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Inhibition of CACNA1H can alleviate endoplasmic reticulum stress and reduce myocardial cell apoptosis caused by myocardial infarction

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Abstract. – OBJECTIVE: In recent years, coronary heart disease (CHD) has become a disease that cannot be ignored by residents of our country, because CHD will not only endanger people's quality of life, but also threaten their lives. Therefore, this research mainly explores the correlation between myocardial infarction (MI) with endoplasmic reticulum (ER) stress and apoptosis.

MATERIALS AND METHODS: First, we constructed a model of myocardial ischemia and hypoxia (I/H) *in vivo* and *in vitro*, and examined the change of CACNA1H expression. At the same time, in order to research the role of CACNA1H, we chose CACNA1H-specific inhibitor ABT-639 to next research and detect changes in heart injury by detecting changes in creatine kinase (CK) content and lactate dehydrogenase (LDH) activity. Next, we used TUNEL staining and immunofluorescence staining to detect changes in apoptosis and ER stress, and analyzed changes in ER stress and apoptotic pathway expression by Western blotting and quantitative Real-Time Polymerase Chain Reaction (qRT-PCR).

RESULTS: At 28 days after MI, the cardiac function of the mice was significantly reduced, the myocardial cell apoptosis rate was dramatically increased, and CACNA1H expression was dramatically increased *in vivo* and *in vitro*. In addition, we treated the model group with the ABT-639, and found that ABT-639 can partially protect myocardial function and relieve myocardial cell apoptosis. At the same time, ABT-639 may reduce H9c2 injury after I/H by reducing the degree of ER stress, because we found that the use of ABT-639 can dramatically reduce ER stress-related factors expression, and can inhibit the expression of apoptosis-related factors Caspase-3 and Caspase-9.

CONCLUSIONS: The CACNA1H inhibitor ABT-639 can alleviate myocardial cell apoptosis caused by MI by reducing the ER stress response.

Key Words:
CACNA1H, ABT-639, Endoplasmic reticulum stress, Apoptosis, Myocardial cells.

Abbreviations

CHD: coronary heart disease; ER: endoplasmic reticulum; LDH: lactate dehydrogenase; CK creatine kinase; AMI: acute myocardial infarction; CAD: coronary atherosclerotic cardiopathy; PERK: protein kinase R-like endoplasmic reticulum kinase; CHOP: transcription factor C-EBP homologous protein; HF: heart failure.

Introduction

Acute myocardial infarction (AMI) is a serious result of coronary atherosclerotic cardiopathy (CAD). In recent years, the morbidity and mortality of CHD has been increasing rapidly in China. It is the fastest rising disease among the cause of death of residents in China[1]. Due to the rapid development of cardiac intervention and bypass surgery, great progress has been made in the treatment of CHD, but there are still many problems that have not been completely resolved, especially the prolonged myocardial ischemia leading to myocardial cell necrosis or apoptosis. Ventricular remodeling occurred in the myocardial infarction area and gradually developed into chronic cardiac insufficiency; and this could significantly increase the disability rate and mortality of patients[2,3]. In recent years, the promotion of surviving myocardial angiogenesis, improved blood supply, and increased myocardial cell activity have provided new ideas and directions for the treatment of CHD, and are currently the main research hotspots[4].

Low-voltage T-type calcium channels are widely distributed in various types of cells, including cardiovascular and neuronal cells. Unlike high-voltage calcium channels, they can be activated when the degree of depolarization is

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close to the resting potential of the membrane. Conducive to the regulation of excitability and electrical response of cardiac pacemakers and neurons when they are close to rest under physiological conditions[5]. We had a deeper understanding of the nature distribution, gene regulation and pharmacology for T type calcium channel in recent years[6] Transgenic animal researches have proven that T-type calcium channels are not only an important drug target for treating renal hypertension[7] but also for arrhythmias[8]. Therefore, we aimed to explore whether the expression of Cav3.2 (CACNA1H) is also potentially associated with MI.

Apoptosis is the main form of myocardial cell death. Some scholars[9] have demonstrated that ER stress is another apoptotic pathway in addition to the mitochondrial and death receptor pathways. Hypoxic-ischemic and other factors can cause cells to aggregate unfolded or misfolded proteins, activate ER-specific molecular chaperone glucose regulator protein 78 (GRP78), and then, initiate ER stress to promote unfolded or misfolded proteins to restore the correct conformation, but if the stress time is too long, it will start the ER stress apoptosis signal pathway GRP78/protein kinase R-like endoplasmic reticulum kinase (PERK)/transcription factor C-EBP homologous protein (CHOP), which induces apoptosis[10].

Materials and Methods

Animal

This study was approved by the Animal Committee of Taizhou People's Hospital. We used 30 males C57BL/6 mice, 8 weeks old, 23-25 g (Peking University Health Science Center, Beijing, China). Mice were fed in Animal Center after purchase, and were free to eat standard feed and water in a light/dark environment for 12 hours. 30 mice were randomly divided into three groups: control group, MI group, and MI + ABT-639 group. Mice in the control group and the ABT-639 group were reared with an equal amount of normal saline or ABT-639 (10 mL/kg, MedChemExpress, Monmouth Junction, NJ, USA) for 1 week. Five days after feeding saline and ABT-639, mice in the MI group and MI+ ABT-639 group underwent ligation of the proximal left descending coronary artery and then continued to be fed with saline or ABT-639 for 2 days.

Model Preparation

One week after the mice were adaptively fed, a model of MI was prepared with reference to the literature[11]. After fasting for 12 hours, the mice were anesthetized intraperitoneally with 2.5% sodium pentobarbital, then, the mice were fixed in supine position, and assisted breathing with a ventilator. We opened the thoracotomy in the left chest longitudinal incision, and carefully separated the pericardium, and gently pressed the right side chest wall to expose the heart. Then, we ligated the proximal end of the left anterior descending coronary artery with a 7-0 (Jinhuan, Shanghai, China) line 1 mm below the left atrial appendage, and the J-point elevation (0.2 mV) of the electrocardiogram after ligation, suggesting successful MI modeling. In the control group, the left coronary artery (LCA) was threaded only at the corresponding site without ligation. We excluded gas and residual blood in the chest, and closed the chest layer by layer. After the mice resumed spontaneous breathing, the trachea was intubated.

Echocardiogram

Four weeks after MI, we randomly selected 6 mice in each group (due to the mortality of MI surgery), anesthetized the mice with 1.5% isoflurane, and used Mylab30CV ultrasound (Biosound Esaote, Guangzhou, China) system to examine the mice in each group by transthoracic echocardiography and analyzed. The minimum and maximum areas of the left ventricle are shown as end-systolic and end-diastolic, respectively. M-mode ultrasound is used to measure left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS). The average was calculated using three consecutive and complete heart cycles.

Specimen Collection

After the echocardiographic examination, the mice were anesthetized intraperitoneally with 2.5% sodium pentobarbital, and then, the heart was removed quickly, the saline was rinsed and the excess water was blotted with filter paper. At the same time, the heart base and apex were excised and stored at -80°C for extraction of protein and RNA. The remaining part was placed in 4% paraformaldehyde for subsequent section staining.

Masson Stain

After the heart tissue was embedded in paraffin, the paraffin block was cut into 5 μm thick

slices with a slicer, and then, placed in an oven at 37°C for 1 day. We then stained them with a Masson kit (Jiancheng, Nanjing, China), and we observed and recorded the results under an optical microscope (Olympus, Tokyo, Japan).

Cell Culture

The H9c2 cells (Cell Culture Center, Shanghai, China) were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Life Technology, Wuhan, China) containing 10% fetal bovine serum (FBS; Life Technology, Wuhan, China), 1% penicillin, and streptomycin (Life Technology, Wuhan, China), and cultured in an incubator at 37°C, 5% CO_2 and 80% humidity. The cells were then grouped and given different stimuli. When the cells grew to a suitable density, H9c2 cells were cultured for 12 hours with I/H (serum-free and anaerobic environment) to construct a model *in vitro*. The cells of the ABT-639 group were treated with (10 μmol/l) ABT-639 and cultured for 12 h before I/H treatment.

Cell Counting Kit-8 (CCK8)

H9c2 cells were planted in a 96-well plate and treated separately according to the experimental group. After 0 h, 6 h, 12 h, 18 h, and 24 h of culture, 10 μL of CCK-8 reagent (Construction Group Co. Ldt., Nanjing, China) was added to each well and incubated for 2 h. The microplate reader was used to measure the light absorption at 450 nm, and the cell activity was calculated according to the formula.

TUNEL

TUNEL staining kit (Construction, Nanjing, China) was performed according to the manufacturer's instructions to determine DNA fragments of apoptotic cells. We observed them with a fluorescence microscope (BX63, Olympus, Tokyo, Japan), and after acquiring images, we used software merge. TUNEL positive cell rate = apoptotic positive cells / total number of cells.

Immunofluorescence

H9c2 cells of a certain density were plated in 6-well plates. When the cell density reached 60%-70%, H9c2 cells were given different treatments and continued to be cultured for 12 hours. Then, we blocked them with 5% goat serum for 2 h, and incubated with primary antibody (CHOP, Abcam, Cambridge, MA, USA, Rabbit, 1:500) at 4°C overnight. Next day, the cells were incubated with secondary antibody for 1 h, and we used DAPI (Construction, Nanjing, China) to stain cell nuclei. Images were taken using a fluorescence microscope (BX63, Olympus, Tokyo, Japan) and the percentage of positive cells was calculated.

Biochemical Indicator Detection

In order to test the heart function of each group of mice, we used CK kit (Jiancheng, Nanjing, China) and LDH kit (Jiancheng, Nanjing, China) and tested the corresponding indicators according to the instructions.

Flow Cytometry

The cells after different treatments were collected and washed 3 times with pre-chilled PBS, and then, the cells were treated according to the instructions of the apoptosis detection kit (KeyGEN Biotechnology, Shanghai, China). The cells were incubated with Annexin V-FITC (fluorescein isothiocyanate) and Propidium Iodide (PI) for 15 minutes each, and sent to the flow center for detection.

Western Blot

The heart tissue or cardiomyocytes were fully lysed with radioimmunoprecipitation assay (RIPA) lysate (Camilo Biological, Nanjing, China), and then, total protein was extracted. The bicinchoninic acid (BCA) method (Camilo Biological, Nanjing, China) was used to determine the protein concentration. After quantification, it was boiled in a water bath for 5 minutes and turned into stable protein. We took 20 μg of denatured protein for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis. The concentration gel (8%) voltage was 80 V, and the separation gel (10%) voltage was 120 V. After electrophoresis, we transferred the protein to a polyvinylidene difluoride (PVDF; Thermo Fisher Scientific, Waltham, MA, USA) membrane and blocked it with 5% skim milk for 2 h. Then, the PVDF membrane was incubated overnight at 4°C with a specific primary antibody (GRP78, Abcam, Cambridge, MA, USA, Rabbit, 1:1000; AFT6, Abcam, Cambridge, MA, USA, Rabbit, 1:2000; PERK, Abcam, Cambridge, MA, USA, Mouse, 1:2000; p-PERK, Abcam, Cambridge, MA, USA, Rabbit, 1:3000; Caspase3, Abcam, Cambridge, MA, USA, Rabbit, 1:2000; Caspase9, Abcam, Cambridge, MA, USA, Rabbit, 1:1000; β-actin, Proteintech, Rosemont, IL, USA, 1:2000). Next day, the PVDF membrane was incubated with secondary antibody (goat

anti-rabbit IgG antibody, Yifei Xue Biotechnology, Nanjing, China, 1:2000) for 1 h, and then, the PVDF membrane was developed with enhanced chemiluminescence (ECL), and observed with a gel imaging system. We finally took pictures. The β-actin was used as the internal reference to compare the gray values.

RNA Isolation and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

We used TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) to extract the total RNA from myocardial tissue and H9c2 cells, and then, we used ultraviolet spectrophotometry to determine RNA purity (Beckman Coulter, Miami, FL, USA). If the RNA purity was in the range of 1.9-2.1, it could be used. Subsequently, reverse transcription and polymerase chain reaction were performed according to the kit (Thermo Fisher Scientific, Waltham, MA, USA) instructions, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal reference, and the mRNA expression level of the target gene was calculated according to $2^{-\Delta\Delta Ct}$ method. Primers used were shown in Table I.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 software (SPSS IBM Corp., Armonk, NY, USA) was used to perform statistical analysis on all experimental data. The measurement data were expressed as mean ± SD (standard deviation). Differences between two groups were analyzed by using the Student's *t*-test. Comparison between multiple groups was done using One-way ANOVA test followed by post-hoc test (Least Significant Difference). $p<0.05$ was considered statistically significant.

Results

Inhibition of CACNA1H can Improve Cardiac Function in MI Mice

First of all, we found that the CK content (Figure 1A) and LDH activity (Figure 1B) of the mice in the MI group were markedly increased by testing the serum of the mice, which indicates that the cardiac function damage of the mice was still severe after MI. At the same time, we also found that mice fed with ABT-639 can alleviate some of the decrease in cardiac function. At the same time, we used echocardiography to test the heart function of the mice in each group, and found that the heart function of EF (Figure 1C) and FS (Figure 1D) of the mice in the MI group were dramatically reduced. Compared with the MI group, the heart functions of the ABT-639 group were significantly increased, but were still lower than the control group. In addition, Masson staining (Figure 1E) also showed that the myocardial fibrosis of the mice in the MI group was evident and the myocardial structure disorder was serious, while the myocardial fibrosis of the mice in the ABT-639 group was significantly reduced and the myocardial structure was also remarkably improved.

CACNA1H Inhibitor Protects Cardiomyocyte Activity and Reduces CACNA1H Expression

First, we tested the change of H9c2 cell activity after I/H treatment by CCK8 method (Figure 2A). As a result, we found that the activity of H9c2 cells significantly decreased with the prolongation of I/H treatment. At the same time, we also tested the effect of CACNA1H inhibitor ABT-639 on the activity of H9c2 cells (Figure 2B). CCK-8 results showed that ABT-639 had the highest activity at 10 μmol/l and 12 h in culture. Therefore, we chose 10 μmol/l for experiments. At the

Table I. Real time PCR primers.

Gene	Forward (5′>3′)	Reverse (5′>3′)
CACNA1H	CGTGACACTGGGCATGTTC	CCACCATCTTGATAACCATCTCC
GRP78	ACTTGGGGACCACCTATTCCT	ATCGCCAATCAGACGCTCC
PERK	GCGTCGGAGACAGTGTTTG	CGTCCATCTAAAGTGCTGATGAT
CHOP	CTGGAAGCCTGGTATGAGGAT	CAGGGTCAAGAGTAGTGAAGGT
Caspase-3	ATGGAGAACAACAAAACCTCAGT	TTGCTCCCATGTATGGTCTTTAC
Caspase-9	TCCTGGTACATCGAGACCTTG	AAGTCCCTTTCGCAGAAACAG
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC

qRT-PCR, quantitative Reverse Transcription-Polymerase Chain Reaction.

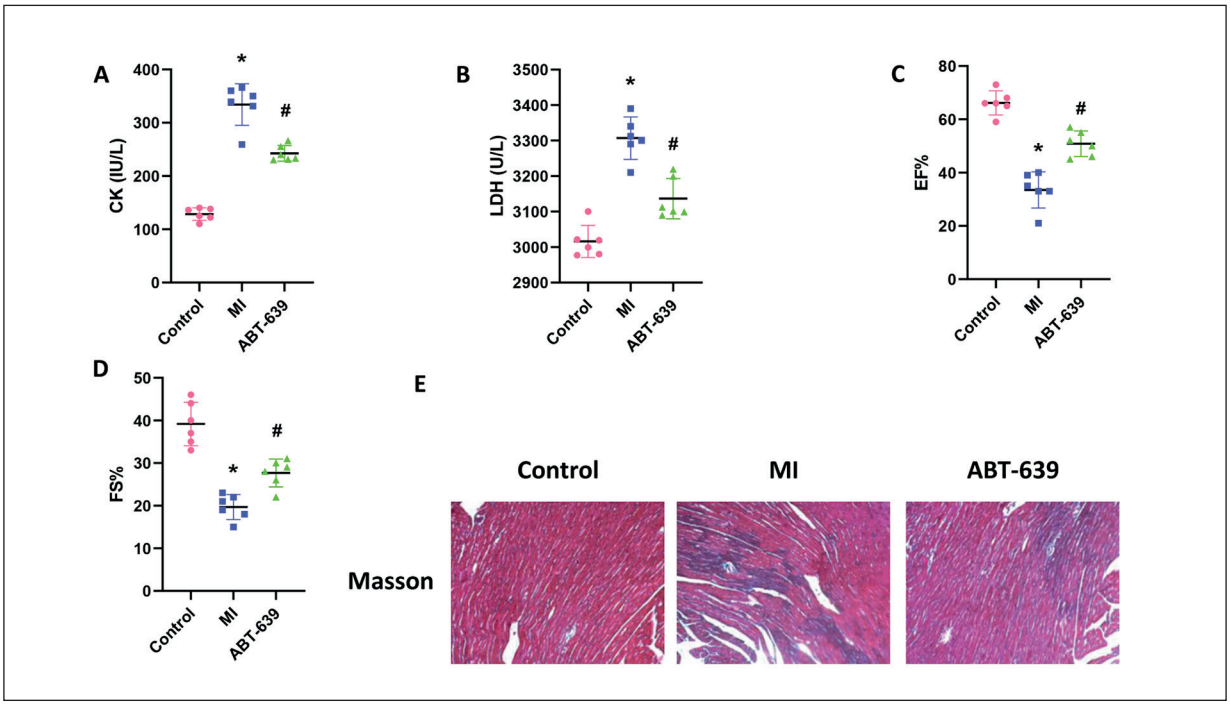

Figure 1. Inhibition of CACNA1H can improve cardiac function in MI mice. (**A**) CK content detection, (**B**) LDH activity detection, (**C**) Two-dimensional echocardiography detection EF value, (**D**) Two-dimensional echocardiography detection FS value, (**E**) Masson stain (magnification: 400×). ("*" indicates statistical difference from the control group $p<0.05$, "#" indicates statistical difference from the MI group $p<0.05$).

same time, we collected myocardial tissue and H9c2 cells, and detected CACNA1H expression by Western blotting and qRT-PCT technology (Figure 2C-2F). The results indicated that the expression of CACNA1H in the model group was significantly higher than that in the control group, both *in vivo* and *in vitro*. After using CACNA1H inhibitors, the expression of CACNA1H was significantly reduced.

Inhibition of CACNA1H can Inhibit I/H Induced Apoptosis and ER Response in H9c2 Cells

At the same time, in order to verify whether the CACNA1H inhibitor ABT-639 has an inhibitory effect on H9c2 cells, we used TUNEL staining (Figure 3A and 3B). The results showed that H9c2 cells had dramatically increased apoptosis after I/H treatment, and the apoptosis rate was markedly higher than the control group. After ABT-639 treated with H9c2 cells, it was found that the apoptosis rate was dramatically reduced. In addition, the results of immunofluorescence staining also found that ABT-639 can effectively inhibit the increase of CHOP into the nucleus induced by I/H treatment (Figure 3C and 3D). Therefore, we speculated that CACNA1H may be involved in myocardial cell apoptosis and ER stress induced by I/H treatment.

Inhibition of CACNA1H can Inhibit Cardiomyocyte Apoptosis and ER Response Caused by MI

In order to research the molecular mechanism of CACNA1H in MI injury, we detected changes in ER stress-related pathways by Western blotting (Figure 4A) and qRT-PCR (Figure 4B-4F). The results showed that the expressions of GRP78, AFT6, p-PERK, Caspase3 and Caspase9 were significantly increased in the MI group, while ABT-639 could effectively inhibit the increase of the expression of these molecules. Therefore, we speculated that MI can induce ER response and apoptosis in cardiomyocytes, and CACNA1H inhibitor ABT-639 can effectively inhibit the ER stress pathway, thereby alleviating apoptosis.

Discussion

AMI caused by acute ischemia of the coronary arteries. It has the characteristics of rapid onset

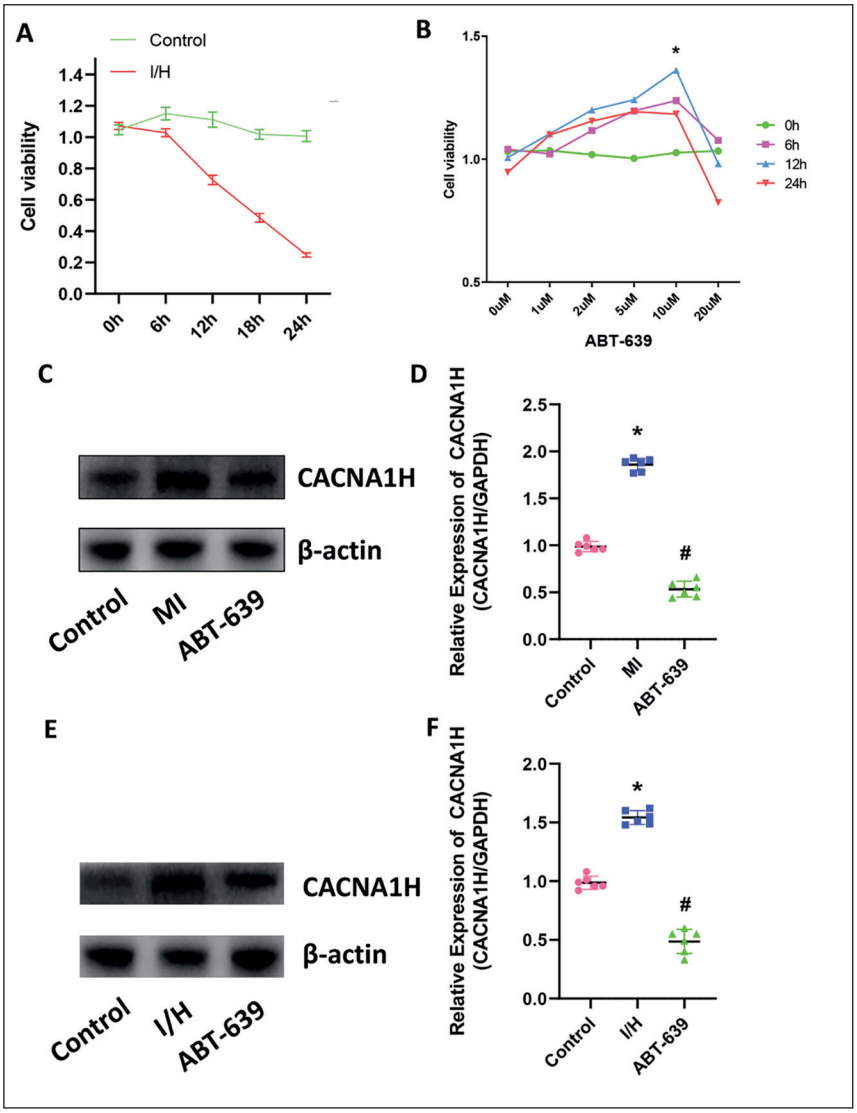

Figure 2. CACNA1H inhibitor protects cardiomyocyte activity and reduces CACNA1H expression. (**A**) CCK8 shows the effect of I/H treatment on H9c2 cells viability, (**B**) CCK-8 detects the effect of ABT-639 on H9c2 cells viability, (**C**) Western blot reveals CACNA1H expression *in vivo*, (**D**) qRT-PCR detects CACNA1H expression *in vivo*, (**E**) Western blot detects CACNA1H expression *in vitro*, (**F**) qRT-PCR discovers CACNA1H expression *in vitro*. ("*" indicates statistical difference from the control group $p<0.05$, "#" indicates statistical difference from the MI or I/H group $p<0.05$).

and rapid progress, has a very high mortality rate, and has a large difference in prognosis, which poses a great threat to patients' lives[12]. MI is one of the most common causes of acute heart injury. Although the treatment of MI has improved in recent years, the oxidative stress and inflammatory response triggered after MI further lead to the apoptosis of a large number of myocardial cells, thus reducing myocardial apoptosis is of great significance for clinical treatment of MI and improving prognosis[13,14].

In recent years, the ER stress pathway has attracted the attention of scholars at home and abroad. ER stress is closely related to apoptosis induced by ventricular remodeling after AMI[15]. The ER is an important organelle that regulates protein folding and Ca^{2+} homeostasis. When cells are exposed to hypoxia, hypoglycemia or ischemic environment, a large amount of free radical would accumulate and Ca^{2+} homeostasis would be destructed, which can cause ER dysfunction and lead to cell apoptosis[16]. The unfolded protein response is an ER marker molecule. Under stress conditions, GRP plays a role in protecting cells. When the stress is excessive and the stress time is too long, the ER stress apoptosis signal pathway GRP78/PERK/CHOP is activated. PERK induces increased expression of CHOP, activates Caspase-12 and promotes apoptosis[17]. In rat models of myocardial hypertrophy and heart failure (HF), GRP78 and CHOP expression remarkably increased[18]. Soejima et al[19] have reported that low glucose can promote the activation of the ER stress pathway, and increase the expression of GRP78, PERK, and CHOP pathway proteins. This research

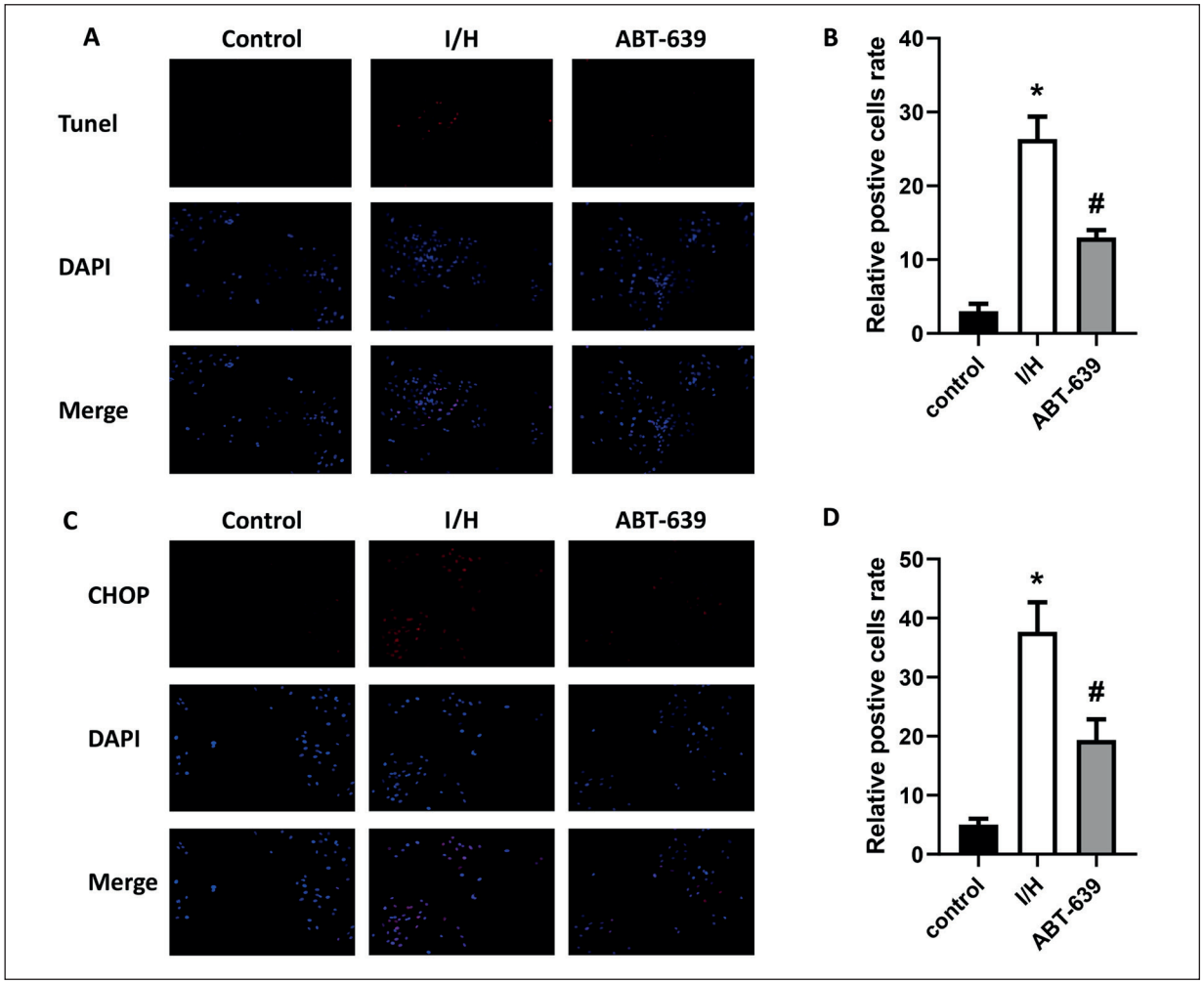

Figure 3. Inhibition of CACNA1H can inhibit I/H induced apoptosis and ER response in H9c2 cells. (**A**) TUNEL staining to detect apoptosis, (magnification: 400×); (**B**) TUNEL positive rate analysis, (**C**) immunofluorescence detection CHOP, (magnification: 400×); (**D**) CHOP positive rate analysis. ("*" indicates statistical difference from the control group $p<0.05$, "#" indicates statistical difference from the I/H group $p<0.05$).

showed that the expression of GRP78, p-PERK, AFT6, and CHOP in the model group dramatically increased. After ABT-639 treatment, the expressions of GRP78, p-PERK, AFT6, and CHOP were remarkably reduced. It suggests that MI promoted the activation of GRP78/PERK/CHOP pathway, while the CACNA1H inhibitor inhibited the activation of GRP78/PERK/CHOP pathway, which indicates that ABT-639 can alleviate apoptosis by inhibiting the ER stress pathway.

Researches have shown that the CHOP apoptotic pathway is closely related to the mitochondrial apoptotic pathway. CHOP can downregulate the expression of the Bcl-2, causing the translocation of Bax to mitochondria and exerting a pro-apoptotic effect[20]. In addition, CHOP can activate the expression of downstream Caspase-3 and Caspase-9 by activating Caspase-12, promoting the occurrence of Caspase cascade and promoting apoptosis[21]. The results of this research demonstrated that the expression of Caspase-3 and Caspase-9 were significantly increased *in vivo*, and the expression of Caspase-3 and Caspase-9 decreased after ABT-639 treatment, suggesting that CACNA1H inhibitor can inhibit cell apoptosis to exert its protective effect on cardiomyocytes.

At the same time, some studies have found that calcium channel inhibitors have a significant effect on regulating myocardial hypertrophy caused by heart failure and cardiac remodeling induced by cardiomyopathy. Calcium channel inhibitor-mibeferdil has a protective

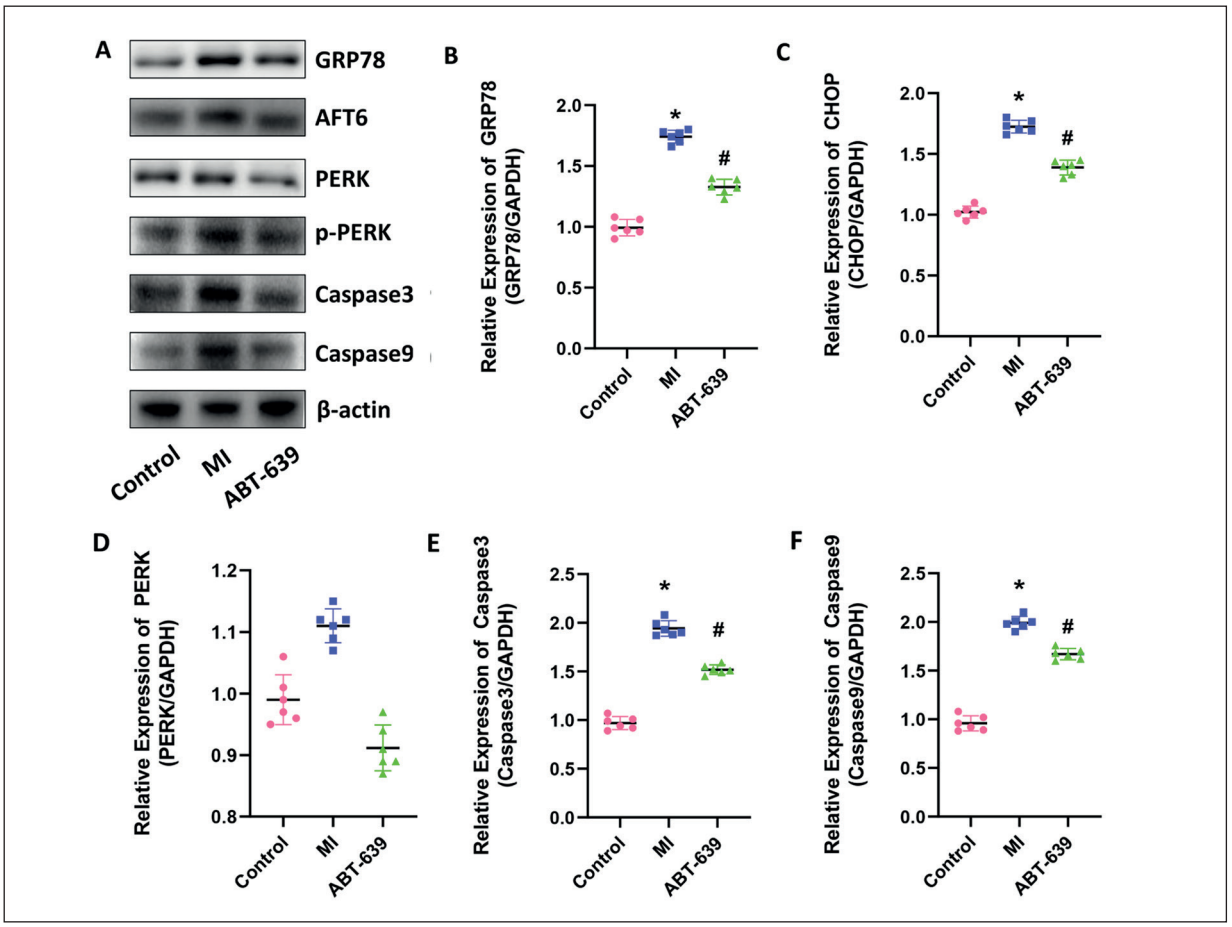

Figure 4. Inhibition of CACNA1H can inhibit cardiomyocyte apoptosis and ER response caused by MI. **A**, Western blot detection of GRP78. AFT6, PERK, p-PERK, Caspase-3, Caspase-9 expression *in vivo*, (**B**) qRT-PCR detection GRP78 expression *in vivo,* (**C**) qRT-PCR detection of CHOP expression *in vivo*, (**D**) qRT -PCR detection PERK expression *in vivo*, (**E**) qRT-PCR detection Caspase3 expression *in vivo*, (**F**) qRT-PCR detection Caspase9 expression *in vivo*. ("*" indicates statistical difference from the control group $p<0.05$, "#" indicates statistical difference from the MI group $p<0.05$).

effect on moderate to severe congestive heart failure[22]. In addition, T-channel blockers also have a role in dilating coronary arteries and cell proliferation[23]. Therefore, we speculate that the use of T-type calcium channel inhibitors in the non-acute phase to treat patients may reduce the symptoms of myocardial infarction. At present, many researchers are still studying whether T-type calcium channels can change heart function after MI. Our results also revealed that when we inhibit the expression of Cav3.2 (CACNA1H), we found that the apoptosis rate was significantly reduced. It is possible to ease the process of cardiac remodeling by regulating mechanical and electrical functions. This will provide new therapeutic targets for the prevention and treatment of MI.

Conclusions

Briefly, ABT-639 can alleviate MI, reduce the number of apoptotic cells, and improve myocardial histopathological changes. It may inhibit the activation of GRP78/PERK/CHOP pathways, and inhibit the apoptosis-related pathways, and play a protective role on myocardial cells.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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References

1) Pollard TJ. The acute myocardial infarction. Prim Care 2000; 27: 631-649.
2) Ersboll M, Valeur N, Hassager C, Sogaard P, Kober L. The association between renal impairment and cardiac structure and function in patients with acute myocardial infarction. Am Heart J 2014; 167: 506-513.
3) Biesbroek PS, Amier RP, Teunissen P, Hofman M, Robbers L, van de Ven PM, Beek AM, van Rossum AC, van Royen N, Nijveldt R. Changes in remote myocardial tissue after acute myocardial infarction and its relation to cardiac remodeling: a CMR T1 mapping study. PLoS One 2017; 12: e180115.
4) Li M, Wang Z, Xia H, Yu L, Hu Z. Vildagliptin and G-CSF improved angiogenesis and survival after acute myocardial infarction. Arch Med Res 2019; 50: 133-141.
5) Perez-Reyes E. Molecular physiology of low-voltage-activated t-type calcium channels. Physiol Rev 2003; 83: 117-161.
6) Chiang CS, Huang CH, Chieng H, Chang YT, Chang D, Chen JJ, Chen YC, Chen YH, Shin HS, Campbell KP, Chen CC. The Ca(v)3.2 T-type Ca(2+) channel is required for pressure overload-induced cardiac hypertrophy in mice. Circ Res 2009; 104: 522-530.
7) Sugano N, Wakino S, Kanda T, Tatematsu S, Homma K, Yoshioka K, Hasegawa K, Hara Y, Suetsugu Y, Yoshizawa T, Hara Y, Utsunomiya Y, Tokudome G, Hosoya T, Saruta T, Hayashi K. T-type calcium channel blockade as a therapeutic strategy against renal injury in rats with subtotal nephrectomy. Kidney Int 2008; 73: 826-834.
8) Le Quang K, Benito B, Naud P, Qi XY, Shi YF, Tardif JC, Gillis MA, Dobrev D, Charpentier F, Nattel S. T-type calcium current contributes to escape automaticity and governs the occurrence of lethal arrhythmias after atrioventricular block in mice. Circ Arrhythm Electrophysiol 2013; 6: 799-808.
9) Iurlaro R, Munoz-Pinedo C. Cell death induced by endoplasmic reticulum stress. FEBS J 2016; 283: 2640-2652.
10) Nie J, Duan Q, He M, Li X, Wang B, Zhou C, Wu L, Wen Z, Chen C, Wang DW, Alsina KM, Wehrens X, Wang DW, Ni L. Ranolazine prevents pressure overload-induced cardiac hypertrophy and heart failure by restoring aberrant Na(+) and Ca(2+) handling. J Cell Physiol 2019; 234: 11587-11601.
11) Reichert K, Colantuono B, McCormack I, Rodrigues F, Pavlov V, Abid MR. Murine left anterior descending (LAD) coronary artery ligation: an improved and simplified model for myocardial infarction. J Vis Exp 2017. Doi: 10.3791/55353.
12) Lu L, Liu M, Sun R, Zheng Y, Zhang P. Myocardial Infarction: symptoms and treatments. Cell Biochem Biophys 2015; 72: 865-867.
13) Neri M, Fineschi V, Di Paolo M, Pomara C, Riezzo I, Turillazzi E, Cerretani D. Cardiac oxidative stress and inflammatory cytokines response after myocardial infarction. Curr Vasc Pharmacol 2015; 13: 26-36.
14) Bezerra OC, Franca CM, Rocha JA, Neves GA, Souza P, Teixeira GM, Malfitano C, Loleiro T, Dourado PM, Llesuy S, de Angelis K, Irigoyen M, Ulloa L, Consolim-Colombo FM. Cholinergic stimulation improves oxidative stress and inflammation in experimental myocardial infarction. Sci Rep 2017; 7: 13687.
15) Zhao N, Mi L, Zhang X, Xu M, Yu H, Liu Z, Liu X, Guan G, Gao W, Wang J. Enhanced MiR-711 transcription by PPARgamma induces endoplasmic reticulum stress-mediated apoptosis targeting calnexin in rat cardiomyocytes after myocardial infarction. J Mol Cell Cardiol 2018; 118: 36-45.
16) He Q, Zhou X, Liu Y, Gou W, Cui J, Li Z, Wu Y, Zuo D. Titanium dioxide nanoparticles induce mouse hippocampal neuron apoptosis via oxidative stress- and calcium imbalance-mediated endoplasmic reticulum stress. Environ Toxicol Pharmacol 2018; 63: 6-15.
17) Yang MY, Wang YB, Han B, Yang B, Qiang YW, Zhang Y, Wang Z, Huang X, Liu J, Chen YD, Ren J, Cao F, Xu Y. Activation of aldehyde dehydrogenase 2 slows down the progression of atherosclerosis via attenuation of ER stress and apoptosis in smooth muscle cells. Acta Pharmacol Sin 2018; 39: 48-58.
18) Ge Y, Li G, Liu B, Guo H, Wang D, Jie Q, Che W, Hou L, Wei Y. The protective effect of lacidipine on myocardial remodeling is mediated by the suppression in expression of GPR78 and CHOP in rats. Evid Based Complement Alternat Med 2015; 2015: 945076.
19) Soejima E, Ohki T, Kurita Y, Yuan X, Tanaka K, Kakino S, Hara K, Nakayama H, Tajiri Y, Yamada K. Protective effect of 3-hydroxybutyrate against endoplasmic reticulum stress-associated vascular endothelial cell damage induced by low glucose exposure. PLoS One 2018; 13: e191147.
20) Seervi M, Rani A, Sharma AK, Santhosh KT. ROS mediated ER stress induces Bax-Bak dependent and independent apoptosis in response to thioridazine. Biomed Pharmacother 2018; 106: 200-209.
21) Lakshmanan AP, Thandavarayan RA, Palaniyandi SS, Sari FR, Meilei H, Giridharan VV, Soetikno V, Suzuki K, Kodama M, Watanabe K. Modulation of AT-1R/CHOP-JNK-Caspase12 pathway by olmesartan treatment attenuates ER stress-induced renal apoptosis in streptozotocin-induced diabetic mice. Eur J Pharm Sci 2011; 44: 627-634.
22) Villame J, Massicotte J, Jasmin G, Dumont L. Effects of mibefradil, a T- and L-type calcium channel blocker, on cardiac remodeling in the UM-X7.1 cardiomyopathic hamster. Cardiovasc Drugs Ther 2001; 15: 41-48.
23) Quignard JF, Frapier JM, Harricane MC, Albat B, Nargeot J, Richard S. Voltage-gated calcium channel currents in human coronary myocytes. Regulation by cyclic GMP and nitric oxide. J Clin Invest 1997; 99: 185-193.