Original Paper

The Involvement of Phosphatase and Tensin Homolog Deleted on Chromosome Ten (PTEN) in the Regulation of Inflammation Following Coronary Microembolization

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Key Words

Coronary microembolization • Inflammation • TNF- α • PTEN

Abstract

Background/Aims: Growing evidence shows that phosphatase and tensin homolog deleted on chromosome ten (PTEN) is involved in regulating inflammation in different pathological conditions. Therefore, we hypothesized that the upregulation of PTEN correlates with the impairment of cardiac function in swine following coronary microembolization (CME).

Methods: To possibly disclose an anti-inflammatory effect of PTEN, we induced swine CME by injecting inertia plastic microspheres (42 μ m in diameter) into the left anterior descending coronary artery and analyzed the myocardial tissue by immunochemistry, qRT-PCR and western blot analyses. In addition, we downregulated PTEN using siRNA. **Results:** Following CME, PTEN mRNA and protein levels were elevated as early as 3 h, peaked at 12 h, and then continuously decreased at 24 h and 48 h but remained elevated. Through linear correlation analysis, the PTEN protein level positively correlated with cTnI and TNF- α but was negatively correlated with LVEF. Furthermore, PTEN siRNA reduced the microinfarct volume, improved cardiac function (LVEF), reduced the release of cTnI, and suppressed PTEN and TNF- α protein expression. **Conclusion:** This study demonstrated, for the first time, that PTEN is involved in CME-induced inflammatory injury. The data generated from this study provide a rationale for the development of PTEN-based anti-inflammatory strategies.

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Introduction

Coronary microembolization (CME) is a major pathogenic outcome of acute myocardial infarction (MI) and is characterized by the spontaneous embolization of thrombotic material and debris or iatrogenic rupture of atherosclerotic plaque-induced obstructions of the coronary microvascular system [1-3]. Accordingly, the resulting coronary microcirculation dysfunction might be determined by perfusion-contraction mismatch, reactive hyperemia, and regional myocardial contractile dysfunction; however, myocardial function partially recovers within minutes [4, 5]. Many studies have shown that CME-induced microinfarcts initiate an inflammatory reaction with increased tumor necrosis factor- α (TNF- α) expression [6, 7], resulting in damage to the myocardium and deterioration of myocardial systolic function [8, 9]. Moreover, our previous studies also verified that microinfarction and ischemia in microembolized myocardium cause a severe inflammatory response with increased TNF- α expression in concert with the impairment of cardiac function [10, 11].

Phosphatase and tensin homolog deleted on chromosome ten (PTEN) dephosphorylates PI-3,4,5-trisphosphate (PIP3) to PI-4,5-bisphosphate (PIP2) and Pi to antagonize the actions of phosphatidylinositol (PI)-3-kinase (PI3K) [12]. Inhibition of PTEN by drugs or knockdown of cardiac-specific PTEN reinforces PI3K activity, inhibits apoptosis, reduces the infarct area, and ameliorates cardiac injury after ischemia-reperfusion [13]. Furthermore, inactivation of cardiac-specific PTEN protects against cardiac failure and myocardial fibrosis in a hypertensive mouse model [14]. PTEN expedites the inflammation response in post-MI remodeling but mitigates the inflammation response in vascular endothelial repair [9, 15]. Nevertheless, the role of PTEN in post-CME myocardial injury has not yet been studied.

Therefore, we focused on the dynamic changes in PTEN expression in myocardium post-CME and concluded that the causal relationship between PTEN and TNF- α exists to prevent post-CME myocardial injury.

Materials and Methods

Animal preparation

Forty miniature swine of either sex (body weight 25-30 kg) were supplied by the Animal Center of the College of Agriculture, Guangxi University (Nanning, People's Republic of China). Throughout all experimental stages, the animals were kept under controlled conditions for temperature, humidity, and light, with pig feed and water available ad libitum. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996). The Clinical and Animal Research Ethics Committees of Guangxi Medical University approved all procedures, and the number and suffering of the animals were minimized according to the guidelines of the American Physiological Society.

Swine models for CME and treatment groups

Fifty miniature swine of either sex (body weight 25-30 kg) were randomly assigned to ten groups as follows: the Sham surgery groups (n=20), which were euthanized at 3 h (n=5), 12 h (n=5), 24 h (n=5), and 48 h (n=5); the CME groups (n=20), which were euthanized at 3 h (n=5), 12 h (n=5), 24 h (n=5), and 48 h (n=5); the CME pre-treated with PTEN siRNA group, which was euthanized at 12 h (n=5); and the CME pre-treated with control siRNA group, which was euthanized at 12 h (n=5). The CME model was induced by manual unrelenting injection of microspheres into the left anterior descending (LAD) artery, as previously described by Carlsson et al. [16]. Briefly, the animals were initially sedated with intramuscular injection of a combination of ketamine (10-15 mg/kg) and atropine (2 mg). After endotracheal intubation, anesthesia was maintained by intravenous administration of diazepam (5 mg/h) into the ear vein. The right femoral artery was dissected, and a 6F vascular sheath (Johnson & Johnson-Cordis Corporation, Miami, FL, USA) was placed in the artery. Prior to coronary cannulation, the animals were anticoagulated with intravenous sodium heparin (200 U/kg), and an additional dose of 100 U/kg was given at hourly intervals to maintain heparinization. A 6F JL 4.0 guiding catheter was used for the left coronary artery system via the right femoral

approach for coronary angiography, and a 1.8F infusion catheter (Johnson & Johnson-Cordis Corporation, USA) was then placed in the LAD artery with the tip between the second and third diagonal branches. Microembolization was induced by manual unremitting injection of 100,000 microspheres with a diameter of 42 μm (Dynospheres; Dyno Particles, Lillestrøm, Norway) into the LAD artery within 40 minutes followed by a 10-mL saline flush. For the sham surgery, the identical procedure was performed, but normal saline was substituted for the microsphere suspension. The systemic blood pressure and heart rate were continuously monitored during the procedure. Twenty swine were subjected to sham surgery ($n=20$), and twenty swine underwent the previously described procedures to create a CME model ($n=20$). The swine from the CME or Sham surgery groups were euthanized at 3 h, 12 h, 24 h, and 48 h post-operatively, resulting in 4 subgroups each ($n=5$ each) and yielding 8 subgroups ($n=5$ each). Because all biomarkers in our preliminary experiment had the maximal alteration when animals were sacrificed at 12 h, the swine in the CME previously treated with PTEN siRNA ($n=5$) and CME previously treated with control siRNA ($n=5$) groups were minimized to five and sacrificed at 12 h. In vivo transfer was performed according to the method described previously [17]. Briefly, the animals in these two groups were handled exactly like the CME model swine, except that 100 μg of PTEN siRNA (PTEN-sus-368; GenePharma, Shanghai, China) or control siRNA (GenePharma) was diluted in the same volume of in vivo transfection reagent (EntransterTM-in vivo; Engreen, Beijing, China), mixed gently by pipetting the solution up and down, and selectively infused into the LAD artery within 1 minute with a flush of 2 mL of saline after the 1.8F infusion catheter (Cordis Inc., USA) was placed in the LAD artery. The CME group pre-treated with PTEN siRNA was then created 72 h after in vivo PTEN siRNA transfer.

Echocardiography

The animals were sedated as previously described and placed on the experimental platform in the right lateral position. One experienced investigator who was blinded to the study protocol performed the transthoracic echocardiography using a GE VIVID 7 system with a 1.5-4.3 MHz transducer. Briefly, the 1.5-4.3 MHz transducer was placed on the left anterior chest wall to obtain the left ventricle end-diastolic dimension (LVEDD), fractional shortening (FS), and cardiac output (CO), and the left ventricle ejection fraction (LVEF) was calculated using a cubic formula. All parameters were averaged from more than three consecutive cardiac cycles. After functional measurement, the animals were sacrificed by intravenous injection of 10 mL of 10% potassium chloride, and the hearts were fixed in 4% paraformaldehyde or quickly frozen at -80°C for use.

Coronary sinus levels of cTnI

EDTA-anticoagulated blood samples were collected from the coronary sinus before the animals were sacrificed. After collection, blood samples were immediately subjected to centrifugation at 4000 rpm for 15 min, and serum samples were stored at -80°C until postmortem analysis. Measurement of the serum cTnI concentration was performed using commercially available electrochemical luminescence kits according to the manufacturer's instructions (Roche, Inc., Switzerland). All measurements were determined in duplicate.

Tissue sampling

After blood sample collection, the hearts were arrested in diastole by the injection of 10 mL of 10% potassium chloride into the ear vein. The heart was immediately removed along with the atrial appendage and the atrium cordis, and the left ventricle was separated into apex and base segments from the midpoint of the left ventricle long axis in a plane parallel to the atrioventricular groove. The apex was rapidly frozen in liquid nitrogen and stored at -80°C until preparation for real-time PCR and western blot analysis. The base was fixed in 4% paraformaldehyde for 12 h, embedded with paraffin, and serially sectioned (4 μm thick; ≥ 12 sections per animal). Three sections containing the same number of microinfarct foci were selected and then stained using Mayer's hematoxylin and eosin (H&E). Hematoxylin-basic fuchsin picric acid (HBFP) staining was used to assess the myocardial microinfarction area on triplicate sections.

Measuring the myocardial microinfarction area

HBFP staining is an important method for the early diagnosis of myocardial ischemia. This stain dyes ischemic cardiac muscle, normal myocardial cytoplasm, and nuclei red, yellow, and blue, respectively. A DMR-Q550 pathological image pattern analysis instrument (Leica, Germany) was used to analyze the

HBFP-stained sections. Briefly, five microscopic visual fields (original magnification $\times 100$) were randomly sampled from each section for observation using the QWin analysis software (Leica, Germany), and the planar area method was used to measure the infarction zone, which was expressed as a percentage of the area of the bulk analysis section and averaged. The relative ischemic area was calculated according to the following formula: ischemic area/total area $\times 100\%$.

Real-time quantitative PCR analysis

Total RNA samples from cardiac tissue blocks were isolated using the TRIzol Reagent kit (GIBCO, USA) according to the manufacturer's protocols. Total RNA (4 μg) was then reverse transcribed using a Reverse Transcriptase Kit (Promega, USA) according to the manufacturer's instructions. Real-time PCR was performed on the ABI PRISM 7000 system (Applied BioSystems, CA, USA) using the sequence-nonspecific SYBR Green I dye (TaKaRa, Japan) for 40 cycles. PTEN and TNF- α in cardiomyocytes were detected using qRT-PCR analysis, with actin as an internal control. PCR was performed in a 25- μl reaction mixture containing 2 μl of cDNA, primers (0.5 μl each), and 12.5 μl of the SYBR Green Master Mix (Applied BioSystems, CA, USA), with water added to the final volume. PCR was initiated by an initial denaturation step at 95°C for 10 minutes; 40 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 15 seconds; a final extension at 95°C for 15 seconds; and then 1 cycle of dissociation at 60°C for 1 minute; and a final dissociation at 95°C for 15 seconds. The primers were 5-GAG CCA TTT CCA TCC TGC AG-3 and 5-GCT GTC ATG TCT GGG AGT CT-3 for swine PTEN, 5-GGC CCA AGG ACT CAG ATC AT-3 and 5-GCA TAC CCA CTC TGC CAT TG-3 for swine TNF- α , and 5-CAC CTT CTA CAA CGA GCT GC-3 and 5-TCA TCT TCT CAC GGT TGG CT-3 for actin (all primers were designed by TaKaRa, Japan). The transcript expression levels were quantified using the Ct value method, where values were normalized to actin as an internal control in the same sample. The specificity of the PCR product amplification was confirmed by their respective melting curves.

Western blot analysis

Tissue blocks (10 mg) of the ventricle anterior wall were homogenized in RIPA lysis buffer (50 mmol/L Tris HCl pH 7.4, 1% NP-40, 0.25% sodium deoxycholate, 150 mmol/L NaCl, 1 mmol/L EDTA, and protease and phosphatase inhibitors) on ice for 30 seconds. Tissue suspensions were incubated on ice for 10 minutes and centrifuged at 10,000 rpm for 10 minutes. Briefly, the protein concentrations were determined with a bicinchoninic acid protein assay kit using bovine serum albumin as the standard. Equal amounts of protein (100 μg) were fractionated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and blotted onto polyvinylidene (PVDF) membranes (Millipore, Bedford, MA, USA). The membranes were blocked for 2 h using 5% non-fat milk in Tris-buffered saline supplemented with Tween-20 (TBST) and probed overnight at 4°C with the following primary antibodies: PTEN (1:1000 dilution, Aviva Systems Biology), TNF- α (1:1000 dilution, Abcam), and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:1000 dilution, PTG). The membranes were then rinsed with TBST and incubated with horseradish peroxidase-conjugated secondary anti-GAPDH (1:8000 dilution, Alexa Fluor® 700 goat anti-rabbit IgG [H+L], or Alexa Fluor® 800 goat anti-rabbit IgG [H+L], Invitrogen) at room temperature for 1 h. Horseradish peroxidase-labeled anti-GAPDH (1:1000) served as an internal control. The blots were exposed to X-ray film, and the integrated absorbance of the identified bands was quantified using the Bio-Rad imaging software (Bio-Rad, Hercules, CA, USA).

Statistical analysis

All quantitative data are expressed as the means (SD) and were analyzed using the SPSS 13.0 software. Two-tailed, unpaired Student's t-tests, one-way ANOVA, and Pearson correlation analysis were used for statistical evaluation of the data. Differences were considered statistically significant when $p < 0.05$.

Results

Animal groups

No statistically significant differences ($p > 0.05$) in body weight or heart rate were identified between all groups (data not shown).

Table 1. Changes in cTnI before and after CME (ng/mL). Data are presented as the means (SD); a, $p < 0.05$ compared with the Sham group; b, $p < 0.05$ compared with the CME group at 12 h; c, $p < 0.05$ compared with pre-operation (0 h)

Parameters	0 h	3 h	12 h	24 h	48 h
Sham (cTnI)	0.041 (0.003)	0.043 (0.002)	0.042 (0.003)	0.042 (0.003)	0.043 (0.004)
CME (cTnI)	0.042 (0.002)	0.100 (0.003) ^{abc}	0.226 (0.013) ^{ac}	0.109 (0.002) ^{abc}	0.070 (0.004) ^{abc}

Table 2. Changes in cardiac function after CME. Each point represents the mean value (SD). The variables were measured in swine by echocardiography at 3 h, 12 h, 24 h, and 48 h after CME or Sham surgery. a, $p < 0.05$ compared with the Sham group; and b, $p < 0.05$ compared with the CME group at 12 h. LVEF, left ventricular ejection fraction; FS, fractional shortening; LVEDD, left ventricular end-diastolic diameters; CO, cardiac output

Variables	LVEF (%)	FS (%)	LVEDD (mm)	CO (L/min)
Sham				
3 h	67.10 (1.25)	43.22 (1.87)	31.56 (1.68)	4.56 (0.98)
12 h	66.76 (2.45)	41.35 (1.58)	32.82 (1.83)	4.29 (0.79)
24 h	67.63 (2.44)	42.84 (1.89)	31.54 (1.59)	4.46 (0.49)
48 h	67.48 (1.72)	40.38 (1.93)	32.84 (1.72)	4.29 (0.68)
CME				
3 h	56.73 (1.64) ^{ab}	31.56 (2.45) ^{ab}	36.19 (1.69) ^{ab}	3.32 (0.85) ^{ab}
12 h	48.14 (1.95) ^a	22.94 (1.54) ^a	39.38 (2.23) ^a	2.54 (0.63) ^a
24 h	58.77 (1.55) ^{ab}	33.27 (2.11) ^{ab}	35.31 (2.04) ^{ab}	3.28 (0.29) ^{ab}
48 h	61.80 (1.18) ^{ab}	37.63 (2.36) ^{ab}	34.21 (1.64) ^{ab}	3.35 (0.76) ^{ab}

Fig. 1. cTnI concentrations before (0 h) and after CME in the CME and Sham groups at 3 h, 12 h, 24 h, and 48 h. The arrow indicates the injection of embolizing microspheres or placebo into the CME or Sham groups, respectively. a, $p < 0.05$ compared with the Sham group; b, $p < 0.05$ compared with the CME group at 12 h.

Serial change of cTnI before and after CME

As shown in Table 1 and Fig. 1, cTnI was significantly higher in the CME group than in the Sham group at each time point post-operation. cTnI rapidly increased as early as 3 h following the surgery, increased to the highest level at 12 h, and then decreased continuously at 24 h and 48 h compared with the Sham group ($p < 0.001$).

Serial change of cardiac function after CME

Cardiac ultrasonography (Table 2) revealed that all parameters, except LVEDD, remained at a high level in the Sham group but were sharply reduced at 3 h and 12 h ($p < 0.05$) and continuously increased at 24 h and 48 h in the CME group ($p < 0.05$). However, compared with the Sham group, significant increases in LVEDD were observed from 3 to 12 h after CME ($p < 0.05$), and LVEDD decreased at 24 and 48 h ($p < 0.05$).

CME histopathology

Slight subendocardial ischemia without infarcts was observed in the Sham group (Fig. 2a). Multiple microinfarction regions were wedge-shaped, locally distributed, and nontransmural in the CME group (Fig. 2b), and these regions were mostly located in the subendocardium of the left ventricular anterior wall and apex of the heart. HE staining

Fig. 2. (a, b, c) HBFP staining showed no necrotic myocardium in the Sham group (a), patchy necrotic myocardium at 12 h after CME (b), and funiform necrotic myocardium when the CME-treated swine were pre-treated with PTEN siRNA (c). Necrosis is indicated by arrows. Normal myocardial cytoplasm, nuclei, and necrotic areas are stained yellow, blue, and red, respectively (HBFP staining, $\times 100$). Figure 2 (d, e) H&E staining showed myocardial necrotic foci with a high level of inflammatory cell infiltration 12 h after CME (d) and localized necrotic myocardium pre-transfected with PTEN siRNA after CME with low-level inflammatory cell infiltration (e). The arrow shows a 42- μ m microsphere following the experimental protocol (H&E staining; $\times 200$).

Fig. 3. (a, b) PTEN and TNF- α mRNA levels were measured in the CME and Sham groups at 3 h, 12 h, 24 h, and 48 h. The arrows indicate the injection of embolizing microspheres or placebo in (a) and (b), respectively. a, $p < 0.05$ compared with the Sham group; b, $p < 0.05$ compared with the CME group at 12 h.

revealed myocardial karyolysis or hypochromatosis with cytoplasmic red dyeing in the area of the microinfarction (Fig. 2d). Furthermore, peripheral cardiac muscle edema and denaturation, peripheral inflammatory cell infiltration, and erythrocyte effusion were also observed (Fig. 2d). The infarct areas after CME were 4.57 (1.38)%, 4.84 (1.27)%, 4.27 (1.31)% and 4.36 (1.58)% at 3 h, 12 h, 24 h, and 48 h, respectively, with no significant differences among the groups.

Fig. 4. (a, b) PTEN and TNF- α protein levels were determined using western blot analysis (normalized to GAPDH). Time course of PTEN and TNF- α protein expression in the CME and Sham groups at 3 h, 12 h, 24 h, and 48 h. * $p < 0.001$ vs. Sham.

Serial changes in PTEN and TNF- α mRNA expression after CME

PTEN mRNA expression was significantly increased in the CME group compared with the Sham group at the corresponding stages (Fig. 3a), with similar trends for the TNF- α mRNA (Fig. 3b). PTEN and TNF- α mRNA in the CME group rapidly increased as early as 3 h following the surgery, increased to the highest levels at 12 h, and then decreased continuously at 24 h and 48 h compared with the Sham group.

Serial changes in PTEN and TNF- α protein expression after CME

PTEN protein expression was significantly increased in the CME group compared with that in the Sham group at the corresponding stages (Fig. 4a), with a similar trend for TNF- α

Fig. 5. PTEN protein expression was positively correlated with TNF- α protein expression ($r=0.863$, $p<0.001$) and serum cTnI levels ($r=0.971$, $p<0.001$) based on Pearson correlation analysis but was negatively correlated with the LVEF ($r=-0.921$, $p<0.001$).

protein expression (Fig. 4b). PTEN and TNF- α protein levels rapidly increased as early as 3 h following the surgery, increased to their highest levels at 12 h, and then decreased continuously at 24 h and 48 h compared with the Sham group.

Pearson correlation analysis

Using Pearson correlation analysis, PTEN protein expression positively correlated with the TNF- α protein ($r=0.863$, $p<0.001$) and serum cTnI ($r=0.971$, $p<0.001$) levels. Additionally, PTEN protein expression negatively correlated with the LVEF ($r=-0.921$, $p<0.001$) (Fig. 5).

Serial changes in a variety of indicators after PTEN siRNA treatment

Table 3 shows that PTEN siRNA treatment reduced the size of the microinfarct area (Fig. 2c), improved cardiac function (LVEF) (Fig. 6b), reduced the release of myocardial injury markers (cTnI) (Fig. 6a), and suppressed CME-induced increases in PTEN and TNF- α mRNA and protein expression (Fig. 6c, d, 7).

Discussion

CME is widely observed with acute coronary syndrome and is considered an iatrogenic complication following coronary interventions in clinical settings [18]. It is thought that CME caused by the embolization of thrombotic material and debris or the rupture of an atherosclerotic plaque generates a transient decrease in coronary blood flow followed by reactive hyperemia and myocardial systolic dysfunction. Rioufol et al. [19] demonstrated

Table 3. Serial changes in a variety of indicators after PTEN siRNA treatment. Each point represents the mean (SD). a, $p < 0.05$ compared with the Sham group; b, $p < 0.05$ compared with CME/pre-treated with control siRNA

Parameters	Sham	CME	PTEN siRNA	Control siRNA
Microinfarct volume (%)	0.00	4.84 (1.27) ^a	2.21 (1.32) ^{ab}	4.62 (1.45) ^a
LVEF (%)	66.76 (2.45)	48.14 (1.95) ^a	61.23 (2.03) ^{ab}	49.12 (1.92) ^a
CTnI (ng/mL)	0.042 (0.003)	0.226 (0.013) ^a	0.087 (0.005) ^{ab}	0.218 (0.015) ^a
PTEN mRNA	0.42 (0.05)	3.08 (0.11) ^a	1.65 (0.08) ^{ab}	2.98 (0.12) ^a
TNF- α mRNA	1.84 (0.08)	4.84 (0.16) ^a	2.36 (0.12) ^{ab}	4.63 (0.17) ^a
PTEN protein	0.23 (0.03)	0.77 (0.07) ^a	0.33 (0.06) ^{ab}	0.73 (0.07) ^a
TNF- α protein	0.20 (0.05)	0.92 (0.06) ^a	0.38 (0.04) ^{ab}	0.87 (0.04) ^a

Fig. 6. Serum cTnI (a), PTEN mRNA (c), and TNF- α mRNA levels (d) were significantly increased in the CME and pre-treated with control siRNA groups compared with the Sham group ($p < 0.05$). Conversely, compared with the Sham group, the LVEF (%) was significantly decreased in the CME and pre-treated with control siRNA groups (b) ($p < 0.05$). Administration of PTEN siRNA reduced PTEN mRNA, TNF- α mRNA, and serum cTnI levels, but not the LVEF. * $p < 0.05$ vs. Sham; # $p < 0.05$ vs. CME/pre-treated with control siRNA.

that the formation of atherosclerosis frequently presented with the rupture and repair of plaques. Therefore, our data further confirmed the pathophysiological manifestations of CME.

Previous studies have demonstrated that the aggregate amount of infarction involved a small area (<5%) of microembolized myocardium in pigs or dogs, as indicated by the typical inflammatory responses with increases in TNF- α expression and leukocyte infiltration [6, 7, 20, 21]. Our preliminary studies showed that a post-CME inflammatory response occurred as a result of the upregulation of TNF- α expression in the myocardium [10, 11]. It has been well established that excessive TNF- α expression is derived from the surviving cardiomyocytes

Fig. 7. PTEN and TNF- α protein expression levels were determined using western blot analysis (normalized to GAPDH). PTEN (a) and TNF- α (b) protein levels were significantly increased in the CME and pre-treated with control siRNA groups compared with the Sham group ($p < 0.05$). However, administration of PTEN siRNA reduced the PTEN and TNF- α protein levels. * $p < 0.05$ vs. Sham; # $p < 0.05$ vs. CME/pre-treated with control siRNA.

surrounding microinfarcts in autocrine or paracrine patterns [6] and is responsible for the detrimental effect on progressive contractile dysfunction [6-8, 20, 22].

Our preliminary experiment revealed that TNF- α was activated after CME and that levels peaked 12 h after CME; thus, we adopted that time point in the present study. Moreover, we found that TNF- α expression in microembolized myocardium increased as early as 3 h post-CME, peaked at 12 h, and then decreased continuously at 24 h and 48 h compared with the Sham group. This dynamic change of TNF- α expression was prolonged up to 48 h in concert with the impairment of post-CME cardiac function, suggesting that TNF- α was markedly elevated at the acute phase and persistently decreased during the subacute and chronic phases, which accounts for the alteration in cardiac function. Twelve hours post-operatively, malignant arrhythmia and high mortality were frequently observed. These data provide a clue regarding the optimal time point for treating extremely severe CME-related complications.

PTEN has been identified as a tumor-suppressor gene and has been associated with maintaining the balance between cell survival and cell death [23]. Growing evidence has revealed that PTEN increases myocardial contractility and is involved in post-MI remodeling by inhibiting the activation of the PI3K/AKT signaling pathway, which is ubiquitously expressed in vascular endothelial cells, vascular smooth muscle cells, cardiomyocytes, and fibroblasts [24]. Partial inactivation of PTEN reduced leukocyte infiltration into the infarcted region. Conversely, PTEN overexpression yielded the opposite effect in mice [15]. However, the inflammatory response was driven by PTEN in rats with bacterial pneumonia rather than bronchial asthma [25]. Moreover, the enhancement of PTEN expression in the myocardium promoted the inflammatory response by upregulating TNF- α expression in post-MI remodeling [9]. Therefore, we concluded that PTEN regulates the post-CME inflammatory response through the upregulation of TNF- α expression.

In our current study, PTEN enhancement of TNF- α activity is required for CME-induced myocardial injury, which resembles the results reported by Skyschally et al. [9], and the positive correlation was confirmed by Pearson correlation analysis. Although the involvement of PTEN in the post-CME inflammatory response was crucial for progressive contractile

dysfunction, PTEN siRNA prevented the expression of myocardial injury markers (cTnI), reduced the microinfarct volume, restored cardiac function, and reduced PTEN and TNF- α mRNA and protein levels. The pernicious effect of PTEN on post-CME inflammatory injury and cardiac function deterioration was attributed to its upregulation of TNF- α expression.

In conclusion, PTEN plays an important role in the CME-induced inflammatory response, which can aggravate cardiac systolic dysfunction by increasing TNF- α expression; therefore, PTEN inhibition may have a protective effect. PTEN siRNA transfection reduced the microinfarct volume, improved cardiac function, and reduced cTnI release. Moreover, PTEN and TNF- α were markedly elevated at the acute phase within 12 h and persistently decreased during the subacute and chronic phases, which was in agreement with the impairment of post-CME cardiac function. Although our study only shows the dynamic changes of these post-CME parameters, it still gives us a potential mechanism through which to prevent CME. Further studies on the mechanism of PTEN in post-CME inflammatory injury still need to be performed.

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Disclosure Statement

The authors declare no conflicts of interest.

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