Inhibiting mTOR enhanced Cardiac STAT3 phosphorylation at site Ser 727 and attenuated Myocardial Ischemia Reperfusion Injury in diabetic rats

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Abstract:

Background: Reduced levels of myocardial STAT3 activity in diabetic hearts may contribute to the increased susceptibility to ischemia-reperfusion injury (I/RI). The protein mammalian target of rapamycin (mTOR) can regulate metabolism and cell processes and plays major roles in the dynamics of I/RI. However, the role of mTOR in the regulation of myocardial STAT3 and thereby its impact on myocardial I/RI in diabetes at relatively late stages of the disease is unknown.

Methods: Diabetes was induced by Streptozotocin in Sprague-Dawley rats. Myocardial I/RI was achieved with coronary occlusion for 30 minutes and reperfusion for 2 hours in absence or presence of the mTOR inhibitor rapamycin. In vitro cardiomyocyte hypoxia/re-oxygenation (H/R) was established in H9C2 cells.

Results: In diabetic rats, the levels of troponin-I (Tn-I), lipid peroxidation products 15-F2t-Isoprostane (15-F2t-Iso) and malondialdehyde (MDA), and the protein expression of mTOR were all significantly increased, while SOD release, the level of phosphorylated STAT3(p-STAT3-Ser727) were both significantly decreased compared to non-diabetic rats. Myocardial I/RI significantly increased the infarct size (IS) and further increased the mTOR activation and decreased p-STAT3-Ser727 compared to diabetic rats. The selective mTOR inhibitor rapamycin reversed these changes and conferred cardioprotective effect. In H9C2 cells, high glucose (HG) significantly increased lactic dehydrogenase (LDH) release, apoptosis cells, ROS production and the activation of mTOR, but decreased p-STAT3-Ser727. H/R further increased cellular injury, mTOR gene knock-down significantly reduced H/R injury.

Conclusion: Myocardial mTOR was enhanced in diabetes and contributed to I/RI. mTOR inhibition attenuated myocardial I/RI through increasing p-STAT3-Ser727.

Key words: Diabetes; myocardial ischemia reperfusion injury; mTOR; STAT3
Introduction:

Myocardial ischemia reperfusion injury (I/RI) is a leading cause of death especially for subjects with diabetes, but its underlying mechanisms are largely unknown. Myocardial infarction is irreversible and is the characteristic consequence of sustained myocardial ischemia with or without reperfusion. Increasing duration of ischemia causes progressive irreversible injury and thus, reducing infarct size is the first goal for I/RI protection. The pathophysiology of I/RI mainly includes autophagy, necrosis, apoptosis, necroptosis, pyroptosis and ferroptosis[1]. A great number of signaling molecules participated in I/RI process, for example, autacoids, such as adenosine and bradykinin; lipid molecules, and these have closed relationship with protein STAT3, a key protein in the cardiac pro-survival RISK and SAFE pathways. These two signaling pathways correlates with each other and play central roles in I/RI and ischemia preconditioning[2]. The mammalian target of rapamycin (mTOR) protein is a 289 KD serine/threonine kinase, which can regulate cell growth and survival[3], mTOR also plays important roles and functions as nutrient energy sensor facilitating the regulation of autophagy[4]. With respect to the heart, mTOR is a necessary component for the physiological regulation of functions related to cardiac structure and cardiac metabolism [3]. mTOR also facilitates completion of maintenance of normal microvascular barrier functions and endothelial permeability. Previous research has indicated that the PI3K/Akt/mTOR signaling pathway is mediated by insulin [4]. The pathway related to inhibition signaling for GSK-3β, the mTOR-dependent angiogenesis signaling pathway, and the mTOR activation-signaling pathway are major signaling pathways related to cardioprotection[5]. Das et al. reported that application of the mTOR inhibitor of rapamycin (0.25 mg/kg, i.p.) prior to ischemia consequently reduced I/RI in mice by inducing the activation of the
Janus kinase 2 (JAK2) Signal Transducer and Activator of Transcription signaling (STAT3). These findings demonstrated that mTOR was a possible regulator of STAT3. STAT3 has also been found to play central roles in maintaining cardiomyocyte function, modulating conditions in the cardiac microenvironment, and is known to communicate with cardiac fibroblasts[6, 7]. STAT3 participated in myocardial preconditioning, and the phosphorylation of STAT3 at sites of tyrosine705 and serine727 have been shown to confer cardioprotection. In Heusch et al studies, they demonstrated the STAT3 phosphorylation at tyrosine705 was significantly greater at 120 minutes reperfusion treatment with remote ischemic preconditioning in pigs. Activation of STAT3 was necessary for ischemic postconditioning in pigs, while RIPC in human needs STAT5 not STAT3, this may be because the underline signaling was different from human[8-11]. Our research has also indicated that STAT3 played crucial roles in both ischemic preconditioning and postconditioning cardioprotection [12, 13]. Furthermore, expression of STAT3 was found to have been reduced in the myocardium of patients afflicted with diabetes, and this may constitute the main reason why diabetes afflicted subjects were more likely to experience myocardial I/RI and were less responsive to protective treatments including pre- and postconditioning [13, 14]. However, whether or not mTOR functions as an upstream effector of STAT3 in cases of diabetic myocardial I/RI is unclear. Therefore, we sought to use streptozotocin (STZ) - induced diabetic rats and high glucose exposed H9C2 cells to facilitate investigations of the roles of mTOR-STAT3 in myocardial I/RI in diabetic conditions. Our main goal was to provide evidence facilitating the identification of novel treatment targets for myocardial I/RI in diabetes.
Methods

In Vivo Studies

Animals and diabetes model

All animal based experimental were examined and given approval by Institutional Committee for Animal Care and Animal Use of Wenzhou Medical University. We purchased 6-8-week-old specific pathogen free (SPF) male Sprague–Dawley rats (260 ± 10 g). We induced diabetes in rats by injection of streptozotocin (STZ; 60 mg/kg, STZ was dissolved in 0.1 M citrate buffer and pH = 4.5; Sigma, St. Louis, MO) into the tail vein and following the protocol as described in more detail previously [15]. Access to food and water for experimental animals was provided ad libitum. Conditions in the room where animals were maintained at a constant air temperature of 23 °C, a constant humidity = 50 %, and a 12hour (h) light and 12 h dark cycle.

Experimental procedure

We randomly assigned rats into following groups: (1) Ctrl: Control (2) D8w: 8-week-old diabetic rats; (3) D8w+I/R: D8w rats with ischemia/reperfusion (I/R); (4) D8w+I/R+Rapamycin: D8w rats treated with Rapamycin (Sigma-Aldrich, St. Louis, MO) before being subjected to I/R. Rats received 1 mg/kg/day of rapamycin via orogastric gavage during the final 4 weeks of experimentation[15].

We established I/R by ligating left anterior descending (LAD) arteries for 30 minutes (min) followed by 2 hours (h) reperfusion in the I/R afflicted groups as described before[16, 17]. TTC (1%, 2,3,5-triphenyltetrazolium chloride) based staining was used to facilitate the determinations of myocardial infarction as described [10]. Upon the completion of reperfusion, normal regions of left ventricles (LV) were defined by way of ligating the left anterior descending and infusing 5 %
Evans Blue (Sigma, St Louis, MO) through the right jugular vein. We then applied an overdose of pentobarbital through injection to euthanize rats. We subsequently dissected and froze hearts for 15 min at -20 °C, and then sliced the hearts into five 1-1.5 mm sections. Slices were submerged in 1% TTC (pH 7.4) for 20 min at room temperature to facilitate delineation of infarcted areas. We used TTC stained area as the targeted region to facilitate measures for area of survival. Images were captured through the use of an Axioplus Image-Capturing System (Zeiss). We subsequently analyzed the images by use of Axiovision Rel. 4.5 Image-Analyzing software.

Assessment of DNA fragmentation through terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

Measures of the levels of fragmentation of DNA were assessed using terminal deoxynucleotidyl transferase nick end labeling (TUNEL) assay kits provided by Roche Applied Science, Indianapolis, IN, USA. In brief, the cells were fixed by using 4 % polyoxymethylene on the slides. Then, the samples were washed thrice with PBS for 5 min each time, followed by 2 min of permeabilization using 0.1 % (v/v) Triton X-100. Samples were further permeabilized by using proteinase K (30 mg/ml, 30 min, 37 °C). We then washed the samples in PBS following all manufacturer’s protocols with respect to detection of apoptotic cells. DNase I was used to facilitate induction of DNA strand breaking and as positive control, whereby we omitted TdT from the mixture and reaction for our negatively oriented control. Positive identifications of TUNEL-based staining were observed and determined under fluorescence microscopy (TE2000U, Nikon, Tokyo, Japan) and by using a B-2A filter (excitation filter of 450-490 nm, 505 nm dichroic mirror, bandpass filter of 520 nm).

Dihydroethidium (DHE) staining

DHE staining was used for reactive oxygen species (ROS) detection following
manufacturer’s protocols. Red fluorescence was emitted when DHE was oxidized by superoxide, and it was detected by fluorescence microscopy (Olympus, Tokyo, Japan).

**mTOR siRNA gene silencing studies in H9C2 cells.**

We cultured H9C2 cardiomyocytes at 37 °C in a constant atmosphere of 5 % CO₂ and Dulbecco's modified Eagle's medium (DMEM) (Gibco Laboratories) containing 10% Fetal bovine serum (FBS) (Gibco Laboratories). We used commercially supplied mTOR siRNA (Santa Cruz Biotechnology, Texas, USA) to knock down mTOR expression following the manufacturer’s protocols. We seeded and assigned 2*10⁵ H9C2 cells each into 4 treatment groups composed as follows: (1) normal levels of glucose (NG): 5.5 mM; (2) high glucose (HG): 30 mM glucose; (3) HG + H/R; (4) HG + H/R + siRNA mTOR. With respect to control siRNA or mTOR siRNA treatment groups, we incubated H9C2 cells in either NG or HG in DMEM for 48 hours (h) prior to subjecting the H9C2 cells to H/R. 6 hours’ of hypoxic conditions were accomplished by equilibration of a humidified plexiglass chamber which contained myocytes and a constant atmosphere of 5%CO₂ and 95% N₂. Hypoxic conditions were confirmed by measurements of O₂ concentrations in chambers and when the oxygen levels fell to ≤ 0.1%. Post-hypoxia, we transferred cells to a CO₂ incubator to facilitate re-oxygenation for 12 hours and then we collected cells and mediums for further analyses.

**Lactate dehydrogenase (LDH) activity**

LDH is a major indicator of myocardial I/RI injury. Thus, we examined levels of LDH in H9C2 culture mediums by LDH Cytotoxicity Assay Kits (Roche, USA) followed the manufacturer’s protocols.

**Western blotting assays**

In our animal-based experiments, we homogenized rats’ LV tissues in 1× lysis
buffer acquired via Cell Signaling Technology (Beverly, MA) and centrifuged samples at 13, 200 g for 30 min, while H9C2 cells in ice-cold 1× lysis buffer were centrifuged for 10 min. The supernatant was collected as total protein. We then used Bradford protein assays to determine the protein concentrations. Then, we sampled equal amounts of protein homogenates, resolved by the SDS-PAGE (7.5-12.5%). Next, we transferred the samples to polyvinylidene nitrocellulose membranes and completed processing as described in previously [9, 10]. We purchased cleaved-caspase3, mTOR, phosphorylation-mTOR (p-mTOR), p-STAT3-Ser727, Total-STAT3, GAPDH antibodies, and secondary antibody all from Cell Signaling Technology (Beverly, MA). We detected protein bands through the standard protocols for the ECL method. Densitometry for protein band assessments was completed in Image J Software (National Institutes of Health, USA). We reported data for normalized protein band density in arbitrary units.

**Statistical analyses**

Analyses were performed in a blinded manner. We used the mean ± the standard error of the mean (± SEM) for values in statistical analyses. We used GraphPad Prism Software (Version 6.0) to complete statistical analyses and used one-way analysis of variance (ANOVA) and Tukey’s tests to facilitate determinations of statistical significance between different treatment groups. The level of significance at which the null hypothesis of no differences between treatment groups would be rejected was p<0.05.
Results:

**mTOR inhibition with Rapamycin attenuated myocardial I/RI in diabetes**

As Fig. 1A indicates, mTOR inhibitor rapamycin significantly reduced sizes of post-ischemic myocardial infarction (IS) (Fig. 1A; 8-week-old diabetic rats (D8w) +I/R+rapamycin vs. D8w +I/R) and significantly reduced the release of Tn-I (Fig. 1B; D8w+I/R+rapamycin vs. D8w+I/R). The levels of cardiac mTOR protein and phosphorylated mTOR (p-mTOR) increased significantly in D8w rats as compared non-diabetic control groups (Figs. 1C and 1D). In contrast, the levels of phosphorylation of STAT3 at Ser727 (p-STAT3-Ser727) were significantly decreased and while total STAT3 did not significantly differ between D8w rats and non-diabetic control, and I/R was further decreased p-STAT3-Ser727 (all \( p < 0.05 \); D8w vs. Ctrl, or D8w+I/R vs. D8w, Figs. 1E and 1F). Treatment with mTOR inhibitor rapamycin reduced the expression of mTOR and enhanced activation of p-STAT3-Ser727 during I/RI.
Fig. 1 The effects of mTOR inhibitor Rapamycin on post-ischemic myocardial injury. A: infarct size (IS); B: serum Tn-I release; C: mTOR protein expression; D: phosphorylated mTOR (p-mTOR) protein expression; E: protein expression of p-STAT3(Ser727); F: STAT3 protein expression. Ischemia reperfusion (I/R) was achieved by 30 minutes ischemia followed by 2 hours reperfusion in diabetic rats. Date are expressed as mean ± SEM (n = 6 per group), *p<0.05.
mTOR inhibition with Rapamycin decreased post-ischemic myocardial ROS production

As Figs. 2A, 2B and 2D indicated, the myocardial lipid peroxidation products 15-F2t-Iso and MDA were significantly increased that was concomitant with increased production of ROS evidenced as increased Dihydroethidium (DHE) staining in diabetic rats, while the SOD level was significantly decreased as compared to control (all \( p<0.05 \); D8w vs. Ctrl), and these changes were further exacerbated by I/R (\( p<0.05 \), D8w+I/R vs. D8w). Treatment with rapamycin reduced the post-ischemia myocardial ROS level induced by I/R and attenuated the increases in 15-F2t-Iso and MDA (all \( p<0.05 \); D8w+I/R+rapamycin vs. D8w+I/R).
Fig. 2 mTOR inhibition with Rapamycin decreased post-ischemic myocardial ROS production. A: 15-F2t-Isoprostone(15-F2t-Iso) release; B: MDA release; C: SOD activity; D: DHE staining. Ischemia reperfusion (I/R) was achieved by 30 minutes ischemia followed by 2 hours reperfusion in diabetic rats. Data are expressed as mean ± SEM (n = 6 per group), *p<0.05.
Effects of mTOR gene knockdown on hypoxia re-oxygenation (H/R) injury in H9C2 cells

Results exemplified in Fig.3A indicated that LDH release increased significantly induced under high glucose (HG) in comparison to normal glucose ($p < 0.05$, HG vs. NG). Moreover, H/R further increased the levels of LDH release and exacerbated cell injury ($p < 0.05$, HG+H/R vs. HG). mTOR gene knockdown via siRNA significantly suppressed H/R induced increases of LDH release and post-hypoxic cell injury under HG (all $p < 0.05$, HG+H/R+siRNA mTOR vs. HG+H/R). As shown in Figs. 3B and 3C, the expression of protein cleaved-caspase3 and numbers of apoptotic cells were significantly increased after HG treatment ($p < 0.05$; HG vs. NG). And, H/R further increased the levels of expression of cleaved-caspase3 and numbers of apoptotic cells ($p < 0.05$; HG+H/R vs. HG). Application of mTOR siRNA decreased the levels of expression of cleaved-caspase3 and numbers of apoptotic cells (Figs. 3B and 3C; all $p < 0.05$; HG+H/R+siRNA mTOR vs. HG+H/R). As exemplified in Fig. 3D, DHE staining indicated that HG induced a significant increase of ROS ($p < 0.05$; HG vs. NG), which was further significantly increased by H/R. mTOR gene silence with siRNA prevented H/R induced further increase of ROS under HG conditions (all $p < 0.05$; HG+H/R vs. HG; HG+H/R+siRNA mTOR vs. HG+H/R). The changes in the patterns of apoptotic cell death largely mirrored the changes in the levels of ROS production assessed by DHE staining (Fig. 3D).
Fig. 3. Effects of mTOR gene knockdown on hypoxia re-oxygenation (H/R) injury in H9C2 cells. A: LDH releasing; B: protein cleaved-caspase3 expression; C: TUNEL staining; D: DHE staining. In HG group, H9C2 cells were subjected to 30 mM high glucose for 48 hours, and then all cells subjected to 6 hours hypoxia and 12 hours reperfusion. Data are expressed as mean ± SEM of two independent experiments each performed in triplicate. n=6 per group. *p < 0.05.
**Effects of mTOR gene knockdown on mTOR and STAT3 protein expressions**

As shown in Figs. 4A and 4B, mTOR and p-mTOR proteins increased significantly under HG compared to NG ($p < 0.05$; HG vs. NG, Figs 4A, 4B) and H/R further exacerbated these increases ($p < 0.05$; HG+H/R vs. HG). After knockdown of mTOR, mTOR and p-mTOR were both significantly decreased ($p < 0.05$; HG+H/R+siRNA mTOR vs. HG+H/R, Figs. 4A, 4B). Fig. 4C demonstrated that total STAT3 was not affected by HG, H/R, or by mTOR gene silencing. The results showed that p-STAT3-Ser727 decreased significantly under HG as compared to NG ($p < 0.05$; HG vs. NG, Fig. 4D), and H/R further decreased p-STAT3-Ser727 in both HG and NG conditions ($p < 0.05$; NG+H/R vs. NG; HG+H/R vs. HG). In contrast, mTOR gene silencing restored the expression of p-STAT3-Ser727 that was otherwise reduced by H/R ($p < 0.05$; NG+H/R+siRNA mTOR vs. NG+H/R; HG+H/R+siRNA mTOR vs. HG+H/R, Fig. 4D).
Fig. 4 Effects of mTOR gene knockdown on mTOR and STAT3 protein expressions. A: protein mTOR expression; B: protein p-mTOR expression; C: protein STAT3 expression; D: protein p-STAT3(Ser727) expression. In HG group, H9C2 cells were subjected to 30 mM high glucose for 48 hours, and then subjected to 6 hours hypoxia and 12 hours reperfusion. Data are expressed as mean ± SEM of two independent experiments each performed in triplicate. n=6 per group. *P < 0.05.
Discussion:

The findings from our study indicated that hyperglycemia induced significant activation of cardiac mTOR and induced reductions in p-STAT3-Ser727 activation. Further, we showed that inhibition of mTOR could reduce myocardial I/R or H/R injury under HG condition through enhancement of p-STAT3-Ser727 activation. To our knowledge, our findings are the first to have characterized the dynamics of mTOR-p-STAT3-Ser727 with respect to myocardial I/RI in hearts impacted by diabetes, this offered additional insights regarding the mechanism of diabetic myocardial I/RI.

mTOR is a type of serine/threonine kinase which functions mainly as a nutrient and energy sensor, and plays essential roles in protein synthesis and autophagy, cell growth and survival[18, 19]. AMPK/mTOR has been identified as a major signaling pathway in the mTOR family and regulates autophagy. However, there is research which that AMPK did not directly regulate mTOR, but instead mTOR could be regulated by Akt [20]. In the current study, we found that the levels of mTOR and p-mTOR were both increased, and other research indicated that mTOR participates in the regulation of autophagy and can be regulated by other upstream effectors, including oxidative stress[21]. It has been reported that mTOR modulated autophagy and protected cardiomyocytes from oxidative stress-induced toxicity[22, 23], whereby vice versa, oxidative stress may provoke mTOR activation and subsequently impair autophagy[24]. Research has also indicated that mTOR played crucial roles in the dynamics underlying the balance of cardiac systems. mTOR has been shown to be essential in preventing apoptosis during ischemic postconditioning mediated cardiac protection in rats[23]. In our study, application of mTOR inhibitor rapamycin in in vivo study or mTOR gene knockdown in H9C2 cells both decreased the myocardial
I/RI or H/R injury, manifested by significantly decreased ROS level that was concomitant with reduced expression of protein cleaved-caspase3 and significant reduction in apoptotic cell death. Continuous activation of mTOR was detrimental to vascular systems[25], and thus reducing the activation of mTOR could reduce vasculopathy and improve microcirculatory coronary flows following cardiac transplantation[26, 27]. Based upon these findings, mTOR not only plays a key role as a regulator of autophagy but also plays an important role with respect to myocardial I/RI through its effect upon other types of signaling. In our study, we found that reduced activation of mTOR consequently decreased post-ischemic ROS levels and oxidative damage manifested as reductions in 15-F2t-Iso and MDA. Thus, we hypothesize that inhibition of mTOR in diabetes may have conferred myocardial protective effects through reducing oxidative stress, although the underlying mechanisms and dynamics merit additional rigorous investigations.

Signal transduction and activation of transcription 3 (STAT3) as the major member of signaling pathway of survival activating factor enhancement pathway (SAFE) has significant roles in myocardial I/RI especially diabetes [12]. The expression of STAT3 decreased in the heart of diabetes which may be the reason why diabetic hearts are prone to myocardial I/RI [22]. STAT3 was essential for ischemic preconditioning (IPC), ischemic postconditioning (IPostC) and remote IPC (RIPC). However, as Heusch et al reported, in separate IPC, IPostC and RIPC studies, the STAT3 phosphorylation at-Tyr705 (p-STAT3-Tyr705) were all significantly increased by IPC, IPostC or RIPC. However, when analyzed the phosphorylation of STAT3 and other signaling proteins in left ventricular biopsies of IPC, IPostC and RIPC in one approach, they only observed the STAT3 phosphorylation at-Tyr705 at early reperfusion was significantly increased along with infarct size reduction by IPC, only trend wise by IPostC and RIPC. Th
ese studies demonstrated that STAT3 phosphorylation at-Tyr705 is necessary conditioning cardioprotection but it may have its own specific time course in myocardial conditioning[2, 9, 10, 16]. Janus kinase 2 (JAK2) is one of the upstream effectors of STAT3 production, and as reported STAT3 Tyr705 was directly regulated by JAK2 whereas STAT3 Ser727 was regulated by ERK/MAPK [23]. Signal-transducing protein kinases are the mitogen-activated protein kinases (MAPK) that could be activated in cardiac cells under stress, it can phosphorylate various substrates including transcription factors such as abovementioned STAT3. MAPK activation needs both tyrosine and threonine phosphorylation, and MAPK may be upstream of ERK, JNK and p38 in myocardial ischemia, reperfusion and ischemic preconditioning as well as in other pathological conditions[28]. Some researchers have reported that STAT3 was also affected by protein kinase C (PKC)[13, 29], while other findings have provided evidence that STAT3 has effect on autophagy by way of serving as the downstream effector of mTOR[30]. In this study, we found that mTOR gene knockdown could subsequently restore I/R or H/R induced reduction in p-STAT3 Ser727 and attenuate myocardial I/RI. Recent research appears to support our findings in that treatments with rapamycin increased the levels of expression of p-STAT3-Tyr705[31]. Thus, our findings suggested that mTOR is an important regulator in myocardial I/RI. However, if mTOR regulates autophagy through STAT3, the dynamics and mechanism underlying this process need further research. Autophagy can have protective effects during ischemia while contrarily plays detrimental roles during reperfusion, thus, the relationship between STAT3 and autophagy deserves more research[32, 33]. Next, we may need to do an in-depth study using the inhibitor of STAT3 or cardiac specific STAT3 knock out to observe the role of STAT3 in diabetic myocardial I/RI. In our another study exploring the effects of remote ischemia preconditioning (RIPC), we demonstrated that STAT3 gene kn
ockdown cancelled RIPC mediated cardioprotective effect, and that RIPC conferred its cardioprotection against myocardial I/RI mainly through PKC-STAT3(Ser727)[13], in this study, we also did not observed the changes of p-STAT3-Tyr705. While, in the study of Heusch et al’ they found that phosphorylation of STAT3 at Tyr705 was essential in ischemia preconditioning, ischemia postconditioning and RIPC. This difference may be due to that we focused on studying different the upstream regulator of STAT3 and that the animal models used were also different. In the current study, we focused on exploring the role of mTOR in diabetic myocardial I/RI. In the following study, we shall do more research to define the roles and interplays in between mTOR and STAT3 and their impact on autophagy in the context of myocardial I/RI in diabetes.

Our study has the limitation that we just used the type 1 diabetes model, while in clinical settings most diabetic patients were type 2 diabetes. Compared to type 2 diabetes, the major pathological change of type 1 diabetes is the insulin deficiency and high glucose was the primary element. The type 2 diabetes was a polygenetic disease, it can be divided into two kinds, with or without obesity, and several genes can predispose individuals to developing the disease, so its mechanism is more diverse. Therefore, on the basis of our initial findings regarding the mechanism of myocardial I/RI in type 1 diabetes, future study shall be extended to study the impacts and mechanisms of type2 diabetes on myocardial I/RI.

Conclusion:

Our study demonstrated that increased activation of mTOR in the heart of type 1 diabetic rats causes subsequent reduction in p-STAT3-Ser727 and exacerbated myocardial I/RI. Further, we found that knockdown of mTOR can increase the expression p-STAT3-Ser727 and reduce cardiomyocyte I/R injury. The mechanistic
insights gained in the current study may help facilitate the development of targeted therapies against myocardial I/RI in diabetes.

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Declarations of interest

No conflicts of interest are declared by the authors.

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