EGCG decreases myocardial infarction in both I/R and MIRI rats through reducing intracellular Ca^{2+} and increasing TnT levels in cardiomyocytes

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Abstract

Background. Myocardial ischemia/reperfusion injury (MIRI) usually induces serious health problems.

Objectives. This study attempted to explore protective effects of (—)-epigallocatechin-3-gallate (EGCG) on MIRI and the associated mechanism.

Materials and methods. Ischemia/reperfusion of an isolated rat heart (I/R model) and the MIRI model were used in this study. Myocardial infarction was measured with staining with 2,3,5-triphenyltetrazolium chloride (TTC). Ca^{2+} and troponin T (TnT) concentrations in coronary perfusion fluid were evaluated using the chromatometry method. Ca^{2+} concentration in cardiomyocytes was determined with detecting Ca^{2+} fluorescence intensity. The ultrastructure of cardiomyocytes was observed using transmission electron microscopy (TEM). β-nicotinamide adenine dinucleotide (NAD^+) of cardiomyocytes was also determined.

Results. The EGCG (I/R+EGCG) significantly reduced myocardial infarction size of isolated rat heart compared to I/R rats ($p < 0.05$), remarkably increased Ca^{2+} and decreased TnT concentrations in coronary perfusion fluid of I/R rats compared to the I/R model ($p < 0.05$), as well as markedly decreased intracellular Ca^{2+} concentration and promoted NAD^+ concentration in cardiomyocytes compared to I/R rats ($p < 0.05$). It also obviously maintained the mitochondrial structure in cardiomyocytes of I/R rats and improved the ultrastructure of cardiomyocytes of MIRI rats. Lonidamine (LND) treatment (I/R+EGCG+LND group) significantly blocked the effects of EGCG on I/R injury compared to the I/R+EGCG group ($p < 0.05$). The EGCG (MIRI+EGCG) significantly decreased myocardial infarction size compared to MIRI rats ($p < 0.05$) and remarkably enhanced Ca^{2+} and reduced TnT concentrations in the pulmonary artery compared to that of MIRI rats ($p < 0.05$).

Conclusions. The EGCG decreased myocardial infarction size in both I/R models and MIRI models by reducing intracellular Ca^{2+} concentration, increasing TnT concentration, promoting NAD^+ concentration, and improving the ultrastructure of cardiomyocytes.

Key words: myocardial infarction, protective effect, cardiomyocytes, EGCG, myocardial ischemia/reperfusion injury

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Background

Myocardial ischemia/reperfusion injury (MIRI) caused by cardiac surgery or myocardial infarction usually induces serious health problems.[1,2] It commonly accompanies coronary heart disorder and has been proven to be a leading factor for heart failure clinically.[3,4] It may also aggravate myocardial dysfunction and induce damage of cardiac cells, causing an overload of calcium, cell apoptosis and inflammatory response in heart tissues.[5] At present, there are many proven risk factors for MIRI, such as excess of reactive oxygen species (ROS) and release of inflammation-associated cytokines or factors, all of which eventually induce cardiomyocyte death resulting in damage to myocardial functions.[6,7] Although plenty of research[8,9] has reported strategies for preventing MIRI and decreasing myocardial infarct size, the clinical outcomes or efficacy for animal models are still unsatisfactory. Therefore, it is critical to discover a promising strategy for preventing MIRI with clinical applications.

Objectives

The (–)-epigallocatechin-3-gallate (EGCG) is an important bioactive ingredient derived from green tea, which has anti-oxidant and free radical scavenging properties.[10,11] Previous studies[10,12] have reported that EGCG plays a series of cardiovascular protective roles, including alleviating heart ischemia/reperfusion (I/R) injury, reducing myocardial ischemia associated dysfunction of the heart, and protecting cardiac muscle of ischemic heart in vivo and in vitro. Kim et al.[13] reported that EGCG modulated Ca^{2+} influx by eliciting extracellular Ca^{2+} in cells. The present study attempted to determine the protective effects of EGCG on I/R injury of the heart and clarify whether Ca^{2+} influx is involved in the protective effect of EGCG.

Materials and methods

Animals

Twenty-four specific pathology-free (SPF) rats weighing 230 ±20 g were provided by the Experimental Animal Department of Zunyi Medical College, China. All rats had free access to water and food and were housed at 25°C with a light/dark cycle of 12 h/12 h. All of the animal experiments or tests complied with the National Institutes of Health (NIH) Guidelines for Usage of Laboratory Animals revised in 1996. This study was approved by the Ethical Committee of The Third Affiliated Hospital of Zunyi Medical University (The First People's Hospital of Zunyi), China.

Ischemia/reperfusion model of isolated rat heart (I/R model)

The ischemia/reperfusion model of isolated rat heart (I/R model) of isolated rat heart was generated as previously described,[14] with some modifications. Twelve Sprague Dawley rats were anesthetized by intraperitoneal injection with pentobarbital sodium (50 mg/kg body weight) and treated with heparin (3000 U/kg body weight) to establish the I/R model. Post-anesthesia, the anterior abdominal wall, diaphragm, and chest were fully cut open to expose the heart. About 5–6 mm remote from the beginning of the aorta, the aorta and the other blood vessels were cut off, and the heart was quickly harvested and pre-cooled at 4°C in KH buffer. Next, the heart was suspended on a Langendorff system through the aortic cannula and fixed with wires. A mixture of 95% O_2 and 5% CO_2 (37°C) was continuously injected into the KH buffer and the heart was perfused at a constant rate of 8 mL/min in a non-circular manner. A small incision was made above the left auricle, and a latex balloon connected to the multi-channel physiological recorder was inserted into the left ventricle through the left atrium and mitral valve. The end diastolic pressure of the left ventricle was adjusted between 8 mm Hg and 12 mm Hg. For the whole process, the volume of the balloon was kept constant. The whole operation, starting from the heart in vitro to the beginning of perfusion, should be completed within 1 min. Attention is needed to prevent air and tissue fragments from entering the coronary artery and causing embolism. The heart started beating a few seconds after perfusion began. The KH buffer was used to balance perfusion for 15 min, and treatment was then performed according to the trial grouping scheme described in the Trial groups and drug regimen subsection. Finally, the heart was ischemic for 30 min and reperfused for 120 min.

MIRI rat model

The MIRI rat model was created as described in a previous study[15] with a few modifications. In brief, a total of 6 Sprague Dawley rats were anesthetized by intraperitoneal injection with pentobarbital sodium (50 mg/kg body weight), intubated using 16-gauge cannulas and connected to ventilators (Zhenghua Bio. Tech., Anhui, China). The MIRI was generated by carrying out coronary artery ligation. Through the left thoracotomy, the heart of the rat was fully exposed to demonstrate the left anterior descending (LAD) coronary artery, which was then ligated for 30 min and followed by 120 min of reperfusion.

Trial groups and drug regimen

There were 3 groups of rats in this study: 12 I/R rats, 6 MIRI rats and 6 control (or sham) rats (a total of 24 rats).

The I/R rats were subdivided into an I/R group (n = 3), I/R+EGCG group (n = 3; I/R rats were perfused with 10 mg

of EGCG for 30 min, induced to global ischemia for 30 min and reperfused for 120 min), I/R+lonidamine (LND) group (n = 3; I/R rats were induced to global ischemia for 30 min, perfused with 30 μM LND for 30 min and reperfused for 120 min), and I/R+EGCG+LND group (n = 3; I/R rats were perfused with 10 mg of EGCG for 30 min, induced to global ischemia for 30 min, perfused with 30 μM LND for 20 min, and reperfused for 120 min).

The MIRI rats were divided into a MIRI group (n = 3; as described above) and MIRI+EGCG group (n = 3; 30 min before MIRI modeling, the femoral vein was injected with EGCG at a dosage of 10 mg/kg body weight).

The control group was also divided into 2 subgroups. Three rats control group rats were perfused with KH buffer, while 3 other were perfused with normal saline.

Measurement of myocardial infarction

Hearts of the rats were rapidly injected with 1% 2,3,5-triphenyltetrazolium chloride (TTC; Sigma-Aldrich, St. Louis, USA; pH 7.4) at 120 min of reperfusion and then stained for 15 min in a 37°C water bath in the dark. At the end of staining, the dye was stopped by washing with distilled water and then the heart was refrigerated at –20°C for 20 min. After fixation, the heart was cut into sections of a 1–2-millimeter thickness. The myocardial infarction area appeared grayish white, while the non-myocardial infarction area was dark red. Finally, myocardial infarction was analyzed using Image Pro-Plus v. 6.0 image analysis software (Media Cybernetics Inc., Bethesda, USA). Myocardial infarction in the rats was represented as the ratio of infarct area (IA)/ischemic area at risk (AAR).

Evaluation of Ca^{2+} and troponin T concentrations in coronary perfusion fluid

The Ca^{2+} and troponin T (TnT) concentrations in coronary perfusion fluid were evaluated using the chromatometry method in this study. A total of 1 mL of coronary perfusion fluid of the rats in each group was collected, treated with anticoagulant, and centrifuged at 4°C and 1000 rpm for 8 min. The isolated serum was stored at –70°C until the experiment. The Ca^{2+} concentration in coronary perfusion fluid was examined using the calcium colorimetric assay kit (Cat. No. K380-250; BioVision, Milpitas, USA) as instructed by the manufacturer. The TnT concentration in coronary perfusion fluid was examined using the TnT assay kit (Cat. No. 48T/96T; RapidBio, Calabasas, USA) according to the protocol of the manufacturer.

Determination of Ca^{2+} concentration in cardiomyocytes

Cardiomyocytes were obtained by homogenizing the heart tissues. After culture of the cardiomyocytes, the medium was removed and washed with Hank's balanced salt solution (HBSS; Gibco, Grand Island, USA). Cardiomyocytes were incubated with Fluo-4/AM (at a concentration of 20 μM; Cat. No. S1060; Beyotime, Shanghai, China) and Pluronic F-127 (at a concentration of 1 μM; Cat. No. ST501; Beyotime) at 37°C for 20 min. Next, cells were washed twice with phosphate-buffered saline (PBS) and cultured for 20–30 min again to confirm that Fluo-4/AM was completely transformed into Fluo-4. The green fluorescence intensity of the cardiomyocytes was detected using laser scanning confocal microscopy (ELX800; Bio-Tek Inc., Winooski, USA). A total of 25 cardiomyocytes were randomly selected as the regions of interest (ROIs). The green fluorescence intensity was analyzed using Las AF software (Leica, Wetzlar, Germany) to evaluate the change in intracellular calcium concentration. The fluorescence intensity of the cardiomyocytes in the control group was assigned a value of 100%. The Ca^{2+} fluorescence intensity in each experimental group was represented as the percentage of the fluorescence intensity of cardiomyocytes.

Measurement of NAD^+

The NAD^+ was measured using the commercial NAD^+/NADH cell-based assay kit (Cat. No. 600480-1; Cayman Chemical, Ann Arbor, USA) as instructed by the manufacturer. In brief, heart was homogenized using the NAD^+ extraction buffer (at a concentration of 100 μL/5 mg heart tissues). The resulting heart extracts were incubated in a 60°C water bath for 5 min, treated with NADH extraction buffer (100 μL) and then centrifuged for 5 min at 12,000 rpm. The supernatants were subsequently mixed with the cell-based assay alcohol dehydrogenase and cell-based assay NAD^+ diaphorase. The optical density at a wavelength of 565 nm was measured and recorded at 0 min (OD0) and 15 min (OD15) using a spectrophotometer. The standard curve was drawn and the content of NAD^+ was analyzed. The NAD^+ concentration was calculated using the following formula: NAD^+ (nM) = [(corrected absorbance-(y-intercept))/slope].

Transmission electron microscopy (TEM)

The cardiomyocytes were fixed using 2.5% glutaraldehyde (Cat. No. G5882; Sigma-Aldrich) at 4°C for 2 h and 1% osmium tetrachloride (Cat. No. S837067; Sigma-Aldrich) at 4°C for 25–30 min, and then washed using PBS 2 times (10 min each time). Subsequently, the cardiomyocytes were dehydrated using a graded series of acetone solutions (50% acetone 1 time, 70% acetone 1 time, 90% acetone 2 times, and 100% acetone 3 times, 12 min per time), cleared in propylene oxide solution, embedded in 3–4 mL of acetone-EPON812-embedding agent for 30 min, and then in pure-embedding agent for 2 h for complete embedding. The embedded samples were baked at 60°C for 24 h to obtain the embedded hard blocks, which were

then cut into sections with a thickness of 1 μm and dried. The sections were stained using methylene blue dye solution (Cat. No. M9140; Sigma-Aldrich) at 60°C for 30 s and then complex dye solution (0.25% sodium borate : 0.5% basic fuchsin = 1 : 1) for 10 s. Subsequently, the sections were re-cut into ultra-thin sections with a thickness of 50 nm. The ultra-thin sections were laid onto 0.45% Formvar pretreated copper grids and stained with uranyl acetate dye solution for 10 min and lead dye solution for 12 min at room temperature. Finally, the ultra-thin sections were observed under a Philips TECNAI-10 transmission electron microscope (TEM; Philips, Amsterdam, the Netherlands).

Statistical analyses

Data are reported as mean ± standard deviation (SD) and analyzed using IBM SPSS software v. 20.0 (IBM Corp., Armonk, USA). Differences between data were analyzed using analysis of variance (ANOVA) with the Bonferroni post hoc test. A p-value <0.05 indicated statistically significant differences.

Results

EGCG reduced myocardial infarction size of the I/R model of isolated rat heart

Myocardial infarction was determined in the I/R model of isolated rat heart (Fig. 1A). The results indicated that the myocardial infarction size of the I/R group was significantly increased compared to the control group (p = 0.000). We found that EGCG treatment (I/R+EGCG group) remarkably reduced the myocardial infarction size compared to the I/R group (Fig. 1B, p = 0.001). Furthermore, LND treatment (I/R+EGCG+LND group) blocked the reductive effects of EGCG (I/R+EGCG group) on myocardial infarction size of the I/R model of isolated rat heart (Fig. 1B, p = 0.003).

EGCG increased Ca^{2+} and decreased TnT concentrations in the coronary perfusion fluid of I/R rats

The results showed that the Ca^{2+} concentration of the I/R group was significantly lower than in the control group (Fig. 2A, p = 0.000). However, EGCG treatment (I/R+EGCG group) remarkably increased Ca^{2+} concentration compared with the I/R group (Fig. 2A, p = 0.000). The LND treatment (I/R+EGCG+LND group) significantly decreased Ca^{2+} concentration compared to the I/R+EGCG group (Fig. 2A, both p = 0.000).

At the same time, TnT concentration for the I/R group was significantly higher compared to the control group (Fig. 2B, p = 0.000). However, EGCG treatment (I/R+EGCG group) significantly decreased TnT concentration compared with the I/R group (Fig. 2B, p = 0.000). The LND+EGCG treatment (I/R+EGCG+LND group) significantly increased TnT concentration compared to the I/R+EGCG group (Fig. 2B, both p = 0.000).

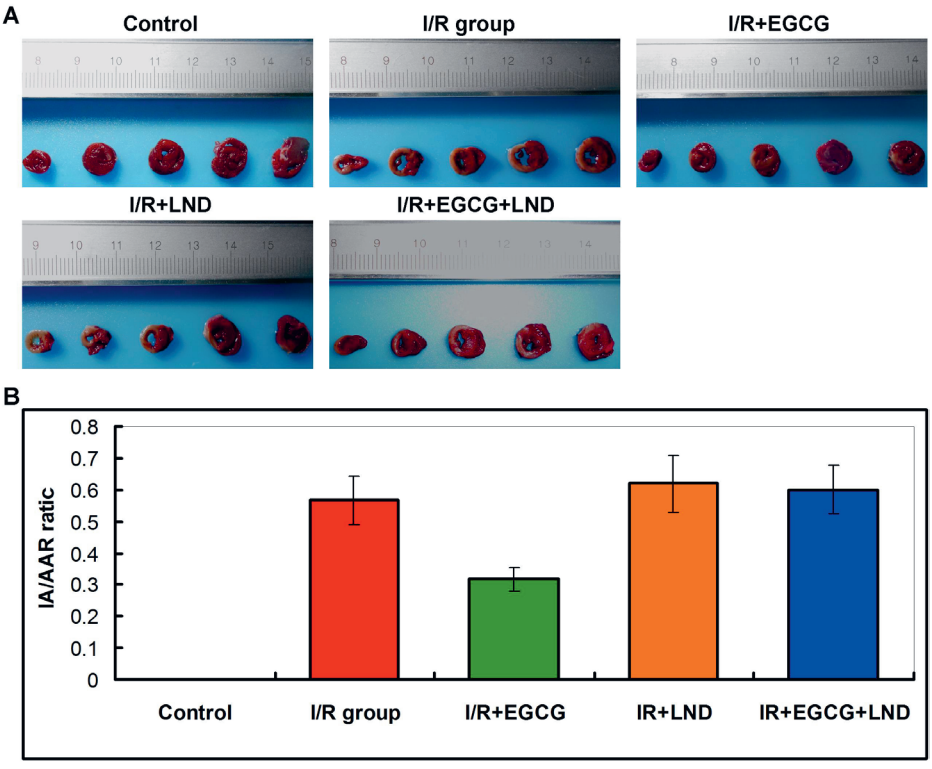

Fig. 1. Effects of EGCG treatment on myocardial infarction in the I/R model of isolated rat heart. A. Images of myocardial infarction in I/R rats undergoing EGCG and/or LDN treatment; B. Determination of the effects of EGCG and/or LDN treatment on myocardial infarction size (IA/AAR ratio) according to statistical analysis. For the I/R group compared to the control group p = 0.000; for the I/R+EGCG group compared to the I/R group p = 0.001; for the I/R+EGCG+LND group compared to the I/R+EGCG group p = 0.003

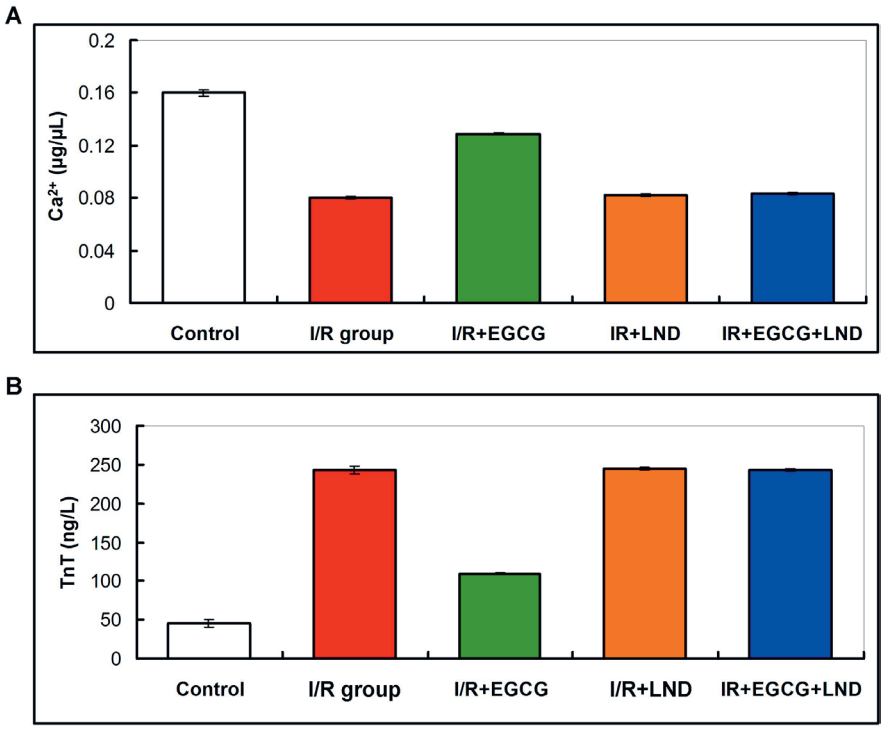

Fig. 2. Enhancive effects of EGCG on Ca^{2+} concentration and reductive effects of EGCG on TnT concentration in the coronary perfusion fluid of I/R rats. A. EGCG treatment enhanced Ca^{2+} concentration in I/R rats. For the I/R group compared to the control group p = 0.000; for the I/R+EGCG group compared to the I/R group p = 0.000; for the I/R+EGCG+LND group compared to the I/R+EGCG group p = 0.000; B. EGCG treatment decreased TnT concentration in I/R rats. For the I/R group compared to the control group p = 0.000; for the I/R+EGCG group compared to the I/R group p = 0.000; for the I/R+EGCG+LND group compared to the I/R+EGCG group p = 0.000

EGCG decreased intracellular Ca^{2+} concentration in cardiomyocytes of I/R rats

Intracellular Ca^{2+} in cardiomyocytes was evaluated using green fluorescence staining in this study (Fig. 3A). The fluorescence findings showed that the intracellular Ca^{2+} concentration in cardiomyocytes of the I/R group was significantly increased compared with the control group (Fig. 3B, p = 0.000), but decreased in the EGCG treatment group (I/R+EGCG group) compared with the I/R group (Fig. 3B, p = 0.000). The LND treatment (I/R+LND group) also increased Ca^{2+} concentration compared with the I/R group (Fig. 3B, p = 0.000). In addition, LND+EGCG treatment (I/R+EGCG+LND group) significantly increased Ca^{2+} concentration compared to the I/R+EGCG group (Fig. 3B, p = 0.000).

EGCG promoted NAD^+ concentration in the cardiomyocytes of I/R rats

According to the NAD^+ measurement findings, rats in the control group exhibited the highest concentration of NAD^+ (Fig. 4). The NAD^+ concentration in the I/R+EGCG group was significantly higher than the I/R group (Fig. 4, p = 0.003). The LND treatment (I/R+LND group) slightly increased NAD^+ concentration compared to the I/R group (Fig. 4, p = 0.113). However, LND+EGCG treatment (I/R+EGCG+LND group) significantly decreased NAD^+ concentration compared to the I/R+EGCG group (Fig. 4, p = 0.006).

EGCG maintained mitochondrial structure in cardiomyocytes of I/R rats

Cardiomyocytes derived from I/R rats were pre-treated using EGCG and/or LND. As shown in Fig. 5, cardiomyocytes derived from rats in the control group demonstrated tightly arrayed cristae structures; by contrast, after I/R injury, the mitochondrial structures were destructed and vacuoles were formed (Fig. 5). However, EGCG treatment (I/R+EGCG group) remarkably improved the mitochondrial structures and only a few smaller vacuoles were observed (Fig. 5). Moreover, there were no obvious improvements in mitochondrial structures in the I/R+LND and I/R+EGCG+LND groups (Fig. 5).

EGCG decreased myocardial infarction size in the MIRI rat model

The myocardial infarction size in MIRI rats was also determined in this study (Fig. 6A). Statistical analysis showed no infarction in the sham group rats (Fig. 6B). The myocardial infarction size (IA/AAR ratio) in the MIRI group was significantly higher compared to the sham group (Fig. 6, p = 0.000). The EGCG treatment (MIRI+EGCG group) remarkably decreased the IA/AAR ratio compared to that of the MIRI group (Fig. 6, p = 0.019).

EGCG enhanced Ca^{2+} and reduced TnT concentrations in the pulmonary artery of MIRI rats

At 120 min post-perfusion, the Ca^{2+} and TnT concentrations in the pulmonary artery of MIRI rats were measured.

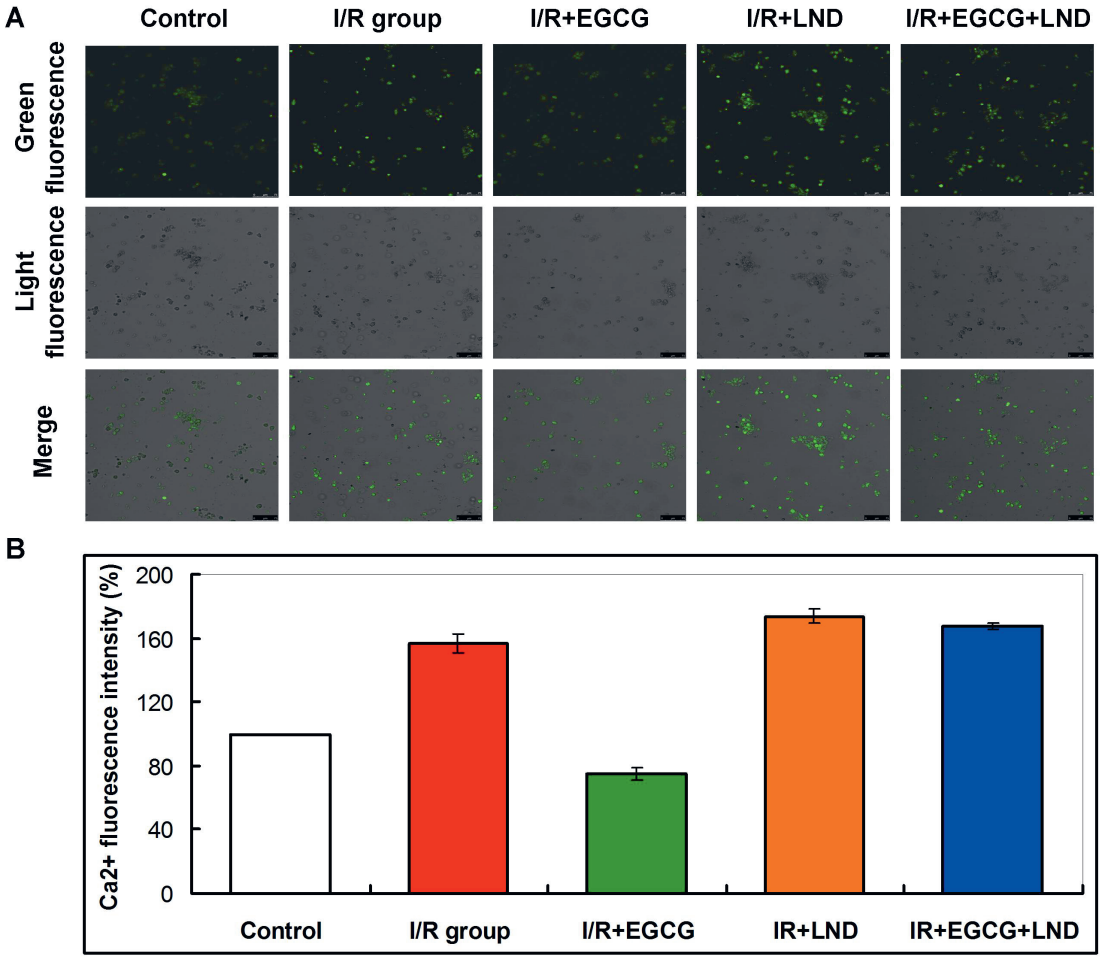

Fig. 3. EGCG decreased intracellular Ca^{2+} concentration in the cardiomyocytes of I/R rats according to Ca^{2+} fluorescence staining results. A. Green fluorescence staining for Ca^{2+} in the cardiomyocytes of I/R rats. Green fluorescence-stained cells represent Ca^{2+} in cardiomyocytes; B. Statistical analysis of Ca^{2+} concentration. For the I/R group compared to the control group p = 0.000; for the I/R+EGCG group compared to the I/R group p = 0.002; for the I/R+EGCG+LND group compared to the I/R+EGCG group p = 0.006

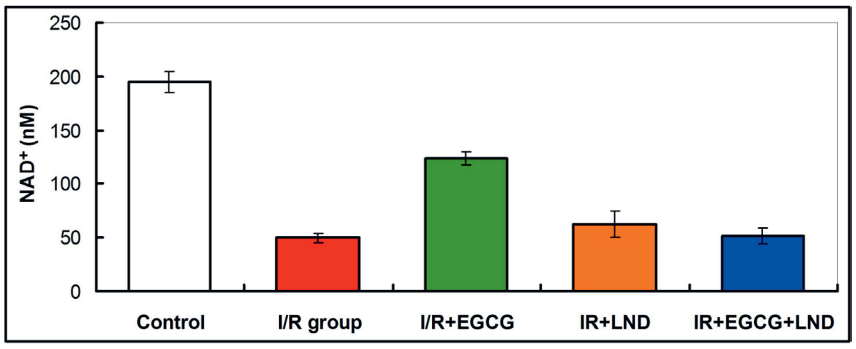

Fig. 4. Evaluation of NAD^+ concentration in the cardiomyocytes of I/R rats administered EGCG and/or LND. For the I/R group compared to the control group p = 0.000: for the I/R+EGCG group compared to the I/R group p = 0.000; for the IR+LDN group compared to the I/R group p = 0.113; for the IR+EGCG+LND group compared to the I/R+EGCG group p = 0.000

The results showed that the Ca^{2+} concentration was significantly lower (Fig. 7A, p = 0.000) and the TnT concentration was significantly higher (Fig. 7B, p = 0.000) in the MIRI group compared to the sham group. However, the EGCG treatment (MIRI+EGCG group) obviously enhanced the Ca^{2+} concentration (Fig. 7A, p = 0.000) and significantly reduced the TnT concentration (Fig. 7B, p = 0.001) compared to the MIRI group.

EGCG improved the ultrastructure of cardiomyocytes of MIRI rats

Cardiomyocytes derived from sham group rats demonstrated a normal and tightly arrayed ultrastructure (Fig. 8). However, the ultrastructure of cardiomyocytes from MIRI rats was destroyed (Fig. 8). Interestingly, EGCG treatment (I/R+EGCG group) significantly improved the ultrastructure of cardiomyocytes (Fig. 8).

Discussion

Clinically, blood flow restoration via the coronary artery can reduce the infarct area and aggravate the outcome post-myocardial infarction; however, reperfusion usually induces damage for the ultrastructure of cardiomyocytes in cell metabolism diseases, followed by inflammation of heart tissues.[6,16] The present research discovered that EGCG can

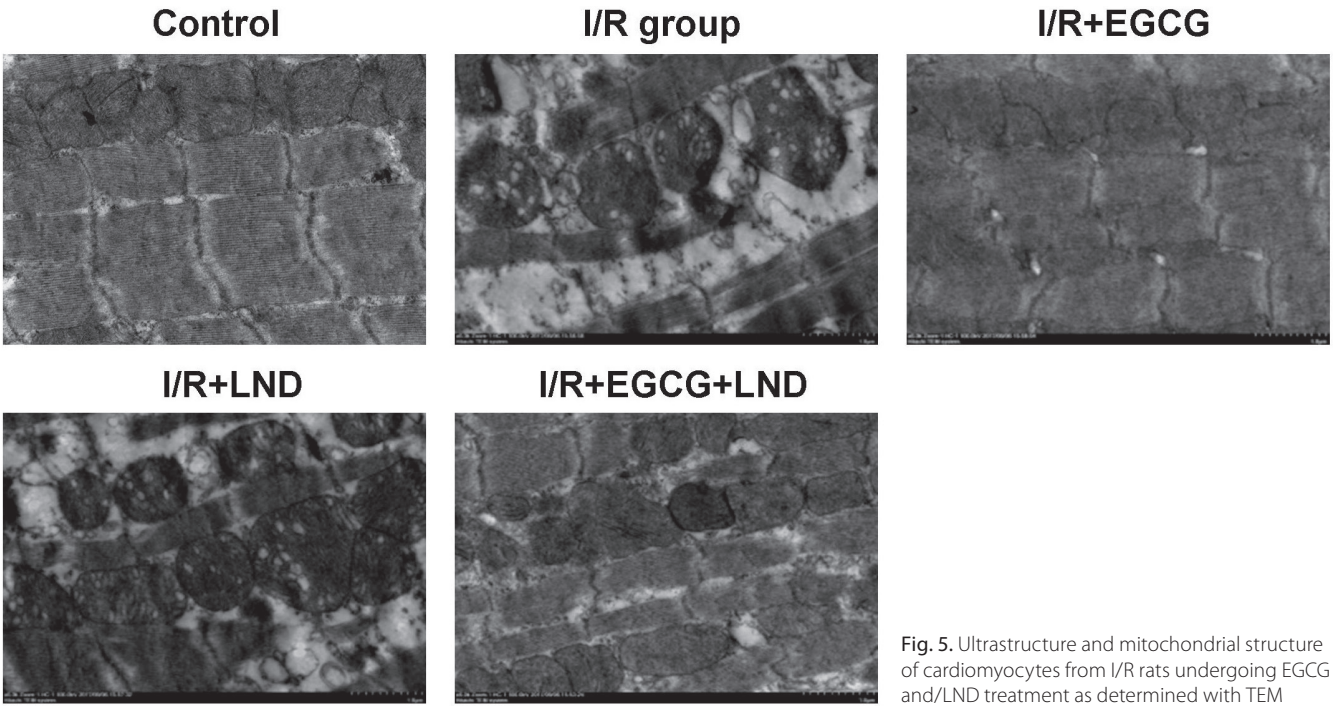

Fig. 5. Ultrastructure and mitochondrial structure of cardiomyocytes from I/R rats undergoing EGCG and/LND treatment as determined with TEM

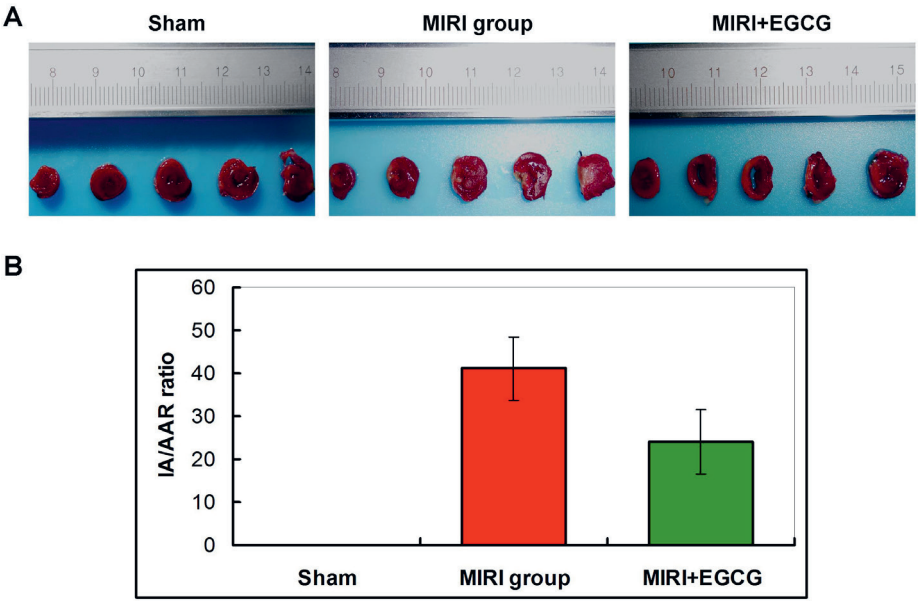

Fig. 6. EGCG treatment decreased myocardial infarction in MIRI rats. A. Images of myocardial infarction in MIRI rats undergoing EGCG treatment; B. Statistical analysis of the effects of EGCG treatment on myocardial infarction size (IA/AAR ratio) in MIRI rats. For the MIRI group compared to the sham group p = 0.000; for the MIRI+EGCG group compared to the MIRI group p = 0.019

significantly inhibit myocardial infarction in I/R or MIRI rats by modulating Ca^{2+} and TnT levels in cardiomyocytes.

The EGCG, which is characterized by a series of biological and pharmacological properties, plays promising protective roles in cardiovascular disorders.[11,17] However, previous studies[18,19] have not clarified the specific mechanisms of the protective functions of EGCG on myocardial I/R injury. Therefore, the present research attempted to explore the protective effects of EGCG on myocardial infarction of rat hearts using both I/R models and MIRI models.

In the present research, both I/R models and MIRI models were created as described in previous studies.[14,15] The treatment dose was 10 mg of EGCG for perfusing the heart for 30 min in both I/R and MIRI rats, which was determined from pre-experimental findings of animal studies by our team. Our findings showed that both I/R and MIRI rats demonstrated serious myocardial infarction compared with rats in the control/sham group. Interestingly, EGCG treatment significantly inhibited myocardial infarction in both I/R and MIRI rats, which suggests that EGCG plays critical roles in protecting against I/R injury. For the I/R and MIRI models used in this study, we must acknowledge that the sample size (rat numbers) of each group is relatively small, which is a limitation of this study.

Previous studies[20,21] have reported that myocardial I/R injury is related to the overload of intracellular Ca^{2+}.

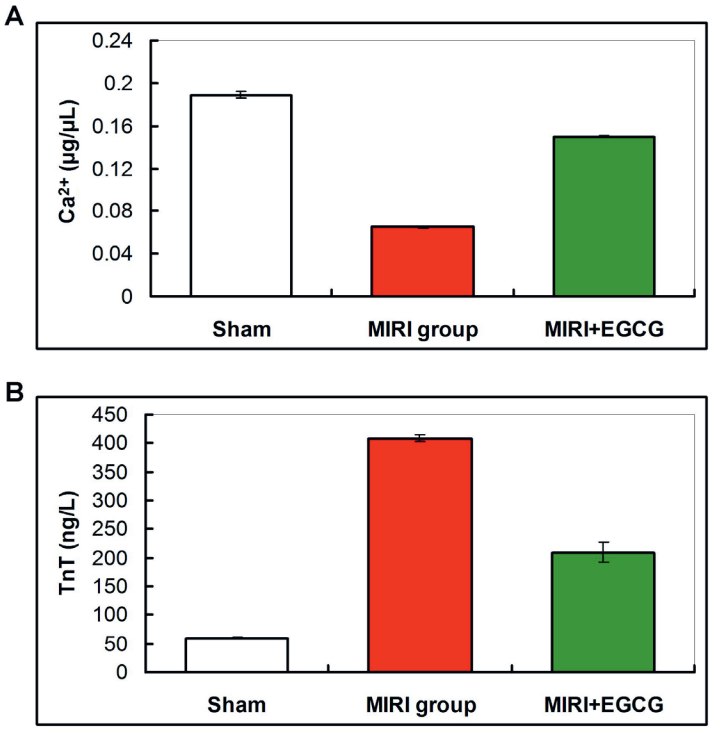

Fig. 7. Ca^{2+} was enhanced and TnT was reduced in the pulmonary artery of MIRI rats undergoing administration of EGCG. A. EGCG administration enhanced Ca^{2+} concentration in MIRI rats. For the MIRI group compared to the sham group p = 0.000; for the MIRI+EGCG group compared to the MIRI group p = 0.000; B. EGCG administration decreased TnT concentration in MIRI rats. For the MIRI group compared to the sham group p = 0.000; for the MIRI+EGCG group compared to the MIRI group p = 0.001

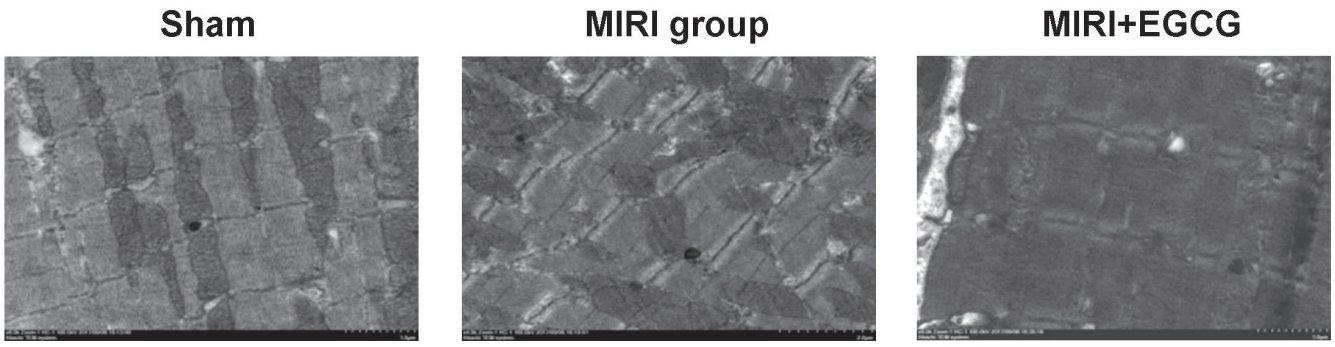

Fig. 8. TEM images illustrating the ultrastructure of cardiomyocytes in MIRI rats

Intracellular Ca^{2+} homeostasis maintains the cardiac functions of the heart, and imbalance of homeostasis is commonly correlated with I/R associated injury.[22] Therefore, we examined the Ca^{2+} levels in coronary perfusion fluid of I/R rats and intracellular Ca^{2+} levels in cardiomyocytes of MIRI rats. The results showed that EGCG treatment significantly increased Ca^{2+} concentration in coronary perfusion fluid of I/R rats and enhanced Ca^{2+} concentration in the pulmonary artery of MIRI rats. Moreover, we found that EGCG decreased the intracellular Ca^{2+} concentration in cardiomyocytes of I/R rats. The EGCG modulated concentration changes of Ca^{2+} suggest that EGCG treatment significantly inhibited the overload of intracellular Ca^{2+} in the cardiomyocytes of I/R rats and MIRI rats. Furthermore, we found that EGCG enhanced the intracellular concentration of TnT, which is a molecule reflecting cardiac functions,[23,24] in cardiomyocytes of I/R and MIRI rats. Our findings show that EGCG treatment significantly decreased TnT concentration in the coronary perfusion fluid of I/R rats and increased intracellular TnT concentration in the pulmonary artery of MIRI rats, which hint that EGCG treatment remarkably improved heart functions in I/R and MIRI rats.

The content of NAD^+ in the myocardium can reflect the opening of mitochondrial permeability transition pores (mPTP): the lower the content of NAD^+, the greater the degree of mPTP opening and the more serious the myocardial injury.[25] Thus, we evaluated NAD^+ levels in the cardiomyocytes of both EGCG-administered I/R rats and MIRI rats. The results illustrated that EGCG treatment promoted NAD^+ concentration in the cardiomyocytes of I/R rats, which suggests that the I/R model-induced myocardial injury was suppressed and cardiac functions were improved. In fact, during myocardial I/R injury, excessive Ca^{2+} is produced and transferred into the mitochondrial matrix, which then causes mPTP opening, production of ROS and ultimately cardiac dysfunction.[26] Our findings prove that EGCG-induced NAD^+ changes are consistent with variation in intracellular Ca^{2+} in the cardiomyocytes of I/R and MIRI rats. The EGCG also maintained the ultrastructure

of mitochondria in cardiomyocytes of I/R and MIRI rats, which suggests that EGCG-triggered cardiac function improvement also involves improvement in the ultrastructure of mitochondria. However, the specific mechanism has not been clarified in this study. Moreover, LND participates in the modulation of conventional chemotherapy and radiotherapy for tumors.[27] In recent years, LND, as a mPTP opener, has been proven to reduce cardiac protection in I/R injury.[28] Therefore, LND was employed as a negative regulator for improvement of I/R injury in this study. However, we found no or only slight effects of LND treatment on myocardial infarction size, intracellular Ca^{2+}, decreased TnT concentration, and the ultrastructure of cardiomyocytes in I/R isolated heart rat models. Moreover, LND treatment significantly blocked the effects of EGCG on cardiac protection in the I/R rat models. This result suggests that EGCG might modulate improvement in I/R injury mediated by mPTP pathways, which should be explored in future research.

Limitations

The sample size in this study was relatively small, but it would be enlarged in the further study. Moreover, except for the Ca^{2+} and TnT participating in EGCG effects, the specific molecule signaling pathway has not been clarified in this study.

Conclusions

EGCG treatment decreased myocardial infarction size in both I/R and MIRI rat models by decreasing intracellular Ca^{2+} concentration, increasing TnT concentration, promoting NAD^+ concentration, and improving the ultrastructure of cardiomyocytes. The EGCG treatment used in this study is a potential source of therapeutic strategies for ischemic stroke-associated diseases.

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References

1. Bartekova M, Barancik M, Ferenczyova K, Dhalla NS. Beneficial effects of N-acetylcysteine and N-mercaptopropionylglycine on ischemia reperfusion injury in the heart. *Curr Med Chem*. 2018;25(3):355–366. doi:10.2174/0929867324666170608111917
2. Kunecki M, Roleder T, Biernat J, et al. Opioidergic conditioning of the human heart muscle in nitric oxide-dependent mechanism. *Adv Clin Exp Med*. 2018;27(8):1069–1073. doi:10.17219/acem/70192
3. Qiao X, Jia S, Ye J. PTPIP51 regulates mouse cardiac ischemia/reperfusion through mediating the mitochondria-SR junction. *Sci Rep*. 2017;7:45379. doi:10.1038/srep45379
4. Ferdinandy P, Schulz R, Baxter GF. Interaction of cardiovascular risk factors with myocardial ischemia/reperfusion injury, preconditioning, and postconditioning. *Pharmacol Rev*. 2007;59(4):418–458. doi:10.1124/pr.107.06002
5. Li Y, Wang X, Lou C. Gastrodin pretreatment impact on sarcoplasmic reticulum calcium transport ATPase (SERCA) and calcium phosphate (PLB) expression in rats with myocardial ischemia reperfusion. *Med Sci Monit*. 2016;22:3309–3315. doi:10.12659/MSM.896835
6. Han MX, Xu XW, Lu SQ, Zhang GX. Effect of olprinone on ischemia-reperfusion induced myocardial injury in rats. *Biomed Pharmacother*. 2019;111:1005–1012. doi:10.1016/j.biopha.2019.01.010
7. Kwak W, Ha YS, Soni N, et al. Apoptosis imaging studies in various animal models using radio-iodinated peptide. *Apoptosis*. 2014;20(1):1–12. doi:10.1007/s10495-014-1059-z
8. Translating cardioprotection for patient benefit: Position paper from the working group of cellular biology of the heart of the European Society of Cardiology. *Cardiovasc Res*. 2013;98(1):7–27. doi:10.1093/cvr/cvt004
9. You L, Pan YY, An MY, et al. The cardioprotective effects of remote ischemic conditioning in a rat model of acute myocardial infarction. *Med Sci Monit*. 2019;25:1769–1779. doi:10.12659/MSM.914916
10. Hsieh SR, Hsu CS, Lu CH, Chen WC, Chiu CH, Liou YM. Epigallocatechin-3-gallate-mediated cardioprotection by Akt/GSK-3beta/caveolin signaling in H9C2 rat cardiomyoblasts. *J Biomed Sci*. 2013;20(1):86. doi:10.1186/1423-0127-20-86
11. Wu Y, Xia ZY, Zhao B, et al. (–)-epigallocatechin-3-gallate attenuates myocardial injury induced by ischemia/reperfusion in diabetic rats and in H9C2 cells under hyperglycemic conditions. *Int J Mol Med*. 2017;40(2):389–399. doi:10.3892/ijmm.2017.3014
12. Salameh A, Schuster R, Dahnert I, Seeger J, Dhein S. Epigallocatechin gallate reduces ischemia/reperfusion injury in isolated perfused rabbit hearts. *Int J Med Sci*. 2018;19(2):628. doi:10.3390/ijms19020628
13. Kim HJ, Yum KS, Sung JH, et al. Epigallocatechin-3-gallate increases intracelullar $[Ca^{2+}]$ in U87 cells mainly by influx of extracellular Ca^{2+} and partly by release of intracellular stores. *Naunyn Schmiedebergs Arch Pharmacol*. 2004;369(2):260–267. doi:10.1007/s00210-003-0852-y
14. Lasukova TV, Zykova MV, Belousov MV, Gorbunov AS, Logvinova LA, Dygai AM. The role of NO synthase in the cardioprotective effect of substances of humic origin on the model of ischemia and reperfusion of isolated rat heart. *Bull Exp Biol Med*. 2019;166(5):598–601. doi:10.1007/s10517-019-04399-y
15. Lin D, Ma J, Xue Y, Wang Z. Penehyclidine hydrochloride preconditioning provides cardioprotection in a rat model of myocardial ischemia/reperfusion injury. *PLoS One*. 2015;10(12):e0138051. doi:10.1371/journal.pone.0138051
16. Cabrera-Fuentes HA, Aragones J, Bernhagen J, et al. From basic mechanisms to clinical applications in heart protection, new players in cardiovascular diseases and cardiac theranostics: Meeting report from the third international symposium on new frontiers in cardiovascular research. *Basic Res Cardiol*. 2016;111(6):69. doi:10.1007/s00395-016-0586-x
17. Yamazaki KG, Romero-Perez D, Barraza-Hidalgo M, et al. Short- and long-term effects of (–)-epicatechin on myocardial ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol*. 2008;295(2):H761–H767. doi:10.1152/ajpheart.00413.2008
18. Kim SJ, Li M, Jeong CW, et al. Epigallocatechin-3-gallate, a green tea catechin, protects the heart against regional ischemia-reperfusion injuries through activation of RISK survival pathways in rats. *Arch Pharm Res*. 2014;37(8):1079–1085. doi:10.1007/s12272-013-0309-x
19. Gebka A, Rajtar-Salwa R, Dziewierz A, Petkow-Dimitrow P. Painful and painless myocaridal ischemia detected by elevated level of high-sensitive troponin in patients with hypertrophic cardiomyopathy. *Postep Kardiol Inter*. 2018;14(2):195–198. doi:10.5114/aic.2018.76413
20. Dykens JA. Isolated cerebral and cerebellar mitochondria produce free radicals when exposed to elevated Ca^{2+} and Na^+: Implications for neurodegeneration. *J Neurochem*. 1994;63(2):584–591. doi:10.1046/j.1471-4159.1994.63020584.x
21. Nath K, Guo L, Nancolas B, et al. Mechanism of antineoplastic activity of lonidamine. *Biochem Biophys Acta*. 2016;1866(2):151–162. doi:10.1016/j.bbcan.2016.08.001
22. Rizzuto R, De Stefani D, Raffaello A, Mammucari C. Mitochondria as sensors and regulators of calcium signalling. *Nat Rev Mol Cell Biol*. 2012;13(9):566–578. doi:10.1038/nrm3412

23. Li C, Zong W, Zhang M, et al. Increased ratio of circulating T-helper 1 to T-helper 2 cells and severity of coronary artery disease in patient with acute myocardial infarction: A prospective observational study. *Med Sci Monit*. 2019;25:6034–6042. doi:10.12659/MSM.913891
24. Bian WS, Tian FH, Jiang LH, et al. Influence of miR-34a on myocardial apoptosis in rats with acute myocardial infarction through the ERK1/2 the ERK1/2 pathway. *Eur Rev Med Pharmacol Sci*. 2019;23(7):3034–3041. doi:10.26355/eurrev_201904_17585
25. Jia P, Liu C, Wu N, Jia D, Sun Y. Agomelatine protects against myocardial ischemia reperfusion injury by inhibiting mitochondrial permeability transition pore opening. *Am J Transl Res*. 2018;10(5):1310–1323. PMID:29887947
26. Giorgi C, Baldassari F, Bononi A, et al. Mitochondrial Ca(2+) and apoptosis. *Cell Calcium*. 2012;52(1):36–43. doi:10.1016/j.ceca.2012.02.008
27. Di Cosimo S, Ferretti G, Papaldo P, Carlini P, Fabi A, Cognetti F. Lonidamine: Efficacy and safety in clinical trials for the treatment of solid tumors. *Drugs Today (Barc).* 2003;39(3):157–174. doi:10.1358/dot.2003.39.3.799451
28. Hao J, Li WW, Du H, et al. Role of vitamin C in cardioprotection of ischemia/reperfusion injury by activation of mitochondrial KATP channel. *Chem Pharm Bull (Tokyo).* 2016;64(6):548–557. doi:10.1248/cpb.c15-00693