Original Research

Protection of Exogenous Phosphocreatine for Myocardium in Percutaneous Coronary Intervention Related to Inflammation

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Abstract

Objectives: Although injury of myocardium after percutaneous coronary intervention (PCI) has been reported, the mechanism and effect of exogenous phosphocreatine (PCr) supplementation on the injury are yet to be elucidated. Biomarkers, such as interleukin-6 (IL-6) and variations in white blood cells for inflammation, and serum cardiac troponin I (cTnI) for myocardial injury are examined. **Methods**: A total of 105 patients undergoing PCI were included and randomly divided into two groups: control (treated with routine hydration therapy) and PCr (treated with additional intravenous infusion of exogenous PCr). The serum levels of biomarkers were detected at administration and 4, 12, 24, and 48 h after PCI, with natural logarithmic ($\log_e$) transformation of data when modeling assumptions were not fulfilled. **Results**: The level of $\log_e$-transformed IL-6 increased in both groups, especially at 12 and 24 h after the operation, and that of PCr group was less than the control group at 48 h. The content of $\log_e$-transformed cTnI was significantly increased in both groups, while that of the PCr group was markedly lower than the control group at all time points after PCI. Moreover, the ratio of neutrophils was elevated at all time points after PCI, while that of the PCr group was lower at 48 h, and the variations in the ratio of lymphocytes showed opposite results. **Conclusions**: Exogenous phosphocreatine reduces stent implantation, triggers inflammation manifested as decreased serum levels of IL-6 and the aggregation of neutrophils, and protects the myocardium of the patients undergoing PCI. These findings provided the potential mechanism and treatment for myocardial injury associated with PCI.

Keywords: percutaneous coronary intervention; myocardial injury; exogenous phosphocreatine; inflammation; interleukin-6

1. Introduction

Cardiovascular disease (CVD) is the main cause of disease burden worldwide, with high morbidity and mortality, and is continuously increasing in most countries [1]. Since Gruentzig successfully performed the first percutaneous transluminal coronary angioplasty (PTCA) in 1977, percutaneous coronary intervention (PCI) has rapidly evolved into the first-line therapy in most patients with acute coronary syndrome [2]. However, PCI causes myocardial injury, inflammatory reaction, and ischemia-reperfusion injury in ischemic and necrotic areas, making some patients suffer from major adverse cardiovascular events (MACEs) after the operation and affecting the therapeutic effect [3]. Therefore, identifying the mechanism and intervention measures of myocardial injury after PCI is essential. Reportedly, stent implantation triggered the acute phase response and systemic inflammation, which was associated with increased plaque burden and pathological features [4].

Phosphocreatine (PCr) is the main form of chemical energy reserve of myocardial cells with a critical role in energy metabolism during myocardial contraction. A declined level of PCr may have adverse effects on myocardial contraction and recovery of cardiac function [5]. Sodium PCr is an efficient energy supplement, which can effectively supply exogenous myocardial energy and play a critical role in reducing myocardial injury and maintaining normal cardiac function [6]. A recent study found that additional sodium PCr treatment improves early cardiac dysfunction and 28-day survival outcomes in patients with septic shock [7]. Thus, PCr is recommended to be involved in inflammation. However, the mechanism and effect of exogenous PCr on myocardial injury after PCI lacks clinical evidence.

In the present study, we hypothesized that exogenous PCr supplementation before operation alleviates systemic inflammation triggered by PCI to protect the myocardium. A series of experiments were designed and performed to test this hypothesis. Interleukin-6 (IL-6) and blood cell count were used to reflect the inflammation. Cardiac troponin I (cTnI) was adopted as a sensitive marker of myocardial injury [8].

2. Materials and Methods

2.1 Study Design and Patients

A total of 105 patients with coronary heart disease, admitted to the Department of Geriatric Medicine, Qilu Hospital of Shandong University (Jinan, China) from August 2019 to June 2021, undergoing PCI and meeting the enrollment criteria, were recruited in this study. The patients were randomly assigned to the control group (n = 51) or PCr group (n = 54) according to a random number table.

2.1.1 Inclusion Criteria

We included patients who had a non-ST-segment elevation of acute coronary syndromes (NSTE-ACS), identified by non-invasive examination or cardiac catheterization with at least 75% stenosis of one major coronary artery suitable for elective PCI, without extreme risk indicators.

2.1.2 Exclusion Criteria

The patients who fulfilled the following criteria were excluded from the study: severe heart valve (mitral or aortic valve) stenosis or hypertrophic/restrictive cardiomyopathy; allergic to contrast agent or exogenous PCr; chronic kidney disease or estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m^2; engaged in other clinical trials within 3 months. The value of eGFR was calculated by a simplified Modification of Diet in Renal Disease Study Group (MDRD) equation.

2.1.3 Sample Size

The minimum number of samples was calculated as 41 in each group, based on Software PASS 11 (NCSS, LLC, Kaysville, Utah). Herein, $\alpha = 0.05$, $\beta = 0.10$, $\mu_1 = 15.84$, $SD_1 = 11.844$, $\mu_2 = 9.26$, $SD_2 = 4.502$ for IL-6 at 48 h after PCI in our preliminary experiment. Considering the loss and refusal to follow-up, a total of 105 patients were enrolled in this study.

2.2 Protocols

The patients received routine pharmacological treatment for coronary artery disease (CAD) as recommended by guidelines in both groups: anti-platelets, nitrates, statins, and beta receptor blockers. Patients with diabetes mellitus ceased metformin administration 24 h before the operation and were injected insulin subcutaneously to control the plasma glucose level. The patients in the control group were infused 100 mL saline intravenously, and those in the PCr group were treated with 4 g of sodium PCr (Harbin Lebotong Pharmaceutical Co., Ltd, Harbin, China) within 30 min before PCI.

The patients in both groups were administered lidocaine for local anesthesia, heparin, nitroglycerin, and nonionic contrast agents during the operation. They also underwent routine electrocardiograph (ECG) examination and bedside ECG monitoring for 24 h after the operation and received secondary prevention of CAD.

2.3 Estimations

Typically, 3–5 mL peripheral venous blood samples were collected from both groups of patients at administration and 4, 12, 24, and 48 h after PCI. The serum was separated by centrifugation of the blood samples at 4 °C, 3000 rpm for 15 min and stored at –80 °C until further use. The levels of serum-sensitive cTnI and IL-6 were determined by chemiluminescence immunoassay (CLIA) system before and 4, 12, 24, and 48 h after PCI, with the reference range <30.0 ng/L and <7 pg/mL, respectively.

Blood cell count was detected by blood routine Sysmex XE2100 hematology analyzer (Sysmex Corp. Kobe, Japan). The parameters were similar to those for the routine blood test. The blood glucose, lipids, and biochemical indicators of the patients were measured in a standard fashion.

The primary endpoint was the evaluation of IL-6 at 48 h after PCI to investigate the mechanism of PCr supplementation. The secondary endpoint was the evaluation of the myocardial injury based on serum cTnI after PCI, as well as the ratio of neutrophils (NEUR) to white blood cells (WBCs) to define the effect of PCr.

2.4 Statistical Analysis

All statistical analyses were performed using SPSS 20.0 software (IBM, Armonk, NY, USA). The quantitative data were assessed for normality and homogeneity of variance. For non-normally distributed continuous variables, the natural logarithmic transformation was carried out, and the normal or approximately normal distribution was confirmed using the quantile-quantile (Q-Q) plot, while the normally or approximately normally distributed data were represented as mean $\pm$ standard deviation ($\pm$ SD). The intra- and inter-group significances among different time points were assessed by analysis of variance (ANOVA) for repeated data, Student's t-test was mainly adopted to compare the differences of primary end point, and also to explore other indicators between groups at different time points, and paired t-test was used to compare the data of each time point postoperatively to the baseline. In ANOVA, if the assumption of Mauchly's test of sphericity was fulfilled ($p > 0.05$), the results of sphericity assumed in tests of within-subjects effects were adopted, otherwise those of Pillai's trace in multivariate tests were used. In multiple t-tests, the Ryan–Holm step-down Bonferroni procedure was performed to adjust the p-values with half-declining α value for the subsequent comparison using the following order for data at different time points: 48 h, 24 h, 4 h, 12 h, and baseline. Because of the potential for type I error caused by multiple comparisons, findings for analyses of secondary end points should be interpreted as exploratory. The qualitative data were expressed as a rate (%), and the comparisons between groups were analyzed using the chi-square test or Fisher's exact test. The statistical significance was considered as $p < 0.05$ (two-sided).

3. Results

3.1 Baseline and Procedural Characteristics of the Patients

A total of 105 patients, aged 64.14 ± 10.84 years, who underwent PCI, were recruited and randomized for the present study. The cohort comprises 51 patients in the control group and 54 in the PCr group, with 65.71% of males. The ultimate disposition is shown in Fig. 1.
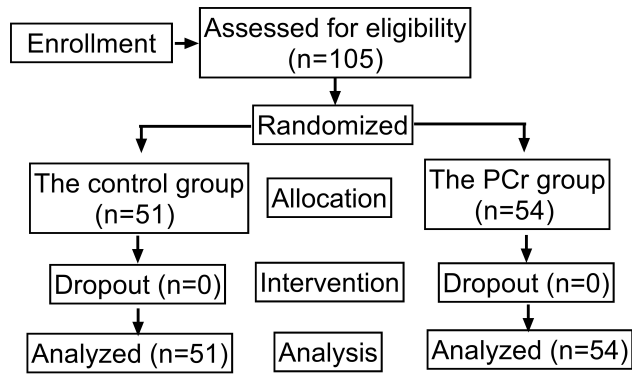
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Enrollment ──► Assessed for eligibility
                        (n=105)
                           │
                      Randomized
              ┌────────────┼────────────┐
              ▼                          ▼
   The control group    Allocation    The PCr group
        (n=51)                            (n=54)
              │                              │
              ▼                              ▼
      Dropout (n=0)     Intervention    Dropout (n=0)
              │                              │
              ▼                              ▼
      Analyzed (n=51)     Analysis     Analyzed (n=54)
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Fig. 1. Consort flow diagram. Patients were randomly assigned to the control and PCr groups. No patient withdrew from the study.

The proportion of patients with the previous diagnosis of hypertension, diabetes, old myocardial infarction, heart failure with New York Heart Association (NYHA) classification of cardiac function worse than grade II, and coronary intervention was 44.76%, 24.76%, 15.00%, 12.38%, and 11.43%, respectively. The clinical characteristics of the patients are summarized in Table 1. The basic fasting blood glucose level and low-density lipoprotein cholesterol (LDL-C) of all patients measured at the time of admission were 5.81 ± 1.44 and 2.25 ± 0.82 mmol/L. The baseline characteristics and the above estimations were similar between the two groups (all $p > 0.05$).

The usage of statins, average amounts of diseased vessels, implanted stents, procedural time, the volume of contrast medium, transarterial approach, and Gensini scores in the major branches of coronary circulation (left main coronary artery, left anterior descending branch, left circumflex branch, and right coronary artery) did not show significant intergroup gaps (all $p > 0.05$) (Table 1).

3.2 Effects of PCr on IL-6

The natural logarithm of IL-6 ($\log_e$IL-6) was used for analysis. As the primary end point, $\log_e$IL-6 in the PCr group was markedly lower than that in the control group at 48 h after PCI (t = 2.187, $p = 0.032$). And no significant difference was detected between the two groups at baseline (t = 0.865, $p = 0.389$). Intragroup gaps were detected among these time points (F = 29.656, $p < 0.001$), while the intergroup differences were not obvious after mixing time factors (F = 2.016, $p = 0.159$). The level of $\log_e$IL-6 ele-

vated significantly at 4, 12, 24, and 48 h in both groups after PCI (all $p < 0.001$) (Fig. 2 and **Supplementary Table 1** in Supplemental Materials).
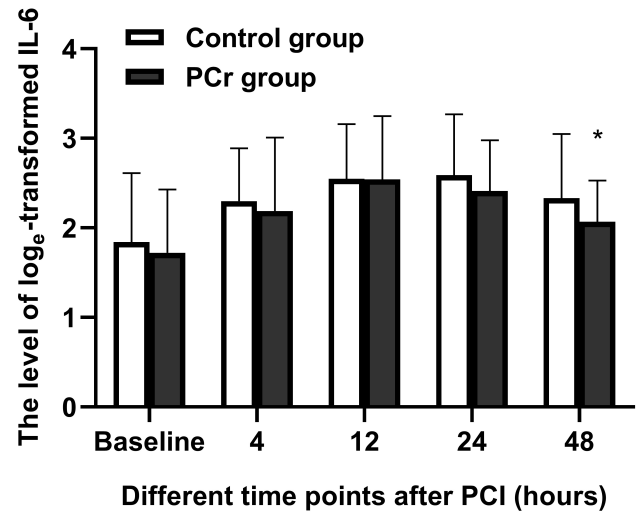

Fig. 2. Effects of PCr on IL-6. The number of patients enrolled was 51 in the control group and 54 in the PCr group. Values are $\log_e$IL-6 presented as mean $\pm$ SD. *$p < 0.05$ for the PCr group compared with the control group at the corresponding time point. All $p < 0.001$ for the control group and the PCr group after PCI compared with corresponding baseline at 4 h, 12 h, 24 h and 48 h (see also **Supplementary Table 1** in Supplemental Materials).

3.3 Effects of PCr on Myocardial Injury

No statistical difference was detected in $\log_e$(cTnI+2) between the two groups at baseline (t = 1.310, $p = 0.194$). However, intra- and inter-group gaps were detected among various time points (F = 86.493, $p < 0.001$; and F = 9.570, $p = 0.003$). The level of $\log_e$(cTnI+2) elevated significantly at 4, 12, 24, and 48 h after PCI in both groups (all $p < 0.001$). Furthermore, the $\log_e$(cTnI+2) in the PCr group was distinctly lower than that in the control group at 4, 12, 24, and 48 h after PCI (t = 2.644, $p = 0.010$ for 4 h; t = 3.082, $p = 0.003$ for 12 h; t = 2.717, $p = 0.008$ for 24 h; and t = 2.371, $p = 0.020$ for 48 h) (Fig. 3 and **Supplementary Table 2** in Supplemental Materials).

3.4 Effects of PCr on WBCs

The WBC count, including neutrophils (NEU), lymphocytes (LYM), eosinophils (EOS), basophils (BAS), and monocytes (MON), and their ratios to WBCs, including NEUR, LYMR, EOSR, BASR, and MONR, did not show any significant difference between the PCr and control groups before PCI (all $p > 0.05$). The intragroup comparisons of WBCs and classifications performed by ANOVA for repeated data were statistically significant among the five time points before and after PCI (F = 7.861 for WBCs, F = 16.960 for NEU, F = 8.268 for LYM, F = 13.130

Table 1. General and procedural characteristics of the patients.

Clinical factors	PCr group (n = 54)	Control group (n = 51)	t/x^2	p value
Age (year, $\bar{x} \pm$ SD)	62.87 $\pm$ 9.29	65.49 $\pm$ 12.21	1.232	0.221
Gender (male) (n, %)	33 (61.11%)	36 (70.59%)	1.046	0.307
BMI (Kg/m^2, $\bar{x} \pm$ SD)	26.30 $\pm$ 3.02	25.72 $\pm$ 2.65	−1.028	0.306
Hypertension (n, %)	24 (44.44%)	23 (45.10%)	0.005	0.946
Diabetes mellitus (n, %)	13 (24.07%)	13 (25.49%)	0.028	0.867
Old myocardial infarction (n, %)	8 (14.81%)	5 (9.80%)	0.607	0.436
Cardiac dysfunction (NYHA >2) (n, %)	5 (9.26%)	7 (13.73%)	0.517	0.472
Previous PCI (n, %)	12 (22.22%)	8 (15.69%)	0.727	0.394
Statins (pitavastatin) (n, %)	29 (53.70%)	26 (50.98%)	0.078	0.780
Diseased vessels (n, $\bar{x} \pm$ SD)	2.46 $\pm$ 0.86	2.63 $\pm$ 0.82	0.998	0.320
Implanted stents (n, $\bar{x} \pm$ SD)	1.50 $\pm$ 0.69	1.73 $\pm$ 0.83	1.517	0.132
Procedural time (minutes, $\bar{x} \pm$ SD)	52.54 $\pm$ 18.53	52.84 $\pm$ 16.74	0.089	0.930
volume of contrast medium (mL, $\bar{x} \pm$ SD)	90.46 $\pm$ 41.86	101.67 $\pm$ 19.66	1.771	0.081
trans-arterial approach (radial artery) (n, %)	49 (90.74%)	46 (90.20%)	0.000	1.000
Gensini scores (per case, $\bar{x} \pm$ SD)	48.94 $\pm$ 37.74	40.49 $\pm$ 21.43	−1.420	0.159

NYHA, cardiac function grading of New York Heart Academy; BMI, body mass index, calculated by dividing weight (Kg) by the square of height (m^2).
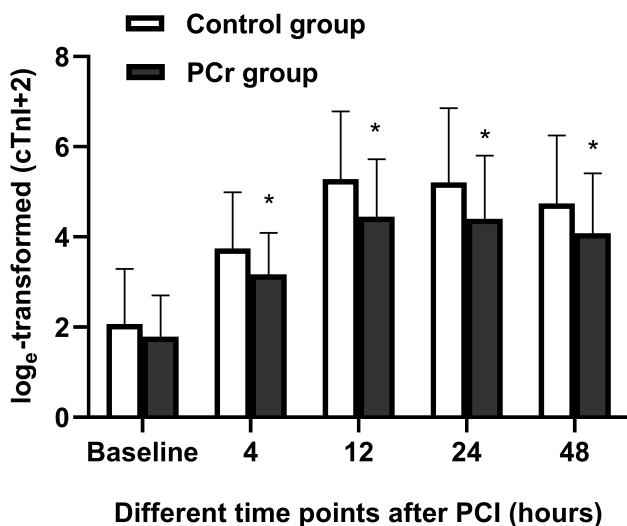

Fig. 3. Effects of PCr on myocardial injury. The number of patients enrolled was 51 in the control group and 54 in the PCr group. Values are natural logarithmic transformed highly sensitive cardiac troponin I plus 2 (log$_e$(cTnI+2)) presented as mean $\pm$ SD. *$p < 0.05$ for the PCr group compared with the control group at the corresponding time point. All $p < 0.001$ for the control group and the PCr group after PCI compared with corresponding baseline at 4, 12, 24 and 48 h (**Supplementary Table 2** in Supplemental Materials).

for EOS, F = 26.016 for BAS, and F = 20.247 for MON; $p < 0.001$). Furthermore, the intergroup comparisons of NEUR and LYMR were significant (F = 4.640, $p = 0.034$ for NEUR; and F = 5.443, $p = 0.022$ for LYMR).

Compared to the baseline at admission, the WBC count in both groups was increased significantly at 4 h and 12 h after PCI, and that in the control group was still higher at 24 h ($p < 0.05$). The NEUs and NEUR in both groups also rose at 4, 12, and 24 h after PCI (all $p < 0.05$), while the NEUs in the PCr group restored at 48 h after PCI. However, the LYMs, LYMR, and other classifications of leukocytes in both groups were decreased after PCI, except for EOS and EOSR at 48 h. Compared to the control group, the NEUR of the PCr group was lower at 48 h after PCI (t = 2.721, $p = 0.008$), while it decreased but not significantly at 24 h (t = 2.176, $p = 0.032 > 0.025$ as adjusted p-values). In addition, the LYMR of the PCr group was higher at 24 and 48 h after PCI (t = −2.377, $p = 0.019$ for 24 h; t = −2.965, $p = 0.004$ for 48 h), similar to LYM at 48 h after PCI (t = −2.345, $p = 0.021$), i.e., LYMR and LYM were close to normal levels in the PCr group (Fig. 4 and **Supplementary Table 3** in Supplemental Materials).

3.5 Effects of PCr on RBCs and PLTs

The indicators of RBCs, including the RBC count, hemoglobin (HGB), hematocrit (HCT), erythrocyte mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW), did not differ significantly between the PCr and control groups before PCI (all $p > 0.05$). The intragroup comparisons of these indicators performed by ANOVA for repeated data were statistically significant among the five time points before and after PCI (F = 31.568 for RBCs, F = 56.186 for HGB, F = 27.213 for HCT, F = 6.965 for MCV, F =
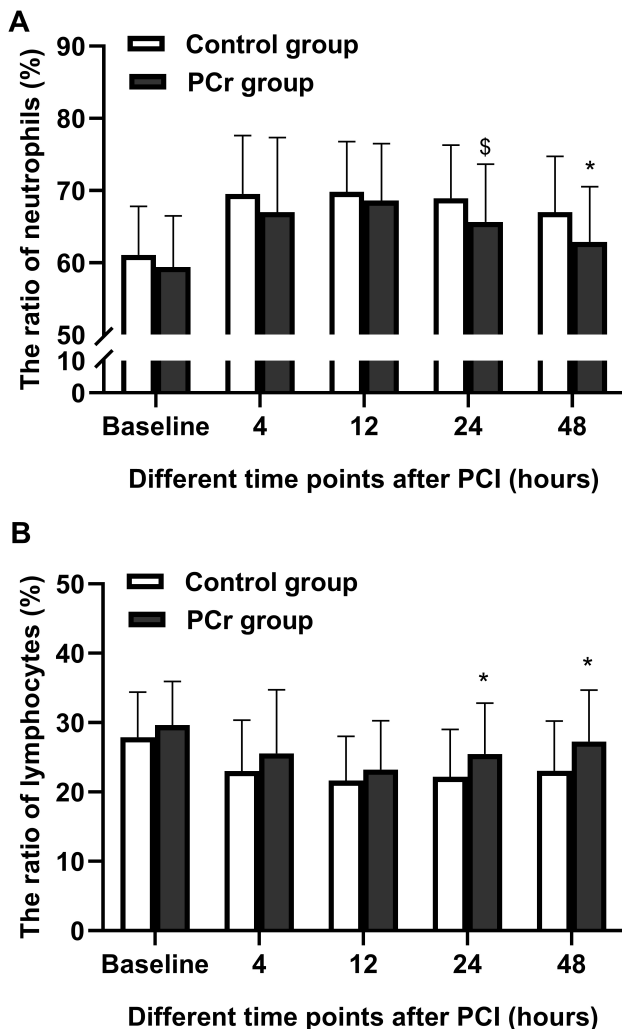
Fig. 4. Levels of NEUR and LYMR of the patients at different time points. The number of enrolled patients was 51 in the control group and 54 in the PCr group. Values are mean ± SD. *$p < 0.05$ for the PCr group compared to the control group at the corresponding time point. $^{\$}p > 0.025$ as adjusted p-values. All $p < 0.05$ for the control group and the PCr group after PCI compared to the corresponding baseline at 4, 12, 24, and 48 h (See also **Supplementary Table 3** in Supplemental Materials).

37.408 for MCH, F = 28.923 for MCHC, F = 10.060 for RDW, and all $p < 0.001$), while, the intergroup differences were not obvious (F = 2.081 for RBCs, F = 0.777 for HGB, F = 0.433 for HCT, F = 2.575 for MCV, F = 0.887 for MCH, F = 1.638 for MCHC, F = 0.113 for RDW; and all $p > 0.05$). A similar phenomenon was observed for the indicators of PLTs, including PLT count, platelet distribution width (PDW), mean platelets volume (MPV), and platelet hematocrit (PCT) (intragroup F = 9.785 for PLTs, F = 103.557 for PDW, F = 132.046 for MPV, F = 49.793 for PCT; and all $p < 0.001$) (intergroup F = 0.109 for PLTs, F = 0.307 for PDW, F = 0.006 for MPV, F = 0.941 for PCT; and all $p > 0.05$).

Compared to the baseline at admission within groups, the RBC count, HGB, HCT, MCH, MCHC, and RDW, PLT count, MPV, and PCT, decreased after PCI in both groups. On the other hand, the MCV and PDW were predisposed to increase after PCI. However, no obvious differences were detected between the PCr and control group in these indicators (all $p > 0.05$) (**Supplementary Table 4** in Supplemental Materials).

3.6 Content of Biochemical Indicators of the Patients

At administration, no statistical alteration was detected in biochemical biomarkers for hepatic function, including alanine transaminase (ALT), γ-glutamyl transferase (GGT), alkaline phosphatase (AKP), total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL), total bile acid (TBA), for renal function, including blood urea nitrogen (BUN) and estimated glomerular filtration rate (eGFR), and N-terminal pro-brain natriuretic peptide (NT-proBNP) for cardiac function between the PCr and control groups. Also, the metabolic-related indicators, such as, blood glucose, uric acid (UA), and blood lipids, including total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG), and inflammation-related indicators, such as C1q and Cys-c, did not show any significant difference between the two groups at baseline (all $p > 0.05$) (Table 2).

3.7 Adverse Events

Recurrent angina pectoris comprised the main cardiovascular adverse events occurring within 48 h after PCI, and no significant difference was observed between the two groups (Fisher's exact test, $p = 1.000$) (Table 3).

4. Discussion

CVD is mainly caused by stenosis or occlusion of the lumen due to atherosclerosis, which leads to myocardial ischemia, hypoxia, or infarction. PCI is one of the major methods to treat CVD by restoring coronary circulation and myocardial reperfusion. Some studies have shown that the myocardium is damaged during PCI, which is one of the main factors of cardiovascular adverse events in postoperative patients [9]. The myocardium is a tissue with high oxygen demand and consumption. When coronary artery stenosis occurs, the blood flow decreases, which destroys the balance of oxygen supply and demand. The primary changes caused by ischemia are the destruction of myocardial cells and vascular endothelial cells [10].

The destruction of intima also decreases nitric oxide production, which disrupts the endothelium-dependent vasodilation function. The myocardial cells also press the microvessels due to cell swelling and ischemia-induced injury, which is due to the decrease in adenosine triphosphate (ATP) caused by long-term ischemia and hypoxia, the inactivation of sodium-potassium-exchanging ATPase on the

Table 2. The contents of biochemical indicators of the patients.

Variables	PCr group (n = 54) ($\bar{x} \pm$ SD)	Control group (n = 51) ($\bar{x} \pm$ SD)	t	p value
ALT (U/L)	18.98 ± 9.64	24.80 ± 21.36	1.783	0.079
GGT (U/L)	26.30 ± 14.99	27.82 ± 19.95	0.445	0.657
AKP (U/L)	72.41 ± 19.43	74.04 ± 20.42	0.420	0.676
TBIL (μmol/L)	10.59 ± 3.86	10.38 ± 4.85	−0.245	0.807
DBIL (μmol/L)	3.62 ± 1.13	3.69 ± 1.60	0.263	0.793
IBIL (μmol/L)	6.97 ± 2.82	6.69 ± 3.40	−0.460	0.646
TBA (μmol/L)	5.99 ± 3.70	6.99 ± 6.39	0.988	0.325
TC (mmol/L)	3.71 ± 0.94	3.99 ± 1.16	1.349	0.180
HDLC (mmol/L)	1.02 ± 0.23	1.07 ± 0.25	1.153	0.252
LDLC (mmol/L)	2.15 ± 0.72	2.36 ± 0.91	1.309	0.194
TG (mmol/L)	1.44 ± 0.66	1.34 ± 0.55	−0.812	0.418
Glu (mmol/L)	5.81 ± 1.49	5.80 ± 1.41	2.072	0.041
BUN (mmol/L)	5.24 ± 1.51	5.58 ± 1.63	−0.021	0.983
Cys-c (mg/L)	0.95 ± 0.20	1.02 ± 0.25	1.127	0.262
UA (μmol/L)	326.15 ± 78.36	329.47 ± 86.23	1.601	0.112
C1q (mg/L)	171.94 ± 31.26	167.62 ± 25.24	0.207	0.837
NT-proBNP (pg/mL)	211.36 ± 388.52	300.93 ± 549.41	−0.777	0.439
eGFR (mL/min/1.73 m^2)	99.31 ± 20.03	92.89 ± 24.30	−1.481	0.142

ALT, alanine transaminase; GGT, γ-glutamyl transferase; AKP, alkaline phosphatase; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; TBA, total bile acid; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; Glu, blood glucoes; BUN, blood urea nitrogen; Cys-c, cystatin c; UA, uric acid; C1q, complement 1q; NT-proBNP, N-terminal pro-brain natriuretic peptide; eGFR, estimated glomerular filtration rate.

cell membrane, the decrease in calcium ion outflow, and the limitation of endoplasmic reticulum's absorption of calcium ions, resulting in the overload of calcium ions in myocardial cells. These changes in myocardial cells are accompanied by the activation of intracellular protease, damaging the myofibrils. The reperfusion injury potentiates the ischemic injury. Previous studies have shown that the increase in serum cTnI level is closely related to the occurrence of MACEs after PCI, which guides the therapeutic strategy [11]. The current results showed that the log$_e$-transformed cTnI increased significantly after PCI and peaked at 24 h. The level of log$_e$-transformed cTnI in the PCr group was lower than that of the control group at 4 h, 12 h, 24 h, and 48 h post-PCI, indicating that PCr intervention significantly alleviates the PCI-induced myocardial injury.

The highly sensitive cardiac troponin I (cTnI) describes the damage of tiny myocardium, and its molecular weight is smaller than that of myocardial enzymology index, with high sensitivity and specificity. When the myocardium is damaged, the intracellular adenosine triphosphate (ATP) level decreases, leading to acidosis and increased permeability of the cell membrane; also, cTnI is easily released into the blood [12]. The supply of energy is vital to relieve myocardial ischemia and PCI-induced injury. PCr is a high-energy phosphate compound found in the brain cells, myocardium and skeletal muscle. It participates in ATP synthesis, forms actin through the hydrolysis effect, and supplies energy for the contraction of the myocardium. The decline in the PCr level in the myocardium affects the oxidative metabolism of myocytes, causes myocardial injury, and slows down the repair process [13]. In addition, as an endogenous substance, PCr can directly enter cardiac myocytes, providing energy for calcium-transporting ATPases and sodium-potassium-exchanging ATPase, facilitating the transport of intracellular Ca^{2+} into sarcoplasmic reticulum that promotes the recovery of myocardial contractility and attenuating the injury [14]. Exogenous PCr is also beneficial in maintaining the ATP level, reducing reactive oxygen species, enhancing membrane stability, preventing damage to the myocardium, and diminishing the cTnI content transferred into the blood [15]. Sodium PCr also reduces the degradation of 5'-mono-, 5'-di-, and 5'-triphosphorylated nucleotides and adenine nucleotides that maintain the state of high energy in cardiac myocytes, prevent PLT aggregation, improve microcirculation, and preserve myocardial function [16].

Recent studies have reported that inflammation is involved in the development of atherosclerotic plaque, and

Table 3. Post-procedural adverse cardiovascular events of the patients.

Variables	Pcr group (n = 54)		Control group (n = 51)	
	n	*%*	*n*	*%*
Recurrent angina pectoris	1	1.85	1	1.96
Malignant arrhythmia	0	0.00	0	0.00
Hypotension	0	0.00	0	0.00
Aggravation of ST-T segment change	0	0.00	0	0.00
Cardiovascular death	0	0.00	0	0.00
Deterioration of cardiac function	1	1.85	0	0.00
Stroke	0	0.00	0	0.00
Sum	2	3.70	1	1.96

There was no significant difference in the incidence of adverse cardiovascular events between the PCr group and the control group (Fisher's exact test, $p = 1.000 > 0.05$).

the release of inflammatory mediators activates the complements, promotes leukocyte aggregation, damages the intima of blood vessels, and induces the formation of plaque and the progression of nontarget lesions [4]. IL-6 is an acute-phase protein of inflammation. Typically, the level of IL-6 is low in the body. Damaged or infected tissue results in a sharp increase in the synthesis of IL-6 and its release into the blood, which in turn promotes the monocytes to release tissue factors, thus increasing the risk of local thrombosis [17]. IL-6 is also involved in the formation of atherosclerosis, causing an intimal reaction in arteries, aggravating inflammation, and possibly causing vasospasm [18]. The higher the level of IL-6, the more serious the inflammation in patients with CVD, which further increases the risk of cardiovascular adverse events that are detrimental to the prognosis of patients [19]. The current results showed that $\log_e$-transformed IL-6 increased after PCI, especially at 12 h and 24 h. However, $\log_e$-transformed IL-6 of the PCr group was lower than that of the control group, and the difference was significant at 48 h after the operation. This phenomenon suggested that the inflammation of the patients increased after PCI. Furthermore, sodium PCr regulates the inflammatory factors stimulated by PCI, restores the serum IL-6 level to normal, and improves the prognosis of patients.

Some debris (foam-like macrophages, aggregated platelets, cholesterol crystals, intimal debris, and thrombus fragments) fell to the distal end of blood vessels due to the rupture of unstable plaques and physical injuries, such as the entry of guidewires, balloon dilatation, and stent implantation during PCI [20]. Although a small amount of debris does not affect the forward blood flow, it induces the aggregation of inflammatory mediators and releases vasoconstrictors, such as thromboxane A2, serotonin, and tumor necrosis factor. Some studies have shown that shed debris can induce NEU aggregation, activate PLTs, and promote thrombosis. The primary mechanism of myocardial damage lies in the aggregation of NEUs and PLTs and the release of reactive oxygen species (ROS). Activated NEUs and PLTs release a large number of vasoconstrictors, inflammatory factors, ROS, and proteases, which further aggregate the NEUs and PLTs, aggravate the inflammatory reaction, form a vicious circle, cause the destruction and edema of the myocardium, and eventually lead to reperfusion injury [21].

NEUs are the main source of ROS during reperfusion and the key inflammatory cells of myocardial injury. In the event of severe inflammation, apoptosis decreases the LYM count while increasing the NEU count [22]. This variation trend was also observed in the present study. The current results showed that after PCI, the counts of WBC, NEU, and NEUR are higher than those before PCI, while those of LYM, EOS, BAS, and MON and the ratios are lower than the baseline. Moreover, the NEUR and LYMR altered at 12 h after PCI. This phenomenon was consistent with the mechanism of reperfusion injury. Therefore, preventing NEU-triggered inflammation during PCI perioperative period may be a key to treating myocardial injury. In this study, NEUR and LYMR in the PCr group were significantly improved compared to those in the control group at 48 h after the operation. This indicated that the intervention of exogenous PCr alleviates the inflammation related to NEUs after PCI, thus protecting the myocardium.

PDW and MPV are crucial features of PLT activation [23]. The current results described that PDW is increased after PCI, while MPV, PLT count, and PCT are decreased. PCr intervention had no effect on the PLT features. These phenomena may be essential to detect sensitive indicators of PLT reactivity [24,25].

HGB is a specific protein that transports oxygen in RBCs. Anemia is related to serious cardiovascular diseases (such as thromboembolism and hemorrhage), which in turn increases the mortality of anemia patients during hospitalization [26]. The disease is also related to inflammation, which increases the release of erythropoietin and cytokines, leading to vascular endothelial damage, accelerating atherosclerosis, activating platelets, putting the body in a hypercoagulation state, and increasing the risk

of thrombosis [27]. The current results showed that RBC, HGB, HCT, MCH, and MCHC were lower after PCI than those before PCI, which was similar to the previous studies. Compared to the control group, these indicators in the PCr group improved slightly, albeit not significantly. Thus, additional studies with larger sample sizes may be needed. Interestingly, the plasma concentration of free hemoglobin (fHGB) was significantly higher in patients with acute myocardial infarction (AMI) between 12 h and 24–48 h time points after stent implantation [28], suggesting that the decrease in HGB may be related to perioperative sterile inflammation and hemolysis.

5. Study Limitations

Nevertheless, the present study has the following limitations. Firstly, this is a single-center, unblinded study, and no confounding factors have been identified to influence the results. Secondly, measuring troponin post-intervention is not clear, and indirect variables for myocardial injury and inflammation are used. Finally, no long-term follow-up was carried out in this study and can be continued in future studies to further evaluate the prediction of the risk of adverse cardiovascular events.

6. Conclusions

In conclusion, a series of operations during PCI in coronary arteries leads to vascular endothelial damage and vasospasm, increases the risk of thrombosis, affects the blood perfusion of distal vessels, aggravates myocardial ischemia and inflammation, and cause myocardial injury and adverse cardiovascular events. Exogenous PCr regulates the levels of serum cTnI, IL-6, NEUR, and LYMR in patients undergoing PCI, which is crucial for improving myocardial perfusion, alleviating inflammation, and regulating the functions of granulocytes and mononuclear cells. Additional studies are warranted to clarify the detailed mechanism and the long-term prognosis.

Author Contributions

MYL performed the analysis, processed the experimental data, and wrote the article. YQX designed and directed the project, performed the statistical analysis, and edited the manuscript. YPS and CL were involved in planning and supervising of the experiments. YPS, ZHW and YW aided in interpreting the results and edited the manuscript. XHL, ZZ and RXZ performed the measurements. LYQ and ML were involved in planning the project and contributed to sample preparation. YLX and HQC aided in interpreting the results. All authors approved the final version to be submitted.

Ethics Approval and Consent to Participate

This study complied with the Declaration of Helsinki, was approved by the Medical Ethics Committee of Qilu

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://www.imrpress.com/journal/RCM/23/3/10.31083/j.rcm2303089.

References

[1] Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, et al. Global Burden of Cardiovascular Diseases and Risk Factors, 1990-2019: Update From the GBD 2019 Study. Journal of the American College of Cardiology. 2020; 76: 2982–3021.

[2] Faxon DP, Williams DO. Interventional Cardiology: Current Status and Future Directions in Coronary Disease and Valvular Heart Disease. Circulation. 2016; 133: 2697–2711.

[3] Silvain J, Zeitouni M, Paradies V, Zheng HL, Ndrepepa G, Cavallini C, et al. Procedural myocardial injury, infarction and mortality in patients undergoing elective PCI: a pooled analysis of patient-level data. European Heart Journal. 2021; 42: 323-334.

[4] Ma J, Liu X, Qiao L, Meng L, Xu X, Xue F, et al. Association between Stent Implantation and Progression of Nontarget Lesions in a Rabbit Model of Atherosclerosis. Circulation: Cardiovascular Interventions. 2021; 14: e010764.

[5] Qiu F, Yuan Y, Luo W, Gong YS, Zhang ZM, Liu ZM, et al. Asiatic acid alleviates ischemic myocardial injury in mice by modulating mitophagy- and glycophagy-based energy metabolism. Acta pharmacologica Sinica. 2021. (in press)

[6] Wang C, Guo J, Di A, Zhu Y, Han W, Cheng A, et al. The Protective Effect of Cx43 Protein-Mediated Phosphocreatine on Myocardial Ischemia/Reperfusion Injury. Cardiology Research and Practice. 2021; 2021: 1–9.

[7] Kang D, Yu J, Xia J, Li X, Wang H, Zhao Y. Effect of norepinephrine combined with sodium phosphocreatine on cardiac function and prognosis of patients with septic shock. International Journal of Immunopathology and Pharmacology. 2020; 34: 205873842095058.

[8] Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, *et al*. Fourth Universal Definition of Myocardial Infarction (2018). Journal of the American College of Cardiology. 2018; 72: 2231–2264.

[9] Mangiacapra F, Bressi E, Di Gioia G, Pellicano M, Di Serafino L, Peace AJ, *et al*. Coronary microcirculation and periprocedural myocardial injury during elective percutaneous coronary intervention. International Journal of Cardiology. 2020; 306: 42–46.

[10] Daniels LJ, Varma U, Annandale M, Chan E, Mellor KM, Delbridge LMD. Myocardial Energy Stress, Autophagy Induction, and Cardiomyocyte Functional Responses. Antioxidants & Redox Signaling. 2019; 31: 472–486.

[11] Shufelt CL, Kim A, Joung S, Barsky L, Arnold C, Cheng S, *et al*. Biometric and Psychometric Remote Monitoring and Cardiovascular Risk Biomarkers in Ischemic Heart Disease. Journal of the American Heart Association. 2020; 9

[12] Qin Z, Kong B, Zheng J, Wang X, Li L. Alprostadil Injection Attenuates Coronary Microembolization-Induced Myocardial Injury Through GSK-3β/Nrf2/HO-1 Signaling-Mediated Apoptosis Inhibition. Drug design, development and therapy. 2020; 14: 4407-4422.

[13] Wang C, Hu L, Guo S, Yao Q, Liu X, Zhang B, *et al*. Phosphocreatine attenuates doxorubicin-induced cardiotoxicity by inhibiting oxidative stress and activating TAK1 to promote myocardial survival in vivo and in vitro. Toxicology. 2021; 460: 152881.

[14] Liu W, Qaed E, Zhu HG, Dong MX, Tang Z. Non-energy mechanism of phosphocreatine on the protection of cell survival. Biomedicine & Pharmacotherapy. 2021; 141: 111839.

[15] Shopit A, Niu M, Wang H, Tang Z, Li X, Tesfaldet T, *et al*. Protection of diabetes-induced kidney injury by phosphocreatine via the regulation of ERK/Nrf2/HO-1 signaling pathway. Life Sciences. 2020; 242: 117248.

[16] Brewster LM. Creatine kinase, energy reserve, and hypertension: from bench to bedside. Annals of Translational Medicine. 2018; 6: 292.

[17] Chen X, Tan Y, Yu S, Lu L, Deng Y. Pinitol protects against ox-LDL-induced endothelial inflammation and monocytes attachment. Journal of Cardiovascular Pharmacology. 2021. (in press)

[18] Zeb I, Jorgensen NW, Blumenthal RS, Burke GL, Lloyd-Jones D, Blaha MJ, *et al*. Association of inflammatory markers and lipoprotein particle subclasses with progression of coronary artery calcium: the multi-ethnic study of atherosclerosis. Atherosclerosis. 2021; 339: 27–34.

[19] Antonopoulos AS, Angelopoulos A, Papanikolaou P, Simantiris S, Oikonomou EK, Vamvakaris K, *et al*. Biomarkers of Vascular Inflammation for Cardiovascular Risk Prognostication. JACC: Cardiovascular Imaging. 2021. (in press)

[20] Tucker B, Vaidya K, Cochran BJ, Patel S. Inflammation during Percutaneous Coronary Intervention-Prognostic Value, Mechanisms and Therapeutic Targets. Cells. 2021; 10: 1391.

[21] Koganti S, Eleftheriou D, Gurung R, Hong Y, Brogan P, Rakhit RD. Persistent circulating platelet and endothelial derived microparticle signature may explain on-going pro-thrombogenicity after acute coronary syndrome. Thrombosis Research. 2021; 206: 60–65.

[22] Lagunas-Rangel FA. Neutrophil-to-lymphocyte ratio and lymphocyte-to-C-reactive protein ratio in patients with severe coronavirus disease 2019 (COVID-19): a meta-analysis. Journal of Medical Virology. 2020; 92: 1733–1734.

[23] Goriki Y, Yoshioka G, Natsuaki M, Shinzato K, Nishihira K, Kuriyama N, *et al*. Simple risk-score model for in-hospital major bleeding based on multiple blood variables in patients with acute myocardial infarction. International Journal of Cardiology. 2021. (in press)

[24] Aiello S, Gastoldi S, Galbusera M, Ruggenenti P, Portalupi V, Rota S, *et al*. C5a and C5aR1 are key drivers of microvascular platelet aggregation in clinical entities spanning from aHUS to COVID-19. Blood Advances. 2022; 6: 866–881.

[25] Zhou S, Wang Z, Liu Z, Mu G, Xie Q, Wang Z, *et al*. Candidate Gene of NOS3, MMP3, AGT, and AGT1R and Pathway Analyses for Platelet Reactivity and Clinical Outcomes of Repeat Revascularization After First PCI in Chinese Patients. Cardiovascular Drugs and Therapy. 2021. (in press)

[26] Minton K. Red blood cells join the ranks as immune sentinels. Nature Reviews Immunology. 2021; 21: 760–761.

[27] Lam LKM, Murphy S, Kokkinaki D, Venosa A, Sherrill-Mix S, Casu C, *et al*. DNA binding to TLR9 expressed by red blood cells promotes innate immune activation and anemia. Science Translational Medicine. 2021; 13: eabj1008.

[28] Jadczyk T, Baranski K, Syzdol M, Nabialek E, Wanha W, Kurzelowski R, *et al*. Bioactive Sphingolipids, Complement Cascade, and Free Hemoglobin Levels in Stable Coronary Artery Disease and Acute Myocardial Infarction. Mediators of Inflammation. 2018; 2018: 2691934.