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Parkin Regulates Mitochondrial Autophagy After Myocardial Infarction in Rats

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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Background: To study the role of Parkin in the regulation of mitochondrial autophagy in the heart by assessing mitochondrial autophagy and changes in Parkin protein expression in rat myocardium after myocardial infarction (MI).

Material/Methods: Rats were randomly assigned to three groups: control, sham, and MI. Four weeks after induction of MI, ultrasonic examination of the rats was performed to measure left ventricular end systolic diameter (LVESD), left ventricular end diastolic diameter (LVEDD), left ventricular ejection fraction (EF), left ventricular fractional shortening (FS), and left ventricular diastolic/systolic volume. Rat myocardium was collected from each group and examined for changes in morphology, size, and amount of mitochondria and autophagosomes by transmission electronic microscopy. A Western blot was performed to analyze the levels of Parkin and the autophagy-related protein LC3.

Results: Four weeks after MI, cardiac function of the MI rats was impaired compared with the control rats. Both LVESD and LVEDD were elevated in the MI rats ($p<0.05$) while EF was decreased, indicating that the MI model was constructed successfully. After MI, increased numbers of mitochondria and autophagosomes were observed in the myocardium ($p<0.05$), and the mitochondrial morphology was destroyed. Chloroquine (CQ) treatment increased the number of autophagosomes in the myocardium of the control rats ($p<0.05$) but not in MI rats ($p>0.05$). In addition, the levels of the autophagy-related proteins LC3II/LC3I were elevated in the myocardium after MI ($p<0.05$) and the activity of Parkin was significantly reduced ($p<0.05$).

Conclusions: Under conditions of chronic MI, mitochondrial dysfunction and disruption of autophagosomal clearance are associated with Parkin expression.

MeSH Keywords: Mitochondria, Heart • Myocardial Infarction • Parkinsonian Disorders

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Background

Autophagy plays a critical role in the degradation of long-lived proteins and damaged organelles in cells, and the autophagy-lysosome pathway is indispensable for normal cardiac function. The process of autophagy consumes and produces energy simultaneously. The fatty acids and amino acids that are generated during the degradation of proteins and organelles can be used for ATP synthesis, which promotes the survival of cardiomyocytes. Disruption or dysfunction of the autophagy pathway flux may lead to serious cardiac events. Disruption of autophagic flux may cause accumulation of proteins and nonfunctional organelles, resulting in cardiac dysfunction and even heart failure [1]. Although autophagy has been implicated in a variety of cardiovascular diseases [2], it has been unclear whether autophagy is an adaptive or adverse response. Autophagy in cardiomyocytes is an important regulatory mechanism, but it can harm the heart under certain conditions. There is no current consensus on the role and mechanism of autophagy in myocardial infarction (MI). Parkin was previously considered to be closely associated with Parkinson's disease [3], and most studies on Parkin are restricted to the brain. These studies have demonstrated that Parkin is closely associated with the autophagy of damaged mitochondria in the brain [4]. Even though Parkin is highly expressed in the heart [5], its role in cardiac function has seldom been studied. Hence, the relationship between Parkin and autophagy in cardiomyocytes needs to be further explored.

Material and Methods

Animals and reagents

Thirty-six male SD rats (4–6 months old), weighing 220±30 g, were purchased from the Animal Experiment Center of Xinjiang Medical University. The experiment was approved by the Animal Ethics Committee of Xinjiang Medical University. The main reagents included anti-Parkin and anti-LC3 I/II antibodies (Cell Signaling Technology, USA), and chloroquine (CQ) (Sigma, USA).

Model construction and grouping

The rats were anesthetized with 10% chloral hydrate by intraperitoneal injection. Tracheal intubation was performed to attach a respirator, and the respiration rate was maintained at 50–60 times/second. A horizontal incision was made between the third and fourth rib of the left chest to open the chest and expose the heart. The anterior descending artery below the left auricular appendage was ligated with 6-0 absorbable suture to the left anterior descending artery (LAD). Upon ligation, the pulse was decreased near the ligation site, and the anterior wall of the ventricle turned pale. In addition, the ECG lead II showed a significant uplift of the ST segment. These phenomena were preliminary evidence of successful MI model construction. The sham operation was performed in the same way, except that the suture was placed at the LAD without ligation [6]. The experimental animals were randomly divided into three groups: control, sham, and MI, with 12 rats in each group.

In order to study whether lysosomal function and autophagic flux were altered after MI, some of the rats from each group received an intraperitoneal injection of CQ, an inhibitor of autophagy, at a dose of 10 mg/kg, one hour prior to the operation. The rats were randomly assigned into the following groups: Control+CQ, Sham+CQ, and MI+CQ.

Examination of cardiac function

Four weeks after the induction of MI, the rats from control, sham, and MI groups underwent ultrasonic examination to measure the following parameters: left ventricular ejection fraction (EF), left ventricular fractional shortening (FS), left ventricular end systolic diameter (LVESD), left ventricular end diastolic diameter (LVEDD), and left ventricular volume (LV Volume). The parameters of each group were analyzed and compared.

Examination of morphological changes in the rat heart by transmission electron microscopy

The heart was fixed in 2% glutaraldehyde and then in 1% osmic acid, dehydrated in ethanol, and embedded in Epon812 epoxy resin. The sample was sectioned into ultrathin slices, positively stained, dried, and observed under a transmission electron microscope. The numbers of mitochondria and autophagosomes were counted in a blinded manner.

Western blot

The left ventricular tissue was collected from each rat, at a weight of approximately 50 mg/ventricle. Protein lysis buffer was added to the minced tissue, followed by sonication and centrifugation. Supernatant was collected, and the protein concentration was determined using a BCA Protein Quantification Kit. Total protein from each sample (50 μg) was separated by 10% SDS-PAGE, and transferred onto a PVDF membrane at 25 V for 60 minutes in a semi-wet transfer apparatus. After rinsing, the membrane was blocked with 5% non-fat milk for two hours at room temperature, rinsed again, and incubated with the primary antibody (1:1000) overnight at 4°C. After three TBST washes, the membrane was incubated with the secondary antibody (1:1000) for two hours, followed by thorough washing and exposure.

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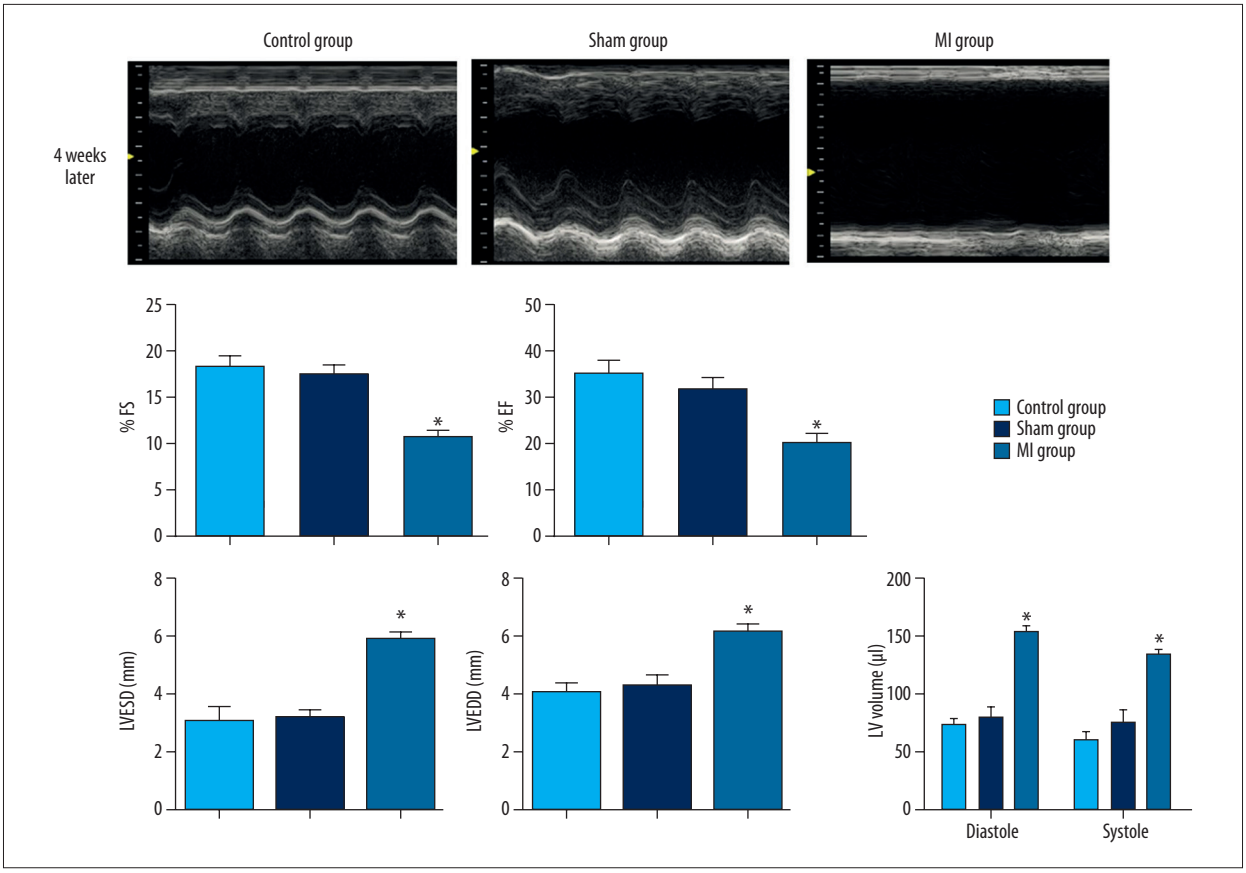

Figure 1. Determination of FS, EF, LVESD, LVESS, and LV Volume in control, sham, and MI groups. * $p<0.05$ when comparing MI and control group.

Statistical analysis

The data were analyzed using the SPSS 13.0 statistical analysis software, and expressed as the mean ± standard error ($\bar{\chi}\pm s$). The data were tested for normality and homogeneity of variance, and the qualified data were analyzed by one-way analysis of variance (ANOVA) for comparisons between multiple groups, and by *t*-test for comparison between two groups. A value of $p<0.05$ indicated a statistically significant difference.

Results

Comparison of the cardiac function

Four weeks after the induction of MI, EF of the MI rats was significantly lower than that of the control rats ($p<0.05$), while EF of the sham group was comparable to that of the control group ($p>0.05$). Moreover, the LV volume, LVEDD, and LVESD in the MI rats were significantly increased compared with the control rats ($p<0.05$). In addition, enlargement of the heart, thinning of the ventricular wall, and ventricular remodeling were observed in the MI rat. These signs of chronic MI indicated that the MI model was successfully constructed in the rats (Figure 1).

Increased number of mitochondria and disruption of mitochondrial morphology in rat myocardium after MI

We observed the network of intracellular collagen fibers in the myocardium of the control rat by transmission electron microscopy. Mitochondria of uniform size and regular morphology were vertically aligned between the myocardial fibers in a zig-zag pattern. By contrast, the myocardial fiber network was disrupted in the MI group, and the number of mitochondria was increased ($p<0.05$). Moreover, the mitochondria became small and round, and were irregularly arranged after MI. These phenomena suggested that the function of myocardial mitochondria was disrupted by MI (Figure. 2)

MI increased the number, and disrupted the clearance, of autophagosomes in rat myocardium

CQ can inhibit the clearance of autophagosomes by interfering with the fusion of autophagosomes with lysosomes, reducing lysosomal degradation. CQ can increase the number

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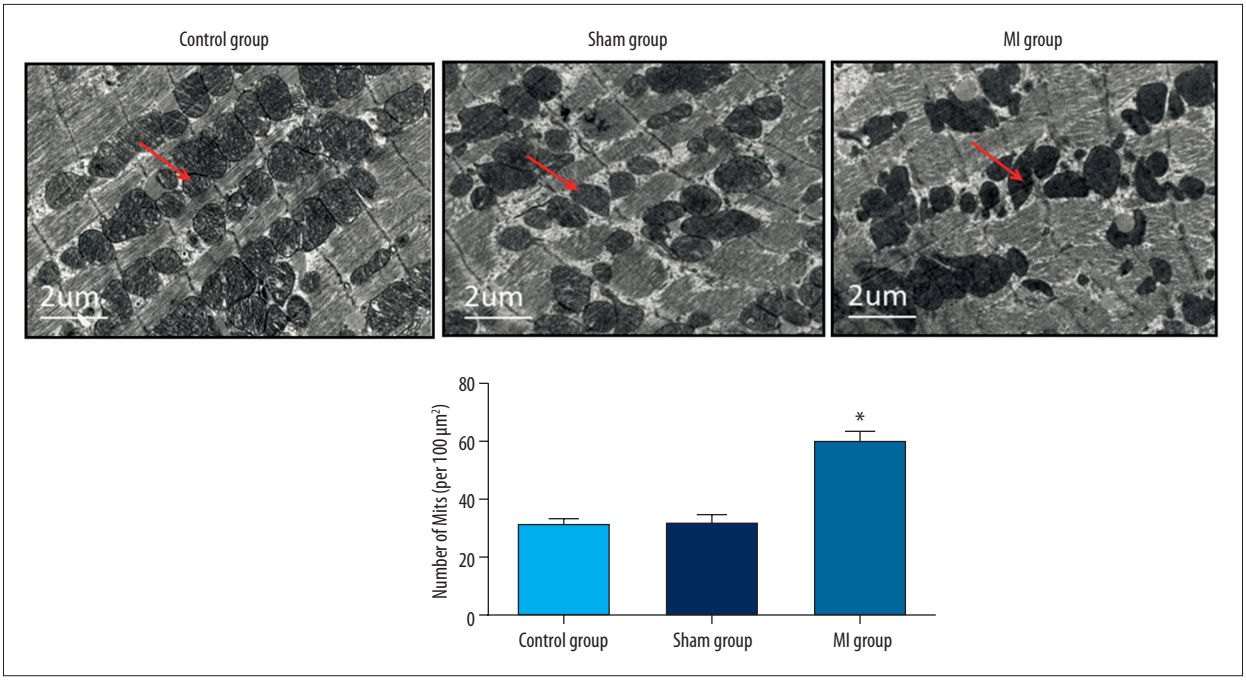

Figure 2. Changes in mitochondrial morphology in control, sham, and MI groups. * $p<0.05$ when comparing MI and control group.

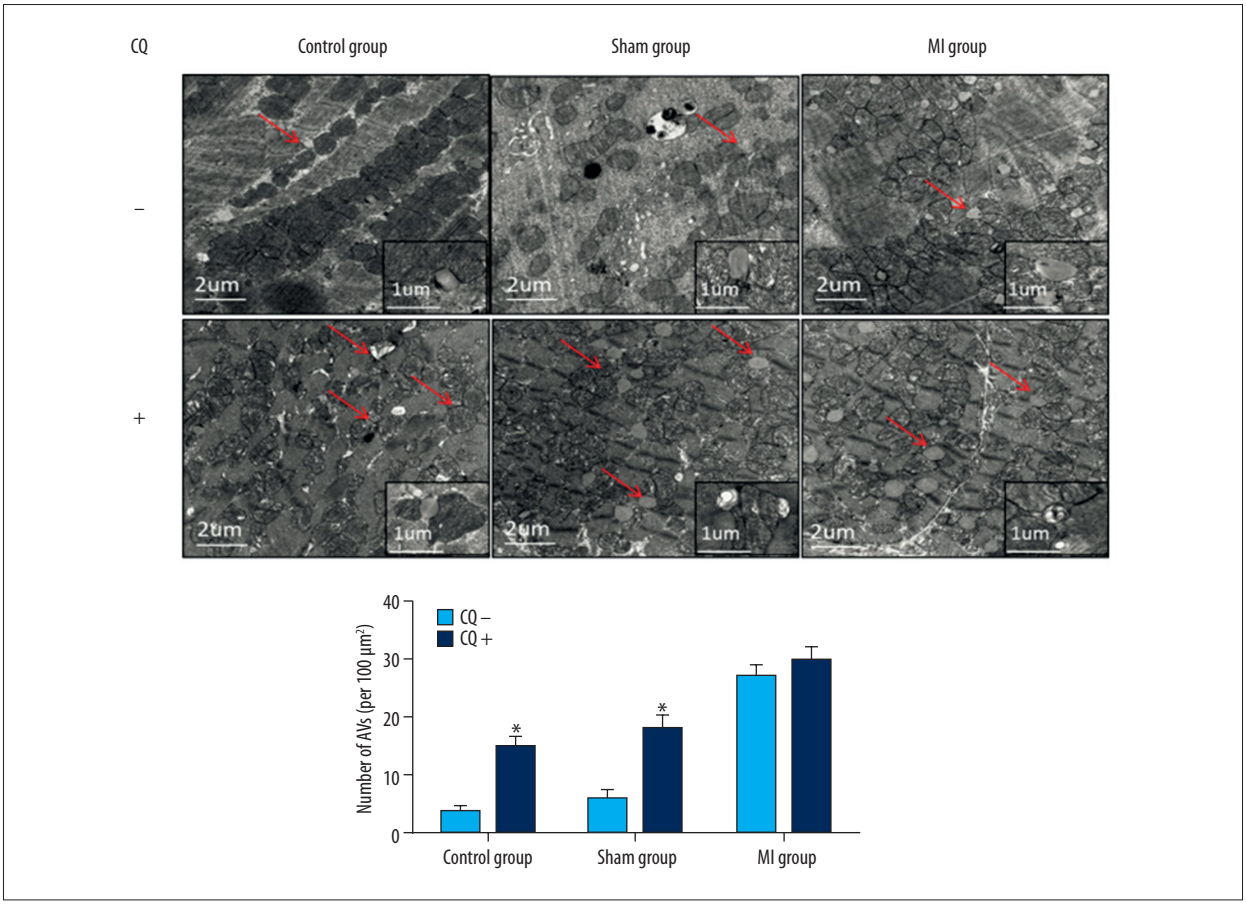

Figure 3. Change in autophagosomes in control, sham, and MI groups. * $p<0.05$ when comparing CQ+ and CQ–.

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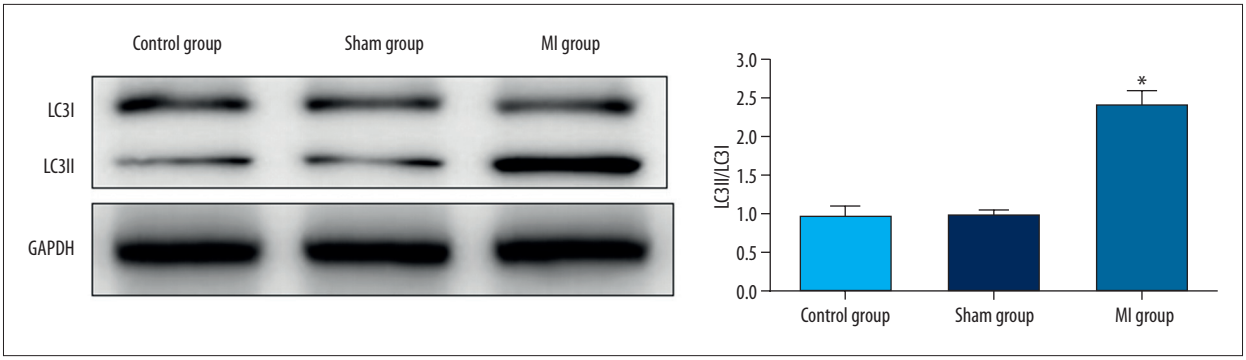

Figure 4. Relative expression of LC3II/LC3I in the myocardium of control, sham, and MI groups. * $p<0.05$ when comparing MI and control group.

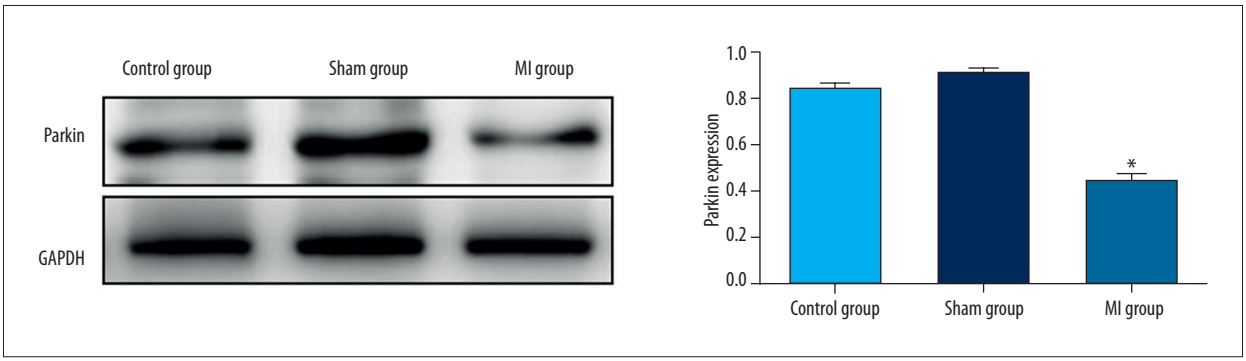

Figure 5. Relative expression of Parkin in the myocardium of control, sham and MI groups. * $p<0.05$ when comparing MI and control group.

of autophagosomes in normal cells, but it does not affect the number of autophagosomes in cells with impaired autophagy. Thus, the number of autophagosomes after CQ treatment can be used to assess whether the clearance of autophagosomes is disrupted.

In the absence of CQ, the number of autophagosomes in the MI rats was markedly increased compared with the control rats ($p<0.05$). The number of autophagosomes was increased in the control rats with CQ treatment (CQ+) ($p<0.05$). However, the number of autophagosomes was not significantly different between the MI rats with CQ treatment and the MI rats without CQ treatment (CQ–) ($p>0.05$) (Figure 3).

MI affected the expression of autophagy-related protein LC3 I/II

Myocardial tissue from the left ventricle was obtained from the rats of the control, sham, and MI groups, and the activity of LC3 I/II was determined. As shown in Figure 4, the LC3 II/LC3 I ratio was higher in the MI group than in the control group ($p<0.05$), suggesting that MI could induce the conversion of LC3 I to LC3 II, and thus increase the level of LC3 II.

Parkin is involved in MI-induced autophagosome dysfunction

The level of Parkin protein in the myocardium of the control, sham, and MI rats was determined by Western blot. Four weeks after MI, the level of Parkin protein in the MI group was significantly lower than in both the control and sham groups ($p<0.05$) (Figure 5).

Discussion

The incidence of coronary heart disease is continuously rising, and MI-induced heart failure has become particularly common. During acute MI, changes in neurohumor modulate myocardial contraction and maintain cardiac output and blood supply to the vital organs. However, such compensatory mechanisms are lost in chronic MI, which not only exacerbates cardiac dysfunction but also causes increase in infarct size, myocardial fibrosis, ventricular enlargement, and a series of symptoms of heart failure [6–8]. Therefore, understanding the pathophysiological process of chronic MI is of great significance.

Autophagy is an important pathway for cells to degrade proteins and organelles (such as mitochondria) in a lysosome-dependent

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manner [9]. Induction and regulation of autophagy is a complicated process and involves a variety of stimulating factors, including nutritional deficiency, hypoxia, ischemia, energy depletion, increased intracellular calcium concentration, and oxidative stress. In addition, a number of signaling molecules are also involved in the regulation of autophagy. Autophagy plays a critical role in the structural and functional homeostasis of cardiomyocytes [10], and it is commonly present in a variety of diseases, such as myocardial ischemia-reperfusion, dilated cardiomyopathy, and heart failure. Danon disease is a lethal myocardial disorder resulting from the deficiency of lysosome-associated membrane protein 2 (LAMP2) [11]. Loss of LAMP2 leads to aggregation of autophagosomes in the cardiomyocytes and impedes the fusion of autophagosome with lysosome [12]. Moreover, it has been shown that conditional disruption of Atg5 in the adult heart causes mitochondrial dysfunction and aggregation, resulting in the development of cardiac insufficiency [13]. Despite these findings, the role of autophagy is still controversial, as most studies have proposed that autophagy is a protective response stimulated by cardiomyocytes. The number of autophagosomes in cardiomyocytes is determined by the dynamic equilibrium between autophagosome synthesis and clearance. In normal cardiomyocytes, CQ can inhibit the function of lysosomes and block the fusion of autophagosomes with lysosomes, resulting in accumulation of autophagosomes in cardiomyocytes. In the present study, the effect of CQ on autophagosome accumulation was lost after MI, implying that MI impeded the clearance of autophagosomes in cardiomyocytes. Thus, changes in autophagosome number during MI might be associated with disrupted autophagic flux and enhanced aggregation of autophagosomes. Further study revealed that MI led to an increased number of autophagosomes and enhanced the conversion of autophagy-related marker LC3 I to LC3 II in cardiomyocytes, suggesting a disruption of lysosomal functions.

Although Parkin was found to be highly expressed in the heart over a decade ago, its function in the heart is still unknown. Our study demonstrated that the level of Parkin was significantly reduced in the myocardium of rats following MI, indicating that Parkin may be involved in post-MI recovery and in the deregulated autophagic clearance of myocardial mitochondria. Parkin was recently demonstrated to mediate the clearance of mitochondria in myocardial ischemic preconditioning in rats [14]. This study showed that ischemic preconditioning induced translocation of Parkin to mitochondria to exert its protective effect, and this protective effect of ischemic preconditioning was compromised in Parkin knockout rats [14]. In addition, treatment with a mitochondrial uncoupler, which reduces mitochondrial membrane potential, was able to induce the translocation of Parkin from the cytoplasm to mitochondria in neurons. In the event of mitochondrial damage, where the membrane potential was lost and mitochondrial fusion was blocked, Parkin selectively translocated to the damaged mitochondria rather than to the normal mitochondria. Moreover, Parkin could mediate selective clearance of mitochondria by autophagy in cells treated with mitochondrial uncoupler for 24 hours [15,16]. These studies demonstrated that damaged mitochondria could be selectively cleared by Parkin for the protection of the organelles. Consistent with previous studies, our results substantiated the conclusion that Parkin plays an important role in the autophagy of mitochondria in cardiomyocytes.

Parkin has broad potential as a therapeutic target. Upregulation of Parkin is useful in MI patients in order to resist cell death through mitochondrial autophagy. Nevertheless, excessive or prolonged autophagy activation is also harmful and may lead to the loss of cardiomyocytes.

Conclusions

Our study demonstrated that Parkin protein is associated with the disruption of mitochondrial function and the clearance of autophagosomes in rat cardiomyocytes after MI. However, the molecular mechanism underlying the activity of Parkin in the autophagy process in cardiomyocytes is still unclear and remains to be further elucidated.

Declaration of interest

All authors have no conflict of interest regarding this paper.

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