European Review for Medical and Pharmacological Sciences 2021; 25: 5490-5499

Mechanism of curcumin against myocardial ischaemia-reperfusion injury based on the P13K/Akt/mTOR signalling pathway

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Abstract. – OBJECTIVE: To investigate the pharmacodynamic mechanism of curcumin against myocardial ischaemia-reperfusion injury by regulating the phosphatidylinositol-3 kinase (PI3K)/protein kinase B (AKT)/rapamycin target protein (mTOR) signalling pathway.

MATERIALS AND METHODS: The left anterior descending coronary artery was ligated for 30 min and reperfused for 3 h to establish an ischaemia-reperfusion injury model. The electrocardiogram (ECG) detection of rats was performed, and the degree of myocardial infarction was determined by 2,3,5-triphenyltetrazolium chloride staining. The expression levels of serum creatine kinase isoenzyme (CK), lactate dehydrogenase (LDH), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), nitric oxide (NO) and other related indicators were detected. The protein expressions of mTOR, phosphorylated (p)-mTOR, AKT and p-AKT were detected by Western blotting, whereas the expressions of Bcl-2 and Bax were detected by real-time polymerase chain reaction.

RESULTS: The results showed that compared with the model group, curcumin could improve the ECG findings, reduce the scope of myocardial infarction, reduce the expression levels of CK-MB, LDH, AST, MDA, NO and increase those of SOD and GSH. Curcumin can also down-regulate the expression of Bax and up-regulate the protein levels of Bcl2, p-mTOR and p-AKT ($p < 0.05$ or $p < 0.01$).

CONCLUSIONS: This study shows that curcumin has a significant protective effect on myocardial ischaemia–reperfusion, and its mechanism may be related to the activation of PI3K/AKT/mTOR signalling pathway and inhibition of inflammation, apoptosis and oxidative stress.

Key Words:
Curcumin, Myocardial ischaemia-reperfusion injury, PI3K/Akt/mTOR signaling pathway.

Introduction

In recent years, cardiovascular disease (CVD) has shown high incidence and mortality and has become a serious threat to human health. According to the World Health Organisation (WHO), CVD is the leading cause of death in the world. Currently, according to incomplete statistics, approximately 16.97 million people die from CVDs globally, accounting for 30.3% of the total global causes of death. CVD is the leading cause of death among Chinese urban and rural residents. According to the '2016 China Cardiovascular Disease Report,' China has 290 million CVD patients[1].

At present, ischaemic heart disease (IHD) and congestive heart failure are the main reasons for the high incidence and mortality of CVDs. Coronary artery recanalisation can effectively reduce the mortality of patients with IHD. However, the recovery of blood perfusion in ischaemic myocardium will further aggravate the damage of myocardial structure and function, including the abnormal function of myocardial cells and cell death. Such a condition is called myocardial ischaemia-reperfusion injury[2] (MIRI). MIRI is widespread in clinics and an important reason for the failure of clinical thrombolytic therapy, coronary ar-

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tery bypass grafting and heart transplantation. However, its mechanism has not been fully elucidated. The research on the pathogenesis and prevention of MIRI has become one of the important research topics in recent years.

Given that cardiomyocytes are terminally and non-renewable differentiated cells, autophagy is used to remove over-aged or excessive proteins and ageing organelles in cells; this process is particularly important for maintaining cell survival and function. Autophagy is involved in the pathological process of myocardial injury and one of the important mechanisms of MIRI[3-5]. Phosphatidylinositol-3 kinase (PI3K)/protein kinase B (AKT)/rapamycin target protein (mTOR) is a classic autophagy pathway in cells, and it can achieve the renewal and self-metabolism of organelles by eliminating damaged intracellular organelles, such as mitochondria[6]. However, Mobeirek et al[5] has shown that autophagy is involved in a variety of myocardial pathological processes, including MIRI, with both beneficial and harmful effects. Thus, the mechanism of autophagy and the protective effect of PI3K/AKT/mTOR signalling pathway on MIRI in rats must be studied[7-9].

Curcumin[10-12] is a kind of natural active ingredient extracted from the dried rhizomes of turmeric. Some studies[11,12] have shown that curcumin has antioxidant, anti-apoptotic and anti-inflammatory activities, and inflammation, apoptosis and oxidative stress are important pathogenic mechanisms of MIRI. This study aimed to observe and investigate the autophagy effect of curcumin preconditioning on MIRI in rats, to explore its effect on the expression of important proteins in the PI3K/AKT signalling pathway and to provide a theoretical basis for the rational clinical application of curcumin.

Materials and Methods

Materials

The materials used in this study included the following: curcumin (Sigma, Sigma-Aldrich Trading Co., Ltd, Shanghai, China), P13Ks (CST, USA), phosphorylated AKT (p-AKT; CST, USA), AKT (CST, USA), mTOR (CST, USA), lactate dehydrogenase (LDH), superoxide dismutase (SOD), malondialdehyde (MDA), creatine kinase isoenzyme (CK-MB), glutathione peroxidase (GSH-Px), nitric oxide (NO) Kit (Nanjing Jiancheng Institute of Biological Engineering, Nanjing, China); 2,3,5-triphenyltetrazolium chloride (TTC), chloral hydrate (Beijing Soleibao Biotechnology Co., Ltd., Beijing, China); B-cell lymphoma/leukaemia-2 (Bcl-2), Bcl-2-related X Protein (Bax) and mRNA Detection Kit (Shanghai Haoran Biotechnology Co., Ltd., Shanghai, China).

Instruments

The following instruments were used in the experiments: HX-300 breathing device (Chengdu Taimeng Technology Co., Ltd., Chengdu, China), XL-200 low-speed centrifuge instrument (Hamen Qilin Bell Instrument Factory, China), Pico-17 low-temperature desktop centrifuge (USA), vertical −80°C refrigerator (Qingdao Haier Instrument Co., Ltd.,), EM-UC6 ultra-thin sectioning instrument (Leica, USA), one-thousandth electronic balance (Ohaus Shanghai Trading Company, Shanghai, China), VE-180 vertical electrophoresis and electroporation instrument (Shanghai Tianneng, Shanghai, China), Bio-Rad gel imaging system (Bio-Rad, USA), decolorising shaker (Hamen Qilin Bell Company, China) and constant-temperature electric heating incubator (Shanghai Qixin Instrument Company, Shanghai, China).

Experimental Animals

A total of 60 healthy male SD rats (SPF grade and weighing 160-200 g) were purchased from the animal experiment centre and raised in the SPF animal laboratory. The indoor temperature was controlled at 22°C-24°C, and the relative humidity was controlled at 60%-64% and maintained at 12 h day and night cycle. Before the experiment, all rats were allowed to eat and drink freely for one week. The experiments complied with the relevant guidelines and regulations of medical ethics.

Methods

Establishment of H9c2 Cardiomyocyte Hypoxia/Reoxygenation (H/R) Model

Rat H9c2 cells were selected to simulate the ischaemia-reperfusion cell injury[13]. With the H9c2 cells in the logarithmic growth phase, and when the cells were fully attached and in good condition, the original medium was discarded, replaced with a sugar-free and serum-free medium and placed in a hypoxic bag. After 4 h, the hypoxic bag was removed, the sugar-free and

serum-free medium was replaced with Dulbecco's Modified Eagle Medium (DMEM) low-sugar complete medium containing 10% foetal bovine serum (FBS). The bag was placed in the incubator for 4 h to reoxygenate. Finally, the H9c2 cardiomyocyte H/R model was established.

Cell Viability Detected by Cell Counting Kit-8 (CCK-8)

CCK-8 was used to detect the effect of curcumin on the viability of H9c2 cells in the concentration range of 1.5-200 μg/mL. H9c2 cells were inoculated in a 96-well plate at a density of 4000 cells per well and incubated in a constant-temperature incubator at 37°C for 24 h. Then, the supernatant was discarded, and a concentration gradient of curcumin solution was introduced as the experimental group. The concentration gradient values were 1.5, 3.125, 6.25, 12.5, 25, 50, 100 and 200 μg/mL, and the cells cultured with the medium only were used in the control group. After 24 h of incubation, the cells were washed with Hank's balanced salt solution buffer. A total of 100 μL CCK-8 solution diluted with the culture medium was added to each well. The wells containing CCK-8 solution only and no cells were used as the blank control group and incubated in a constant-temperature incubator at 37°C for 1.5 h. The absorbance (optical density (OD) value) was measured at 450 nm with a microplate reader, and the cell viability was calculated in accordance with the following formula: Cell viability (%) = (OD value of test well-OD value of blank well)/(OD value of control well- OD value of blank hole) × 100%.

SOD, LDH and MDA Detection

Each group (curcumin concentration: 12.5, 25 and 50 μg/mL) used a cell culture fluid to detect the LDH activity. The cardiomyocytes were collected and sonicated, and the levels of MDA and SOD in the cardiomyocytes were detected.

Animal Grouping and Administration

Exactly 50 SD rats were randomly divided into the Sham operation (Sham), model (IRI) and curcumin (Cur) groups, with ten rats included in each group. The Cur group was given curcumin (25, 50 and 100 mg/kg) intraperitoneally 30 min before operation. The Sham and IRI groups were given an equal volume of normal saline through intraperitoneal injection.

Establishment of the Rat Ischaemia-Reperfusion Model

Before surgery, the rats were fasted for 12 h and allowed to drink water. On the 7th day, the rats were administered with their corresponding treatments, and 30 min later, the surgery was performed. Before the operation, the rats were weighed, anesthetised with 10% chloral hydrate (3.5 mL/kg) intraperitoneally and fixed in the supine position. The electrodes were inserted under the skin of the rat's limbs, and the BL-420F biological function experiment system was connected to observe the electrocardiogram (ECG) under normal conditions. The No. 18 intravenous indwelling needle was inserted into the trachea through the mouth. Then, the small animal ventilator was connected, the breathing rate was set to 80 times/min, the tidal volume was set to 80 mL/kg, and the breathing ratio was 1.5:1. The skin was cut at the left heart of the sternum, and the muscles were bluntly separated until the ribs became visible. Then, the thoracic cavity was opened through the 3rd to 4th intercostal space, and the pericardium was torn carefully and marked with the accompanying left coronary vein at the lower edge of the left atrial appendage 1 at ~2 mm. The left anterior descending coronary artery was ligated with No. 6-0 surgical suture through the myocardium surface. A slip knot was tied to block the blood supply, and the ligature was loosened after 30 min. The reperfusion for 3 h caused myocardial ischaemia and reperfusion, resulting in the perfusion injury model. The ECG ST elevation and myocardial cyanosis in the ischaemic area were signs of successful ligation. In the Sham group, the left anterior descending coronary artery was threaded without ligation.

Measurement of Myocardial Infarction Area in Rats

After reperfusion, the heart was removed, washed with 4°C normal saline, frozen at −20°C for 20 min, retrieved and cut into 2-3 mm slices. The slices were placed in the TTC dye solution and incubated in a 37°C water bath for 20-30 min. The slices were turned over every 5 min. After staining, the slices were fixed with 10% formaldehyde solution for 24 h. Then, images were captured and analysed with Image Pro Plus 6.0 software to calculate the area rate of myocardial infarction.

IS denotes the infarct size, and AAR is the area at risk of myocardial ischaemia. Myocardial infarction area (IS/AAR)% = (sum of infarct area/whole heart area) × 100%.

Western Blot Detection of Autophagy Protein Expression in Rat Myocardial Tissue

Based on the number of tissues, 9 volumes of phosphate-buffered saline (1:9) were added. The tissues were crushed with a homogeniser and centrifuged at 12000 rpm for 10 min. The supernatant, which is the cytoplasmic protein sample required for the experiment, was obtained. The total protein was extracted with radioimmunoprecipitation assay (RIPA) lysis buffer containing protease inhibitor and phosphatase inhibitor, and the proteins were separated on 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a polyvinylidene difluoride membrane (PVDF; Millipore, Billerica, MA, USA). The membrane was blocked with BSA at 25°C for 1 h, incubated with primary antibodies overnight at 4°C glyceraldehyde-3-phosphate dehydrogenase (GAPDH), mTOR, p-mTOR, Akt, p-Akt, P13K and p-P13K) and then coupled with horseradish peroxidase at 25°C. The combined secondary antibodies were incubated for 1 h. Finally, the electrochemiluminescent liquid was added to the film in a dark room for 5 min. After scanning the image, the band was analysed by ImageJ software (National Institutes of Health, Maryland, MD, USA). The OD ratio of the target protein to the inner GAPDH band was calculated.

Determination of the Activity of Serum Myocardial Injury Marker Enzymes CK-MB and LDH

A total of 6 mL blood was drawn from the abdominal aorta of the rat, and it was allowed to stand for 1 h at 25°C and centrifuged at 3500 rpm for 10 min at low temperature. The serum aliquot was obtained and stored at −80°C for later use. The serum was removed during the measurement and thawed quickly. The serum CK and LDH were determined in strict accordance with the kit instructions.

SOD, GSH, NO and MDA Detection

The kit instructions were strictly followed to determine the levels of SOD, GSH-Px and MDA. The NO content and nitric oxide synthase (NOS) activity were determined in accordance with the instructions. The enzyme-linked immunosorbent assay was used to detect the NO content in the serum, and ultraviolet spectrophotometry was used to determine the NOS activity.

Detection of the Expressions of Bcl-2 and Bax mRNA by Real-Time Polymerase Chain Reaction (PCR)

The apical myocardial tissue was obtained, chopped and ground. The total RNA was extracted by TRIzolf, and the purity and integrity of the RNA were detected by agarose gel electrophoresis. The sample with absorbance A260/A280 $\geq$ 1.80 was considered a qualified sample. Using the TaqMan Reverse Trascription Reagents kit, the diluted RNA sample was subjected to reverse transcription to synthesise cDNA, and the PCR was performed afterward.

The primer sequences are as follows: β-actin (189 bp), upstream primer 5′-CCACCATGTACCCAGGCATT-3′ and downstream primer 5′-CGGACTCATCGTACTCCTGC-3′; Bcl-2 (90 bp) upstream primer 5′-GATTGTGGCCTTCTTTGAGT-3′ and downstream primer 5′-CACAGAGCGATGTTGTCC-3′; Bax (85 bp), upstream primer 5′-TGAGCTGACCTTGGAGCA-3′, downstream primer 5′-GTCCAGTTCATCGCCAAT-3′.

The PCR programme was as follows: 95°C for 5 min; 95°C for 10 s and 60°C for 30 s; 40 cycles. β-Actin was set as the internal control. The relative expression of mRNA was calculated using the $2^{-\Delta\Delta}$Ct method.

Statistical Analysis

SPSS 20.0 statistical software (IBM, Armonk, NY, USA) was used for the analysis. The data are expressed as mean ± standard deviation, and comparisons between multiple groups were performed by one-way analysis of variance (ANOVA). $p < 0.05$ indicated a statistically significant difference.

Results

Cell Viability Detected by CCK-8

The CCK-8 method reflects cell viability through mitochondrial activity. Figure 1 shows the results of CCK-8 detection and the cell viability map of H9c2 cells after the treatment with curcumin solution with final concentrations of 1.5, 3.125, 6.25, 12.5, 25, 50, 100 and 200 μg/mL for 36 h. The figure shows that the survival rate of H9c2 cells treated with curcumin had a certain dose effect, that is, as the concentration of curcumin increased, the survival rate of cells gradually decreased. When the concentration reached 100 μg/mL, the absorbance decreased slightly. However, compared with the control group, the

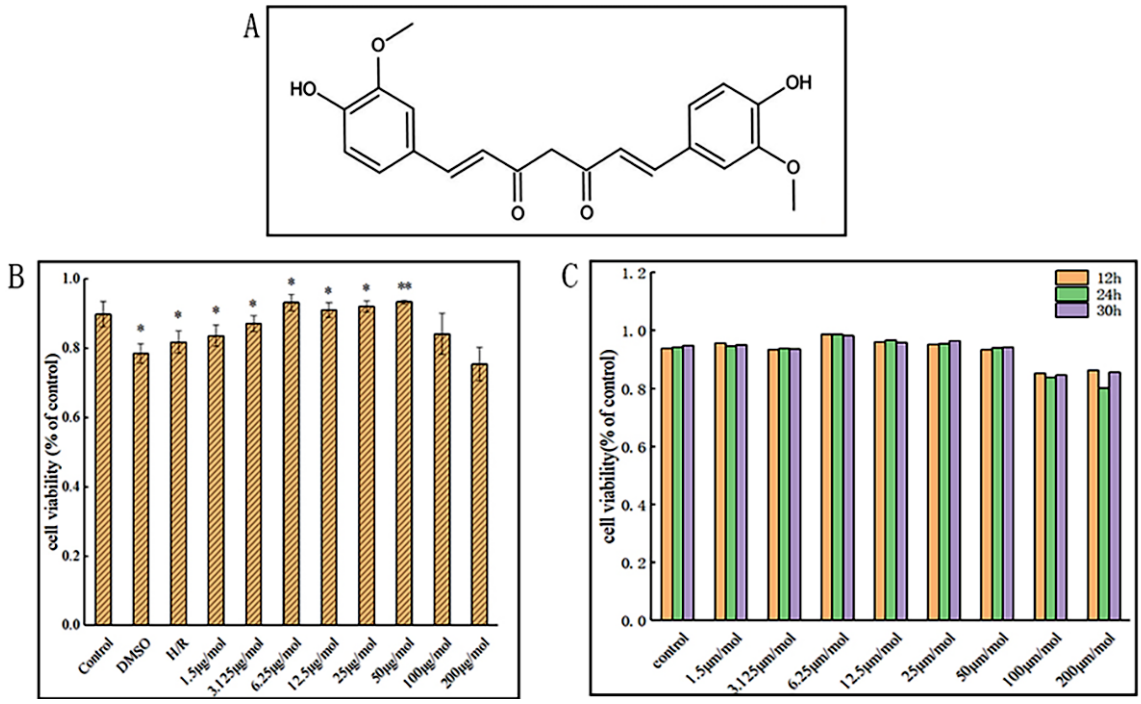

Figure 1. The cell viability diagram of H9c2 cells after curcumin acts on H9c2 cells for 36 h. **A**, the structural formula of curcumin. **B**, **C**, detection of the effect of curcumin on the viability of H9c2 cells by CCK-8 method.

absorbance of the treatment group was significantly higher. The results showed that curcumin had no significant effect on the cell proliferation rate.

We observed that curcumin exhibited no effect on cell viability. Curcumin was positively correlated with the cell survival rate at 100 μg/mL. A curcumin concentration of 200 μg/mL was positively correlated with the cell survival rate, indicating that high concentrations of curcumin had a certain damaging effect on cardiomyocytes.

Detection of Cell Viability Damaged by Hypoxia and Reoxygenation

For verification, we further studied the effect of different concentrations of curcumin on the viability of H/R-injured cardiomyocytes. After the cardiomyocytes were successfully cultured for 2-3 days, the experiment was divided into (1) control group; (2) DMSO; (3) H/R model group; (4) different concentration Cur+H/R group. After reoxygenation, the cell viability of each group was detected by CCK-8. As shown in Figure 2, compared with the normal control group, the cell viability of the IRI group was significantly reduced ($p < 0.05$). Compared with the IRI group under each dose of curcumin, the cell survival rate was significantly improved ($p < 0.05$). Compared with the blank control group, 1.5 μg/mL curcumin exhibited a certain protective effect, and curcumin at a concentration of 200 μg/mL showed an evident cell-killing effect.

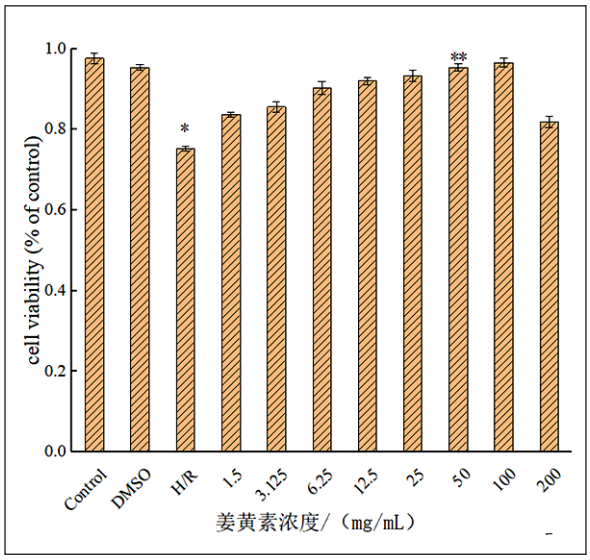

Figure 2. Curcumin's effect on the vitality of H9c2 cells damaged by hypoxia and reoxygenation.

Expression Levels of SOD, LDH and MDA in Cells Injured by Hypoxia and Reoxygenation

As shown in Figure 3, after reoxygenation, the levels of lactate dehydrogenase (LDH) in each group of cell culture medium were detected. Compared with those in the control group, LDH and MDA of the cells injured by hypoxia and reoxygenation (H/R)group (100 μg/mL)significantly increased, whereas SOD significantly reduced. Such results were due to the large amount of oxygen free radicals released after H/R, severely lipidated biomembrane and increased permeability of the cell membrane, suggesting that H/R aggravated the damage of cardiomyocytes. The activities of LDH and MDA in the treatment group decreased, whereas that of SOD increased, indicating that cell damage was inhibited after pre-protection (Figure 3). The effect was the most significant at the drug concentration of 100 μg/mL, which is consistent with previous experimental results.

Rat Myocardial Infarction Area

TTC staining is a common method for detecting ischaemic infarction in mammalian tissues. TTC reacts with nicotinamide adenine dinucleotide (NADH) in normal tissues and turns red. However, the cells that died after single ischaemia lost their cell membrane integrity and therefore also lost the NADH content. This condition resulted in the pale appearance of the infarcted tissue in the heart muscle. The heart of rats from the Sham group showed red staining, whereas the anterior wall of the myocardium of the heart in the IRI group was white. Compared with the IRI group, the white matter of myocardium in the turmeric group was reduced. The area of myocardial infarction and the risk area in the IRI group were significantly higher than those in the administration group (Figure 4). The area of myocardial infarction in the IRI group was 31.33% ± 3.21%. After modelling, different concentrations of curcumin can effectively reduce the area of myocardial infarction by 24.73% ± 2.79%, 18.53% ± 4.15% and 10.32% ± 1.98% (compared with the MIRI group, $p < 0.01$) (Table I). Compared with the Sham group, the area of myocardial infarction in the IRI group was significantly increased ($p<0.001$). Compared with the IRI group, the area of myocardial infarction in Cur group (100 mg/kg) was significantly reduced ($p<0.001$).

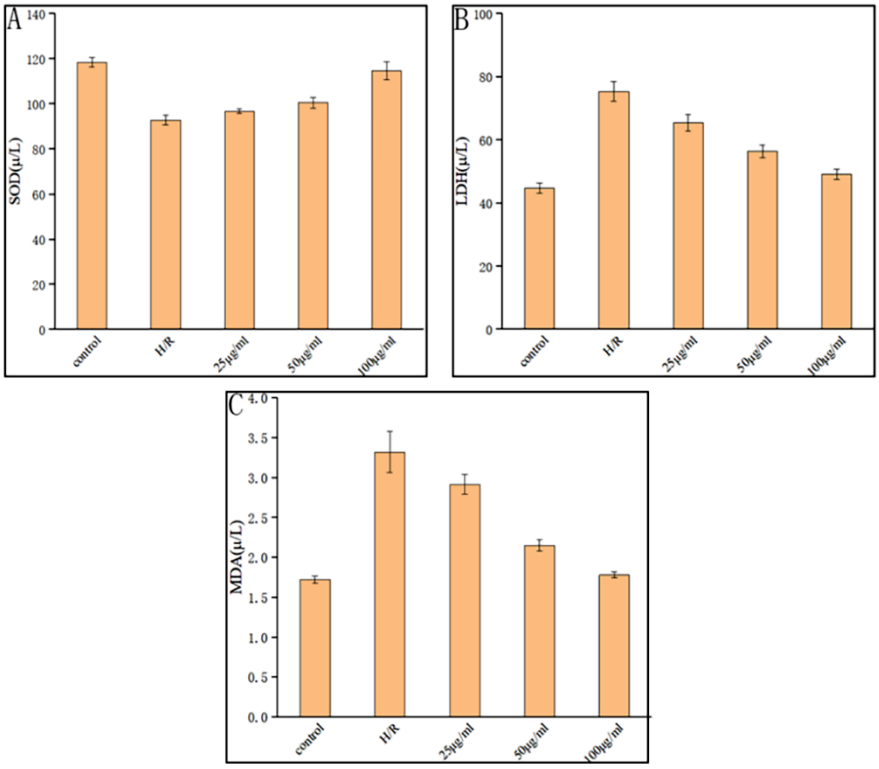

Figure 3. The effect of curcumin on SOD, LDH and MDA at the end of hypoxia/reoxygenation.

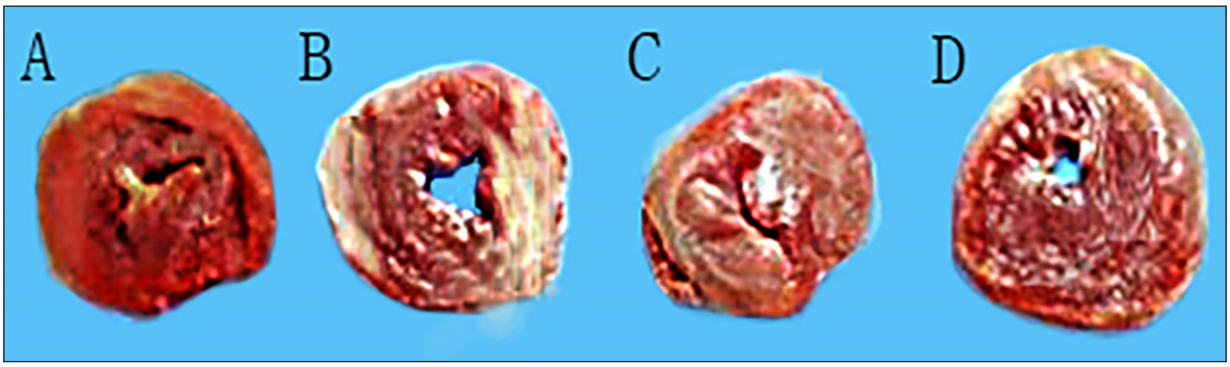

Figure 4. The effect of curcumin on the area of myocardial infarction in MIRI rats. **A**, Sham operation group; **B**, Model group; **C**, Curcumin group (50 mg/kg); **D**, Curcumin group (100 mg/kg).

Effect of Curcumin on the Expressions of P13K, mTOR, AKT and p-AKT Proteins in Rat Myocardium

The PI3K/Akt/mTOR signalling pathway is the most classic signalling pathway in cell apoptosis. Western blot was used to detect the expressions of PI3K/Akt/mTOR-related proteins in each group.

Compared with those in the Sham group, the expressions of PI3K, p-PI3K, Akt, p-Akt and mTOR proteins in the IRI group were reduced (p<0.001). The levels of mTOR, P13K and p-Akt in the heart of the Cur group were significantly higher than those in the IRI group, indicating that curcumin can upregulate m-TOR The expression of P13K and p-Akt proteins, reduce the level of autophagy, improve the anti-apoptotic ability and protects the myocardium (Figure 5). Compared with the IRI group, the expressions of P13K, p-Akt and mTOR in the Cur group were further enhanced. Compared with the Sham group, the levels of mTOR, P13K and p-Akt in the Cur group were slightly lower. The levels of mTOR, P13K and p-Akt in the Cur group were significantly higher than those in the IRI group (Figure 5). The results suggest that curcumin can activate and regulate the PI3K/Akt/mTOR signalling pathway, thereby regulating the level of autophagy. It shows that m-TOR, P13K and AKT are involved in the protective effect of curcumin on cardiomyocytes (Figure 5).

Table I. The effect of curcumin on the area of myocardial infarction in MIRI rats ($\bar{x} \pm s$, n = 10).

Groups	Dose (mg/kg)	Rat myocardial infarction area/%
Sham operation group	–	1.23 ± 0.14
Model group	–	31.33 ± 3.21
Curcumin group	25	24.73 ± 2.79
	50	18.53 ± 4.15
	100	10.32 ± 1.98

Effect of Curcumin on the Expressions of Serum Myocardial Injury Marker Enzyme CK-MB and Activity of LDH

The activities of serum CK-MB and LDH in the IRI group were significantly higher than those in the Sham group ($p < 0.05$). The effects of curcumin on the activities of serum myocardial injury marker enzymes CK-MB and LDH were determined. Compared with the Sham group, the serum CK-MB and LDH activities of the ischaemia-reperfusion group were significantly higher than those of the IRI group, and the myocardial tissue CK-MB and LDH activities of the Cur group were significantly lower than those of the IRI group. The effect of low-dose curcumin on CK-MB activity was also significantly different from that of the IRI group. However, as shown in Table II, no significant difference was observed in the effect of curcumin on LDH activity.

Effect of Curcumin on the Expressions of Serum SOD, GSH and NO and MDA Activity

As shown in Table III, compared with that in the Sham group, the MDA content of the IRI group significantly increased, whereas the antioxidant enzyme (SOD, GSH and NO) levels significantly decreased. Compared with the IRI group, curcumin can significantly increase the activity of myocardial tissue SOD and GSH-Px and reduce the content of MDA. Comparing the

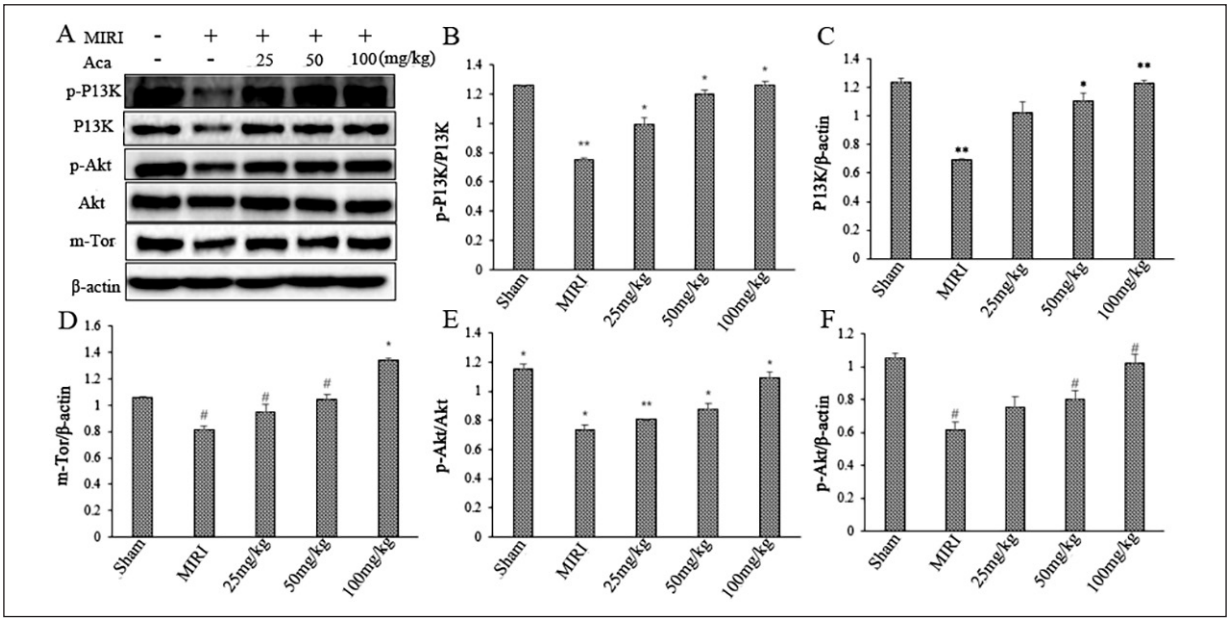

Figure 5. Curcumin reduces MIRI by regulating PI3K/Akt/mTOR signaling pathway (#: $p<0.05$ compared with the control group,*: $p<0.01$ compared with the model group).

Table II. Comparison of CK-MB and LDH activities in myocardial tissues of rats in each group ($\bar{x} \pm s$, n = 10).

Group	CK-MB (U/L)	LDH (U/L)
Sham operation group	1125.41 ± 98.61	567.88 ± 39.63
Model group	5639.63 ± 400.72	1988.73 ± 267.19
Curcumin group (25 mg/kg)	4007.42± 368.16	1263.54 ± 206.48
Curcumin group (50 mg/kg)	3216.37 ± 297.45	1083.33 ± 167.28
Curcumin group (100 mg/kg)	2016.72 ± 254.84	875.42 ± 79.31

two groups, the results were statistically significant ($p < 0.01$). Thus, curcumin can increase the activity of antioxidant enzymes in reperfused rats (Table III).

Effect of Curcumin on the Expressions of Bcl-2 and Bax mRNA in the Myocardium of Rats with Myocardial Ischaemia-Reperfusion

The process of cell apoptosis is related to numerous genes. Bcl-2 is the main anti-apoptotic gene, and its homologous apoptosis-inducing gene Bax inhibits the expression and activity of Bcl-2. Bcl-2 and Bax are a balanced system. The overexpression of Bax will increase cell apoptosis.

Compared with that in the Sham group, the expression of Bcl-2 mRNA in the IRI group was significantly reduced ($p < 0.01$). The expressions of Bcl-2 mRNA and Bax mRNA in the IRI group significantly increased, and the expressions of Bcl-2 mRNA and Bcl-2/Bax in the Cur group were significantly higher than those in the IRI group (Table IV). Thus, curcumin can up-regulate

Table III. Comparison of SOD, GSH, NO and MDA levels in myocardial tissues of rats in each group ($\bar{x} \pm s$, n = 10).

Group	SOD (U/mL)	GSH (mol/L)	NO (μmol/L)	MDA (μmol/L)
Model group	120.62 ± 23.78	1.28 ± 0.28	3.57 ± 0.96	6.83 ± 2.91
Curcumin group (25 mg/kg)	166.79 ± 30.46	1.39 ± 0.11	4.93 ± 0.68	5.23 ± 0.50
Curcumin group (50 mg/kg)	179.68 ± 24.17	1.45 ± 0.09	6.67 ± 1.34	4.07 ± 1.06
Curcumin group (100 mg/kg)	186.77 ± 18.96	1.57 ± 0.06	8.02 ± 1.39	3.78 ± 0.76

Table IV. The effect of curcumin on the expression of Bax and Bcl-2 mRNA in MIRI rats.

Group	Bcl-2	Bax	Bcl-2/Bax
Sham operation group	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Model group	0.29 ± 0.01	2.03 ± 0.47	0.15 ± 0.03
Curcumin group (25 mg/kg)	0.47 ± 0.08	1.78 ± 0.23	0.26 ± 0.01
Curcumin group (50 mg/kg)	0.63 ± 0.03	1.66 ± 0.41	0.39 ± 0.08
Curcumin group (100 mg/kg)	0.78 ± 0.11	1.54 ± 0.19	0.51 ± 0.01

the expression of Bcl-2 and inhibit the expression of Bax, thereby reducing the level of autophagy and improving the ability of anti-apoptosis.

Discussion

Clinically, IHD is the first killer of human health, and the key to its treatment is the restoration of blood supply. However, myocardial injury is often aggravated after the restoration of blood supply, a condition known as ischaemia-reperfusion injury. Reperfusion therapy essentially relieves myocardial ischaemia, but it may also cause irreversible cardiomyocyte apoptosis and cardiac remodelling. Therefore, MIRI is the main cause of IHD[14]. Preventing cardiomyocyte apoptosis is considered to be one of the important ways to prevent MIRI. However, an inappropriate MIRI treatment can cause complications, such as heart failure, ventricular remodelling and cardiac insufficiency, which seriously endanger human health[15]. Schauer et al[13] has shown that curcumin can effectively reduce the damage of H9C2 cells induced by HMP-R through the regulation of various processes. However, the mechanisms by which curcumin protects cardiomyocytes are still unclear, and the role of the P13K/Akt/mTOR pathway in cardiomyocyte injury is not fully understood.

Our study showed that the P13K/Akt/mTOR signalling pathway plays an important role in the protective effect of curcumin on H9C2 cell death induced by hypoxia/reperfusion. The results of in vivo experiments revealed that curcumin could significantly reduce the area of myocardial infarction and reduce changes in heart function. The content of MDA can reflect the degree of damage to cells by free radicals[16]. SOD and GSH-Px can inhibit reactive oxygen species and oxidative stress, effectively protecting cardiomyocytes and avoiding damage. Curcumin can significantly reduce the content of MDA, increase the activity of SOD and GSH-Px and protect the myocardium. In addition, curcumin can improve the antioxidant capacity of the myocardium and inhibit the production of lipid peroxidation products. The protective effect of curcumin on cerebral ischaemia-reperfusion injury may be related to its antioxidant effect. CK-MB and LDH are important marker enzymes reflecting the degree of myocardial cell damage. Detecting the activities of these marker enzymes can be used to assess the degree of myocardial necrosis and evaluate the degree of myocardial damage. Curcumin can inhibit the increase in CK-MB and LDH activity, suggesting that it can prevent the leakage of myocardial enzymes.

Conclusions

In summary, this study confirmed that curcumin could significantly improve the antioxidant capacity of myocardial tissue, promote NO synthesis, up-regulate the expression of Bcl-2, reduce the expression of Bax, inhibit myocardial cell apoptosis and effectively combat MIRI. Its protective effect is related to changes in the P13K/Akt/mTOR pathway.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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