Effects of Theaflavin on *Beclin-1/Vps34* Complex Regulation during Autophagy in Myocardial Ischemia-Reperfusion Injury in Rats

Donghui Z, Qian F and Jinghua L*

Department of Cardiology, Beijing An Zhen Hospital, Capital Medical University, and Beijing Institute of Heart, Lung and Blood Vessel Disease, Beijing, China

**Abstract**

To elucidate the protective effects of Theaflavin (TF1) on myocardial Ischemia-Reperfusion (I/R) injury. A rat myocardial I/R injury model was prepared by constriction of the anterior descending branch of the coronary artery and recanalization. Intracellular contents of reactive oxygen species and reactive nitrogen species were detected by flow cytometry and ELISA. Protein expression levels of autophagy-related proteins were determined by western blotting. Application of TF1 20 μmol/L inhibited the changes in heart rate, weight/body weight ratio, and left ventricular thickness observed in the I/R group. In particular, the change in infarct area was significantly reduced. Compared with the I/R group, the TF1 group had slightly disorganized myocardial fibers with better morphology, relatively clear nuclei, cell transverse stria, and intercalary discs, and only small amounts of necrotic areas and inflammatory cell infiltration. The ROS increase in the I/R group was decreased by application of TF1. Furthermore, treatment with TF1 reduced that the iNOS activity, NOX level, and nitrotyrosine content observed in the I/R group. The apoptosis index of myocardial cells in the I/R group was significantly alleviated by TF1 from 38.4% ± 3.4% to 21.3% ± 2.7%. TF1 also inhibited the peak time of the intracellular calcium transient as well as the 90% decay time. Protein expression of LC3-II was markedly reduced by TF1. The increases in *Beclin-1* and Vps34 protein expression observed in the I/R group were significantly inhibited by TF1. TF1 plays an important role in autophagy regulation through up-regulation of *Beclin-1/Vps34* protein expression.

**Keywords:** Theaflavin; Cardiac ischemia reperfusion; Autophagy; *Beclin-1/Vps34* complex

**Introduction**

There are still major challenges to be faced in the prevention and treatment of cardiovascular diseases. Cardiovascular diseases currently affect 290 million people worldwide, including 11 million with coronary heart disease in China [1,2]. Direct percutaneous coronary intervention is the most effective strategy for revascularization in ischemic heart disease, and saves the lives of many patients [3]. However, reperfusion therapy can cause secondary damage to the myocardial tissue while opening occluded blood vessels and restoring the cardiac blood supply, thereby worsening the prognosis of patients. The symptoms are mainly manifested as myocardial depression, severe fatal ventricular arrhythmia, and lack of coronary regurgitation, which directly threaten the health of patients [4]. The biggest problem faced by medical workers is how to reduce or prevent myocardial ischemia and subsequent injury. Thus, strategies to reduce or prevent myocardial Ischemia-Reperfusion (I/R) injury are urgently needed.

Reperfusion injury is an independent risk factor for cardiac remodeling and heart failure. The mechanism involves hypoxia of tissues, decreased scavenging ability of free radicals, and increased Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) such as superoxide, hydrogen peroxide, hydroxyl radical, ONOO−, and its proton form HOONO− [5]. These factors cause changes in intracellular pH, degrade ATP, destroy cellular DNA and RNA, disrupt normal cell structure and function, and lead to necrotic cell death. At the same time, they induce intracellular calcium overload, mitochondrial dysfunction, and myocardial injury aggravation through dysfunction of Na+/Ca2+ exchangers on the myocardial cell membrane surface, abnormal intracellular calcium ion uptake and release, abnormal opening of mitochondrial mPTP osmotic pores, and excessive intracellular Ca2+ accumulation [6]. *Beclin-1* expression is up-regulated during reperfusion, leading to excessive autophagy and cell death as one of the main mechanisms for myocardial I/R. Under
physiological conditions, Bcl-2 inhibits the cellular autophagy process by forming a complex with Beclin-1. ROS lead to an imbalance in the Beclin-1/Bcl-2 interaction during reperfusion, wherein Bcl-2 dissociates from Beclin-1 complexes and accelerates the occurrence of autophagy. The interaction of Beclin-1 and Vps34 (mammalian class III phosphatidyl inositol-3 kinase) to form Beclin-1/Vps34 complexes for autolysosome membrane synthesis and transport plays a key role in autophagy [7-9].

Theaflavin (TF1) is one of the natural polyphenols found in black tea [10]. It possesses a broad spectrum of biological functions, such as free radical scavenging, anti-inflammatory and cardiovascular protection activities [11,12]. Theaflavin has a protective effect on focal cerebral and heart ischemia reperfusion injury in rats, and its mechanism may be related to antioxidation, inhibition of apoptosis and inflammatory reaction [13,14]. The protection effect of TF1 on I/R hearts, however, mechanisms of autophagy in particular is far from clear. For this study, a rat I/R model was prepared, and TF1 was applied for intervention to observe its effect on the myocardium after I/R, as well as its effect on ROS, RNS, intracellular Ca²⁺, and apoptosis in myocardial cells. The effects of TF1 on Beclin-1/Vps34 regulation were also evaluated. We further aimed to elucidate the molecular mechanism of the effect of TF1 on myocardial I/R injury.

**Methods**

**Animal groups and model preparation**

Specific pathogen-free Sprague-Dawley rats aged 8 weeks with body weight 200 g ± 10 g were selected. All experiments were performed as approved by the institutional animal care and use committee. At −4°C, the hearts were quickly removed and randomly divided into three groups:

1. I/R group: After stabilization for 30 min with KH solution, the hearts were subjected to 30 min no-flow global ischemia followed by 120 min of reperfusion.

2. TF1 group: The hearts were treated with 20 μmol/L TF1 (That was purchased from Chromadex Inc., U.S.) for 10 min before ischemia and reperfusion, respectively.

3. Sham group: The hearts were reperfused for 150 min with KH solution.

**Echocardiographic assessment**

Cardiac function was noninvasively monitored using a Vivid 7 Dimension Echocardiographic System (GE Healthcare). Briefly, the rats were anesthetized with 10% chloral hydrate, and echocardiograms were performed on the anesthetized rats.

**Myocardial infarction area assessment**

The heart was taken and frozen at −20°C for 20 min. The heart is sliced into 1 mm to 2 mm slices from base to apex. The slices were stained for 15 min in 1% TTC at 37°C. The slices were fixed for 48 h with 10% formaldehyde solution. The section of the heart was scanned and analyzed by image software.

**Hematoxylin-eosin (HE) staining**

The ventricle was immersed in 4% paraformaldehyde for 3.5 h, and then transferred to 70% ethanol. Individual lobes of ventricular material were placed in processing cassettes. Some 4-μm thick tissue sections were dewaxed in xylene, washed in PBS, and subjected to HE staining. The sections were dehydrated through increasing concentrations of ethanol and xylene. Photomicrographs were obtained using a BX53 microscope (Olympus, Tokyo, Japan).

**Immunohistochemistry**

Rat cardiac tissue samples (ischemic regions) were homogenized in PBS and centrifuged at 12,000 x g for 30 minutes at 4°C. The total protein concentrations in the supernatants were measured by the bicinchoninic acid method. The specific concentrations of Malondialdehyde (MDA), Myeloperoxidase (MPO), and Glutathione (GSH) were also measured by the bicinchoninic acid method. The concentrations of nitrotyrosine, the accepted footprint for in vivo ONOO− formation, were determined with a commercial ELISA kit (Cell Sciences Inc., Canton, USA).

**Flow cytometric detection of apoptosis**

ROS were measured using an Annexin V-FITC/PI Apoptosis Kit (Invitrogen). Briefly, the cells were harvested, and incubated with 5 μl of FITC-annexin V and 1 μl of PI working solution (100 μg/ml) for 15 min at room temperature in the dark. Fluorescence was measured by flow cytometry using a FACS Calibur (BD Biosciences, USA).

**Single atrial myocyte preparation**

Immediately after removal, rat hearts were perfused with Tyrode solution in a Langendorff apparatus to maintain a pH of 7.4. The composition of the Tyrode solution (mmol/L) was: NaCl 113, KCl 4.7, KH₂PO₄ 0.6, Na₂HPO₄ 0.6, MgSO₄ 1.2, HEPES 10, glucose 5). Briefly, the isolated hearts were perfused for 21 min to 25 min with Tyrode solution containing 1.2 mg/ml collagenase II and 0.3 mg/ml trypsin. The ventricle was cut into pieces to obtain single ventricular myocytes.

**Confocal Ca²⁺ imaging**

Confocal imaging of Ca²⁺ fluorescence was used to detect intracellular Ca²⁺ by recording Ca²⁺ spike, the time to Ca²⁺ peak of Ca²⁺ waves and the Ca²⁺ decay time. The atrial myocyte intracellular Ca²⁺ level reflected by Ca²⁺ fluorescence was quantified with Fluo-4 AM (Thermo Fisher Scientific) in a bath solution for 20 min. A laser scanning confocal microscope (SP5; Leica Microsystems) was used to record basal Ca²⁺, Ca²⁺ release events, and Ca²⁺ cycling under different conditions. Fluorescence was excited at 488 nm and light was emitted at >500 nm.

**Western blotting**

For protein isolation from cells, RIPA buffer supplemented with protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA) was used. Equal amounts of cell lysates were electrophoresed by 10% SDS-PAGE and transferred to PVDF membranes. The membranes were blocked for 1 hour in Tris-Buffered Saline with 0.05% Tween-20 (TBST) containing 5% skimmed milk, and incubated overnight with the following primary antibodies at 4°C: anti-Beclin-1 (1:1000), anti-LC3-II (1:1000), anti-LC3-I (1:1000), anti-p62 (1:1000), anti-Vps34 (1:1000), anti-UVRAG (1:1000), anti-Vps34 (1:1000), anti-Vps15 (1:1000), and anti-GAPDH (1:1000). After three washes with TBST, the membranes were incubated with a secondary antibody (alkaline phosphatase-conjugated IgG; 1:500) at room temperature for 1 h and washed again with TBST. Images of the antibody-bound target proteins were detected with a Chemilum HRP Substrate Kit (Millipore). The band intensities were normalized by the intensity of signals for GAPDH. All experiments were independently repeated at least three times.

**Statistical analysis**

SPSS 21.0 statistical software was used to process the data. Data were expressed as mean ± S.D. Paired or two groups of continuous
measurement data are analyzed by a t-test, multiple groups were analyzed by one-way ANOVA, and comparisons among multiple groups were conducted by an SNK-q test. The level of accepted statistical significance was set at $P<0.05$.

**Results**

**Effects of TF1 on heart pathology in I/R injury**

Compared with the control group, significant differences were found for heart rate, heart weight/body weight ratio, and left ventricular peripheral thickness in the I/R group. In particular, the infarct area was significantly increased. After application of 20 μmol/L TF1, all index changes in I/R were inhibited, with the infarct area in particular being significantly reduced ($P<0.01$, $n=10$, Table 1). In the I/R group, Left Ventricular End-Diastolic Pressure (LVEDP) was significantly increased ($P<0.01$, $n=10$), while Left Ventricular End-Systolic Pressure (LVESP) and cardiac Fractional Shortening (FS) were significantly decreased. With application of 20 μmol/L TF1, the abnormal changes in LVEDP, LVESP and FS in the I/R group were significantly inhibited, while FS was significantly increased ($P<0.05$, $n=10$, Table 1).

HE staining revealed that the arrangement of myocardial fibers in the I/R group was disorganized and wavy, with unclear local transverse stria and widened myocardial fiber spaces. Inflammatory cell infiltration and hemorrhage were observed. In the TF1 group, the myocardial fibers were slightly disorganized, with better morphology than the I/R group, relatively clear nuclei, cell transverse stria, and intercalary discs, and only small amounts of necrotic areas and inflammatory cell infiltration (Figure 1).

**Effects of TF1 on myocardial infarction area in I/R injury**

The infarcted area of the heart in the I/R group was detected. TF1 20 μmol/L significantly reduced the infarcted area of the heart. The AAR/LVA (Left Ventricular Area) ratio changed slightly, which was not statistically significant.

**Effects of TF1 on ROS and RNS in I/R injury**

ROS increased from 24.3 ± 3.2 to 80.7 ± 7.8 ($P<0.01$, $n=10$) after I/R, but decreased to 41.4 ± 5.7 after application of TF1 20 μmol/L (P<0.01, n=10, Figures 2a, 2b). The I/R group showed increased levels of MDA and MPO, and decreased levels of GSH. After application of TF1 20 μmol/L, MDA and MPO were decreased, while GSH was increased (Figures 2c-2e). The I/R group exhibited significantly increased iNOS activity and levels of NOx and ONOO⁻ in the myocardium. The indexes of the three groups were 0.005 pmol/min/g ± 0.002 pmol/min/g (protein), 150.4 mol/g ± 24.3 mol/g (tissue), and 0.51 nmol/g ± 0.02 nmol/g (protein). The corresponding indexes in the Sham group were increased to 0.623 pmol/min/g ± 0.021 pmol/min/g, 1333.4 mol/g ± 97.5 mol/g, and 3.81 nmol/g ± 0.38 nmol/g, respectively. Following treatment with TF1 20 μmol/L, the above indexes were reduced to 0.314 pmol/min/g ± 0.003 pmol/min/g, 802.5 mol/g ± 78.4 mol/g, and 2.55 nmol/g ± 0.18 nmol/g, respectively (all $P<0.01$, n=10, Figures 2f-2h).

**Effects of TF1 on apoptosis in I/R injury**

The apoptosis index of cells after reperfusion in the I/R group was 38.4% ± 3.4%, and significantly higher than that in the Sham group (12.5% ± 1.2%). The TF1 group showed alleviation of the above changes, with an apoptosis index of 21.3% ± 2.7% (Figure 3a, 3b). Compared with the Sham group, Bcl-2 expression in the I/R group was significantly up-regulated, while BAX expression was down-regulated. Compared with the I/R group, the changes in these two proteins were significantly inhibited in the TF1 group ($P<0.05$, n=3, Figures 3c-3e). There was no significant difference in caspase-3 expression among the three groups (Figure 3c, 3f).

**Effects of TF1 on intracellular calcium transients in I/R injury**

Compared with the Sham group, the peak time of the intracellular calcium transient in the I/R group was shortened from 72.3 ms ± 6.21 ms to 42.9 ms ± 4.6 ms ($P<0.05$, n=10), indicating increased intracellular calcium release. The 90% decay time of the calcium transient was extended from 219 ms ± 15.7 ms to 523 ms ± 19.8 ms.

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**Table 1: Effects of Theaflavin on Cardiac Basic and Functional Indicators.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sham</th>
<th>I/R</th>
<th>TF1</th>
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<tbody>
<tr>
<td>Heart rate (time/min)</td>
<td>226 ± 25</td>
<td>256 ± 31</td>
<td>235 ± 22</td>
</tr>
<tr>
<td>Heart/weight ratio (g/kg)</td>
<td>3.87 ± 0.14</td>
<td>3.42 ± 0.21</td>
<td>3.57 ± 0.17</td>
</tr>
<tr>
<td>Thickness of the left ventricular (mm)</td>
<td>1.44 ± 0.41</td>
<td>2.42 ± 0.51</td>
<td>1.57 ± 0.37</td>
</tr>
<tr>
<td>Relative infarct area (%)</td>
<td>0</td>
<td>17.2 ± 3.2</td>
<td>9.6 ± 2.2</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>3.6 ± 1.1</td>
<td>10.4 ± 2.3</td>
<td>6.4 ± 1.0</td>
</tr>
<tr>
<td>LVESP (mmHg)</td>
<td>95.7 ± 19.3</td>
<td>67.5 ± 15.8</td>
<td>88.6 ± 14.7</td>
</tr>
<tr>
<td>FS (%)</td>
<td>50.5 ± 2.9</td>
<td>35.9 ± 1.4</td>
<td>42.3 ± 3.5</td>
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</table>

I/R: Ischemia-Reperfusion; LVESP: Left Ventricular End-Systolic Pressure; LVEDP: Left Ventricular End-Diastolic Pressure; FS: Cardiac Fractional Shortening

*P<0.05, **P<0.01, vs. Sham group; *P<0.05, **P<0.01, vs. I/R group

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**Figure 1:** The myocardial fibers in the Sham group showed clear nuclei, transverse stria, and intercalary discs. The myocardial fibers in the I/R group were disordered and wavy with unclear local transverse stria and widened myocardial fiber spaces. The myocardial fibers in the TF1 group were slightly disorganized, with relatively clear nuclei, transverse stria, and intercalary discs.
Application of TF1 20 μmol/L decreased the peak time of the intracellular calcium transient and the 90% decay time (P<0.05, n=10, Figure 4).

Effects of TF1 on autophagy-related proteins in I/R injury

We evaluated the expression of autophagy marker proteins (LC3-II and LC3-I) and p62 before and after TF1 treatment. Expression of LC3-II was up-regulated, while expression of p62 was down-regulated in myocardial cells after I/R. The protein expression changes in LC3-II and p62 in the I/R group were markedly inhibited by TF1 (P<0.01, n=3, Figure 5a, 5c, 5d).

Effects of TF1 on *Beclin-1* and other autophagy-related molecules in I/R injury

We observed the effects of TF1 on *Beclin-1* and autophagy regulatory molecular complexes. Increased protein expression of *Beclin-1*, Vps15, and Vps34 was observed in the I/R group. The up-regulation of *Beclin-1*, Vps15, and Vps34 in the I/R group was significantly inhibited by application of TF1 20 μmol/L (P<0.05 or P<0.01, n=3, Figures 5b, 5e-5g). These findings suggested that the decrease in the autophagy process induced by TF1 after myocardial I/R injury was closely related to *Beclin-1/Vps34* complex regulation. TF1 had little effect on expression of *UVRAG* (Figure 5b, 5h).
Table 2: Effects of Theaflavion Myocardial Infarction area in I/R injury (n=10).

<table>
<thead>
<tr>
<th>Groups</th>
<th>MIA</th>
<th>AAR</th>
<th>LVA</th>
<th>MIA/AAR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>2.12 ± 0.25</td>
<td>69.51 ± 2.82</td>
<td>178.62 ± 6.68</td>
<td>3.1</td>
</tr>
<tr>
<td>I/R</td>
<td>38.52 ± 2.75**</td>
<td>74.42 ± 3.83</td>
<td>176.61 ± 5.04</td>
<td>5.2</td>
</tr>
<tr>
<td>TF1</td>
<td>25.82 ± 1.82**</td>
<td>72.57 ± 2.56</td>
<td>180.43 ± 7.30</td>
<td>3.6</td>
</tr>
</tbody>
</table>

MIA: Myocardial Infarction Area; AAR: Area-at-Risk; LVA: Left Ventricular Area; **P<0.01 vs. Sham group; ***P<0.01 vs. I/R group

Figure 4: The peak time of the intracellular calcium transient in the I/R group was shortened, while the 90% decay time was extended. Application of TF1 20 μmol/L inhibited these changes in the peak time of the intracellular calcium transient and the 90% decay time. *P<0.01, vs. Sham group; **P<0.05, ***P<0.01, vs. I/R group.

Figure 5: In the I/R group, LC3-II expression was up-regulated and p62 expression was down-regulated. The protein expression changes in LC3-II and p62 in the I/R group were markedly inhibited by TF1 (a, c, and d). The up-regulated changes in Beclin-1, Vps15, and Vps34 in the I/R group were inhibited by application of TF1 20 μmol/L (b and e to g). TF1 had little effect on UVRAG expression (b and h). *P<0.01, vs. Sham group; **P<0.05, ***P<0.01, vs. I/R group.
Discussion

In this study, it was first found that effect of TF1 reduced ROS damage to ischemic reperfusion myocardium through autophagy process after I/R in rats. It is well known that I/R can induce the mitochondria of myocardial cells to produce large amounts of ROS or oxygen free radicals. Oxygen free radicals damage enzymes on the cell membrane or bind to receptors. The resulting increases in membrane permeability and brittleness cause inflammatory reactions and aggravate cell autophagy disorders, inducing cell apoptosis. TF1 comes from natural polyphenols found in black tea. It reported that the polyphenols possessed inhibiting the oxidative stress on myocardial cells arising from I/R and reduce the activity of oxygen free radicals generated [15].

The present results showed that TF1 significantly reduced LC3-II protein expression and increased p62 expression in rat myocardial cells after I/R, and inhibited excessive autophagy. Moreover, autophagy was inhibited by TF1 through reductions in ROS and intracellular Ca2+ overload. With an increase in reperfusion, ROS are continuously produced and the mitochondrial membrane permeability is enhanced, resulting in increased autophagy, which in turn accelerates apoptosis of myocardial cells and causes cardiac dysfunction. LC3 is located on the autophagosome membrane and involved in the formation of autophagy. It can be expressed in two forms: LC3-I and LC3-II. When autophagy occurs, LC3-I becomes linked with phosphatidyl ethanolamine in a ubiquitin-like reaction mode to form LC3-II. In turn, LC3-II binds to and is adsorbed on the autophagosome membrane, and its content is proportional to the content of autophagosome vesicles [16]. The present study demonstrated that TF1 can not only indirectly inhibit autophagy by reducing ROS, but also inhibit autophagy by directly reducing LC3-II. Binding of p62 to LC3 plays a key role in autophagy regulation. p62 forms a complex with ubiquitinated proteins and LC3-II protein on the autophagosome membrane to complete their degradation in the autophagosome and is continuously degraded during autophagy. Therefore, the p62 expression level is negatively correlated with the autophagy level. It was also reported that p62, as a marker of autophagy activation, may not accurately reflect true autophagy [17,18]. In the present study, p62 was found to be decreased during cardiac I/R in rats, consistent with the mainstream view that TF1 can increase the expression of p62. Many studies have shown that excessive intracellular ROS or extracellular oxidative stress can lead to cell death by up-regulating autophagy. ROS may be a strong inducer of Beclin-1 that mediates autophagy during reperfusion [19]. In 1993, Gordon et al. [20] reported that it inhibited autophagy by chelating intracellular calcium. Our results showed that Ca2+ can activate autophagy, and aggregation of intracellular Ca2+. Furthermore, studies have shown that increased intracellular Ca2+ can induce autophagy through multiple methods, including CaMKII-mediated activation of AMP-activated protein kinase. Autophagy can be induced or initiated by promoting the binding of Phosphatidylinositol-3-Phosphate (P13P, Vps34) to the autophagosome membrane. Based on the above analyses, it is not difficult to conclude that TF1 has a strong theoretical basis for reducing autophagy by reducing the overload of ROS and intracellular Ca2+.

Further, another finding is that TF1 plays a role in autophagy regulation by up-regulating beclin-1/Vps34 protein complex formation. Beclin-1 is a key protein regulator for autophagy body formation and autophagy protein production. Beclin-1 overexpression was found to increase autophagy activity in vitro. In contrast, beclin-1 knockout mice showed reduced autophagy activity in myocardial cells during reperfusion [8]. It primarily works with class III phosphatidylinositol excitation (PI3K, Vps34) to increase other proteins involved in complex formation, promote autophagy body membrane formation, and guide localization of other Atg proteins in the autophagy body membrane, thereby triggering autophagy. Beclin-1 is also a key factor that regulates the balance between autophagy and apoptosis, and determines cell survival or cell death. A previous study showed that the myocardial cell apoptosis rate in Beclin-1 knockout mice was lower than that in wild-type mice [21].

Beclin-1 contains a BH3 domain structure and has a Bcl-2 site. Beclin-1 also contains an ECD structure domain (with a class III Vps34 binding site) and a CCD domain structure, with the CCD structure of the UVRAG domain able to form dimers [22]. Autophagy is also regulated by the interaction of phosphatidylinositol-3-phosphokinase (Vps34). Recent studies have shown that Beclin-1 forms complexes with other related molecules to participate in autophagy with the Beclin1/Vps34 complex. The beclin-1/Vps34 complex is involved in autophagy regulation, and promotes autophagosome formation by repositioning Atg proteins to the pre-autophagosome structure [23]. The present results showed that TF1 had effects not only on beclin-1, but also on Vps34. In addition, it is reasonable to believe that TF1 mainly regulates autophagy after myocardial I/R injury in rats through Beclin-1/Vps34 complex formation and inhibits excessive autophagy.

Limitations

This study only explored the mechanism of TF1 based on the changes in cardiac I/R injury in rats, and did not examine other polyphenols. It needs to be further verified that whether the results and conclusions of the present study have broader significance. Furthermore, many molecules are involved in beclin-1 regulation of complex formation, but we only selected the main key molecules for the present study, and examined their protein molecular interactions for complex formation. Finally, autophagy is also regulated by mTOR and other signaling methods, and further studies are required to determine the effects of TF1 on multiple signaling methods [24].

Clinical Significance

The current treatment strategies for coronary artery disease include drugs, interventional therapy, and coronary artery bypass grafting. These methods often cause I/R injury, arrhythmia, myocardial infarction, and heart failure. I/R injury seriously affect cardiac function and can even lead to death. Therefore, prevention and treatment of I/R injury is important in the treatment of coronary heart disease. This study clarified the mechanism of TF1 from the molecular regulatory mechanism of autophagy, and thus provides not only theoretical and experimental support for the clinical efficacy of this drug, but also a good reference for studies on the common mechanism of action of polyphenols.

Acknowledgment

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Ethical Approval

This study was performed in strict accordance with all applicable
international, national, and/or institutional guidelines for the care and use of animals.

References