, myocardial ischemic preconditioning, post-processing and medical treatments can all relieve the reperfusion injury (5). Puerarin is an isoflavone compound extracted from the Kudzu root; its cardiovascular vessel-protective effects have been confirmed by various pharmacological actions (6). This study was designed to evaluate the effects of puerarin preconditioning on the expression levels of creatine kinase isoenzyme MB (CK-MB), troponin I (cTnI) and inflammatory factors in patients undergoing cardiac valve replacement.

Patients and methods

General data. We enrolled 50 patients undergoing cardiac valve replacement surgery with CPB in Department of Cardiovascular Surgery from March 2017, to September 2017, and randomly separated them into a puerarin ($n=25$) and a control group ($n=25$). All patients had ASA II or III classification levels. We excluded patients with serious primary diseases of important organs and patients with surgical contraindications. The patient's baseline age, sex and ASA levels and other general characteristics were comparable ($P > 0.05$).

The present study was approved by the Ethics Committee of The Second Affiliated Hospital of Zhengzhou University

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Table I. Comparison of general data between patients in the two groups.

Groups	Age (years)	Men/women (cases)	Height (cm)	Weight (kg)	EF (%)	MAP (mmHg)	ASA grading (II/III)
Puerarin	48.08±2.27	8/17	159.7±2.52	53.28±2.89	65.19±1.25	88±15	13/12
Control	48.92±2.77	11/14	158.9±1.96	55.24±2.18	63.62±2.30	89±13	15/10

Table II. Comparison of operation conditions between the two groups.

Groups	Sufentanil (μ g)	CPB time (min)	Aorta clamping time (min)	Operation time (min)	Electric defibrillation times (case)
Puerarin	506.70±37.60	118.2±10.30	86.00±7.77	208.20±12.61	6.22±1.09
Control	475.00±27.18	110.3±10.16	74.82±8.04	221.90±15.33	6.81±0.84

CPB, cardiopulmonary bypass.

Table III. Comparison of postoperative conditions between the two groups.

Groups	Contractility score 24 h after operation (μ g/kg.min)	Assisted respiration time after operation (h)	ICU hospitalization time (/h)	Extubation time (/h)	Adverse events after operation (case)
Puerarin	7.79±1.25 ^a	19.89±2.33 ^a	55.01±9.56 ^a	21.46±2.27	1
Control	6.01±1.36	24.78±2.45	65.91±8.98	25.21±3.27	2

^aP<0.05, compared with the control group.

(Zhengzhou, China) and signed informed consents were obtained from all participants.

Research methods. After entering the operating room, the patients underwent routine anesthesia induction and maintenance. Prior to CPB establishment the patients in the puerarin group were administered an intravenous injection of 4 mg/kg of puerarin (Zhejiang Zhenyuan Pharmaceutical, Zhejiang, China), while the patients in the control group were administered an injection of an equivalent volume of normal saline. The central venous pressure (CVP) and other life signs were closely monitored.

Test index and methods. Radial artery blood samples were extracted before anesthesia induction (T0), 30 min after aortic declamping (T1), 4 h after aortic declamping (T2) and 8 h after aortic declamping (T3). The samples were centrifuged at 3,200 x g for 10 min, the supernatants extracted and kept at -80°C until further use. A 7600 auto-chemistry analyzer (Hitachi, Ltd., Tokyo, Japan) was used to determine the levels of CK-MB and cTnI in serum. A CyFlow Cube 8 flow cytometry (Sysmex Europe GmbH, Norderstedt, Germany) was used to determine the expression levels of inflammatory factors NF- κ B, IL-6 and IL-8 in neutrophils. We recorded the following monitoring indexes during and after the operations: Aorta clamping time, CPB time, electric defibrillation time,

and contractility score 24 h after operation, assisted respiration time after operation and ICU hospitalization time.

Statistical analysis. We used the IBM SPSS 19.0 statistical software (IBM Corp., Armonk, NY, USA) for data analysis. Measurement data were expressed by mean \pm standard deviation (SD). We analyzed comparisons between two groups using the t-test, and comparisons among multiple groups using analysis of variance (ANOVA) and Least Significant Difference test. Correlations were established using the Pearson's correlation analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

The differences revealed by the comparison of general data of patients in the two groups before the operation were not statistically significant (P>0.05) (Table I).

Comparison of indexes between two groups before and after the operation. We found patients in the puerarin group had improved aorta clamping, CPB, electric defibrillation, and operation times than patients in the control group, but the differences were not significant (P>0.05). Compared with the control group, the mean contractility score 24 h after the operation of the puerarin group was significantly improved,

Table IV. Comparison of mean CK-MB and cTnI levels between the two groups at different time-points.

Index	Groups	T0	T1	T2	T3
CK-MB(U/l)	Puerarin	12.01±3.31	81.94±11.51 ^b	71.24±10.92 ^{a-c}	61.34±13.00 ^{a-c}
	Control	12.23±2.98	83.24±12.10 ^b	85.19±12.40 ^b	75.66±14.28 ^{b,c}
cTnI (μg/l)	Puerarin	0.67±0.14	2.95±0.87 ^b	2.58±0.89 ^{a,b}	1.30±0.22 ^{a-c}
	Control	0.70±0.17	3.06±1.09 ^b	3.19±1.01 ^b	2.44±0.31 ^{b,c}

^aP<0.05, compared with the control group at the same time-points; ^bP<0.05, compared with the same group at T0; ^cP<0.05, compared with the same group at T1. CK-MB, creatine kinase isoenzyme MB; cTnI, troponin I.

Table V. Comparison of mean levels of NF-κB, IL-6 and IL-8 between the two groups at different time-points.

Index	Groups	T0	T1	T2	T3
NF-κB	Puerarin	1.96±1.31	2.89±1.41	4.45±2.53 ^b	5.01±2.20 ^{a,b}
	Control	2.03±1.59	3.66±2.00	5.74±2.52 ^b	7.72±2.35 ^{b,c}
IL-6	Puerarin	1.79±1.05	2.65±0.32	4.03±2.11 ^b	4.52±2.22 ^{a,b}
	Control	1.77±1.53	2.88±2.26	5.15±2.39 ^{b,c}	6.57±3.71 ^{b,c}
IL-8	Puerarin	2.26±1.15	3.00±1.02	4.04±1.89 ^b	4.96±2.65 ^{a,b}
	Control	2.23±1.08	3.49±1.09	5.18±2.18 ^{b,c}	6.35±2.94 ^{b,c}

^aP<0.05, compared with the control group at the same time-points; ^bP<0.05, compared with the same group at T0; ^cP<0.05, compared with the same group at T1. NF-κB, nuclear factor κB; IL-6, interleukin 6.

Table VI. Comparison of mean NF-κB, IL-6 and IL-8 levels between the two groups at different time-points.

Index	NF-κB	IL-6	IL-8
CK-MB			
r value	0.136	0.325	0.197
P-value	0.005	0.002	0.003
cTnI			
r value	0.150	0.331	0.109
P-value	0.004	0.001	0.026

NF-κB, nuclear factor κB; IL-6, interleukin 6; CK-MB, creatine kinase isoenzyme MB; cTnI, troponin I.

and the assisted respiration and ICU hospitalization times were significantly shorter (P<0.05) (Tables II and III).

Comparison of serum CK-MB and cTnI levels between the two groups. We found the CK-MB and the cTnI levels of patients in the two groups increased significantly at T1. With time, the serum myocardial injury markers in patients of the puerarin group decreased gradually, and the levels at T2 and T3 were significantly lower than that at T1. The control group levels were significantly higher than those of the puerarin group after T1 (P<0.05) (Table IV).

Mean expression levels of inflammatory factors NF-κB, IL-6 and IL-8 of the two groups. The positive expression levels of

NF-κB, IL-6 and IL-8 in neutrophils in both groups increased over time after T0. However, the values in the control group were increased to a significantly higher degree than those in the puerarin group. The expression levels of NF-κB, IL-6 and IL-8 at different time-points in the patients of the puerarin group were lower than those in patients of the control group, and the difference was statistically significant at T3 (P<0.05). These results suggest that puerarin preconditioning can inhibit the release of inflammatory factors IL-6 and IL-8 during the process of ischemia-reperfusion injury (Table V).

Analysis of the correlation between inflammatory factors and the myocardial injury markers. Pearson correlation analysis results showed that inflammatory factors NF-κB, IL-6 and IL-8 in the serum of all patients undergoing cardiac valve replacement were positively correlated with CK-MB and cTnI (P<0.05) (Table VI).

Discussion

Ischemia-reperfusion injury occurs often in the process of CPB during cardiac surgery. It can lead to heart failure and sudden death in serious cases (7). Clinically, myocardial cell injury is graded after monitoring the perioperative dynamic changes in serum CK-MB and cTnI after the CPB procedure (8). The mechanisms of myocardial ischemia-reperfusion injury, caused by aorta clamping and declamping during the cardiac valve replacement operation under CPB, is related to the activation, adhesion, accumulation and release of inflammatory mediators in neutrophils. The expression of

NF- κ B, as a transcriptional regulator of inflammatory genes is increased (9,10). The generation of IL-6 occurs as an acute reaction, and it can stimulate the expression of inducible nitric oxide synthase, increase the level of myocardial cGMP and reduce the level of myocardial cAMP (11-14). IL-8 is the most powerful chemotaxis factor of neutrophils. It can strengthen chemotactic activity and stimulates release of large amounts of inflammatory mediators, while inhibiting apoptosis and prolonging inflammation (15,16). Research on ischemia/reperfusion injury protection includes the application of a cardiac arrest technique, cardiac cryogenics, ischemic preconditioning and pharmacological preconditioning (17,18). Puerarin exerts various effects in cardiovascular diseases, it can dilate coronary arteries, relax vessels, improve ischemic myocardium metabolism, and slow-down the heart rate, and reduce myocardial ischemia (19). Animal experiments have shown that puerarin reduces myocardial ischemia-reperfusion injury (20).

This study evaluated the effects of puerarin preconditioning on acute myocardial ischemia-reperfusion injury due to CPB during cardiac valve replacement. Our findings confirmed the surgical procedure increases the levels of CK-MB1 and cTnI of patients, as seen by the increased levels in both groups at T1. We showed how the levels decreased over time in the puerarin group. The levels of NF- κ B, IL-6 and IL-8 were lower in the puerarin group than those in the control (with a significant difference at T3). Collectively this suggests that puerarin preconditioning can inhibit the release of inflammatory factors IL-6 and IL-8 in the process of ischemia-reperfusion injury. Our results also show that after puerarin preconditioning, the clinical markers of patients after the operation were better than the same markers in the control group, suggesting that puerarin can protect myocardial cells and promote their recovery after the operation. The reduction in the levels of inflammatory cytokines in serum was consistent with the reduction in the myocardial injury markers levels. Moreover, our correlation analysis revealed that the inflammatory cytokines NF- κ B, IL-6 and IL-8 in patients with cardiac heart valve replacement were positively correlated with CK-MB and cTnI ($P < 0.05$). Our results indicate that puerarin preconditioning before cardiac valve replacement can relieve myocardial ischemia-reperfusion injury by effectively inhibiting the expression of inflammatory factors and reducing the release of myocardial enzymes.

In conclusion, puerarin preconditioning can reduce the NF- κ B activation and overexpression of IL-6 and IL-8 in neutrophils, and it can inhibit the release of myocardial enzymes cTnI and CK-MB, suggesting myocardial protective effects. Further studies with puerarin are warranted given its potential clinical application value.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YZ and LL were responsible for cardiac valve replacement. CG and NL recorded and analyzed the index. YZ and XF helped with statistical analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of The Second Affiliated Hospital of Zhengzhou University (Zhengzhou, China). Signed written informed consents were obtained from the patients or the guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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