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Nicotinamide pretreatment alleviates mitochondrial stress and protects hypoxic myocardial cells via AMPK pathway

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Abstract. – OBJECTIVE: The aim of this study was to explore the protective effect of nicotinamide on hypoxic cardiomyocytes and to investigate its possible mechanism.

MATERIALS AND METHODS: Primary cardiomyocytes were used as study subjects. They were divided into three groups, including the blank group, control group and nicotinamide pretreatment group. Cell counting kit-8 (CCK-8) was used to detect cell viability. Lactate dehydrogenase (LDH) was used to detect cytotoxicity and flow cytometry was used to detect cell apoptosis. Polymerase Chain Reaction (PCR) and Western blot were used to measure the expressions of genes in adenosine monophosphate-activated protein kinase (AMPK) pathway. JC-1 detected the levels of mitochondrial membrane potential and reactive oxygen species (ROS). NAD was used for nicotinamide adenine dinucleotide (NAD)+, and NAD phosphate (NADP)+ levels. Adenosine triphosphate (ATP) assay was performed for the detection of intracellular energy metabolism.

RESULTS: In the absence of oxygen, nicotinamide had a protective effect on primary cardiomyocytes. Meanwhile, nicotinamide could markedly inhibit the increase of caspase3 mRNA in cardiomyocyte apoptosis pathway, and suppress the expression of apoptotic proteins. Furthermore, it could significantly induce the increase of intracellular ATP and activate the AMPK pathway. The detection of mitochondria indicated that nicotinamide alleviated hypoxic cardiomyocytes. In addition, the mitochondrial membrane potential disrupted and inhibited mitochondrial oxidative stress levels.

CONCLUSIONS: Nicotinamide pretreatment protects hypoxic cardiomyocytes and reduces intracellular mitochondrial stress. This protection may be related to the induction of the AMPK pathway and the increase of intracellular energy production.

Key Words:
Nicotinamide, Myocardial hypoxia, Mitochondrial stress, AMPK.

Introduction

The cardiovascular disease has become one of the most common diseases with the highest incidence in modern times[1]. Systemic or local myocardial hypoxia exists in many cardiovascular diseases, such as cyanotic congenital heart disease, coronary atherosclerotic heart disease or high-altitude heart disease[2]. Hypoxia is a common, basic pathological process. It has been demonstrated that hypoxia often refers to abnormal changes in cellular metabolism, function and morphological structure due to the reduction of tissue oxygen supply or oxygen consumption disorder. The core of hypoxia is the apparent contradiction between oxygen supply and oxygen demand of tissue cells[3]. Adenosine monophosphate-activated protein kinase (AMPK) is a kind of highly-conserved protein in cells[4]. AMPK belongs to the serine/threonine kinase, which is a key regulator of intracellular energy homeostasis[5]. When adenosine triphosphate (ATP) content in cells declines due to various metabolic stress states, threonine 172 in AMPK molecule will be phosphorylated, and AMPK will be activated. This regulates various metabolic reactions, inhibits anabolism and promotes catabolism and ATP production, ultimately restoring intracellular energy homeostasis[6].

After ischemia and hypoxia, the metabolism of myocardial cells is significantly inhibited.

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Deoxyribonucleic acid (DNA) transcription and translation, protein assembly and transport processes are blocked. Meanwhile, cell viability is greatly declined. At the same time, increased production of reactive oxygen species (ROS), increased permeability of mitochondrial inner and outer membrane, release of cytochrome C (cyt C), calcium overload and opening of mitochondrial permeability transition pore lead to decreased production of mitochondrial ATP and energy metabolism disorder, eventually activating apoptosis and necrosis pathways[7]. Mitochondrion is a major organelle for energy metabolism in cell respiration. Nicotinamide adenine dinucleotide $(NAD)^+$ and NAD phosphate $(NADP)^+$ are involved in the electron transport and ATP synthesis of respiratory chain complexes. The mitochondrial membrane potential reflects the integrity of the structure and function of the mitochondrial membrane. Meanwhile, mitochondrial homeostasis maintains the normal functioning of the cell life process. Previous studies have found that the mitochondrion is the core target organelle of cellular hypoxia-ischemia injury. Furthermore, mitochondrial damage is the most important link in hypoxia-ischemia injury[8]. Therefore, the exploration of the mechanism of drug protection against mitochondrial damage is of great significance in preventing cell death induced by ischemia and hypoxia after burn.

Nicotinamide (NAM), also known as niacinamide, is an amide compound of the B-vitamin niacin. It is also a component of coenzyme I and coenzyme II, involved in cellular respiration and energy synthesis[9]. Clinically, NAM is mainly used in the treatment of cardiac conduction block and viral myocarditis. The curative effect of NAM is evident. NAM has been showed to significantly alleviate neuronal hypoxia-ischemia injury and cerebral edema, which has a protective effect on the nervous system[10]. According to recent studies, cardiac hypoxia-ischemia injury can be relieved in mice fed with NAM-rich fodder. However, the mechanism of myocardial protection remains unclear[11].

In this experiment, NAM was used to establish the hypoxic-ischemic rat model of primary myocardial cells *in vitro*, and the protective effect of NAM on myocardial cells after hypoxia-ischemia injury was observed. Meanwhile, the possible underlying mechanism was explored, to provide an experimental basis for the clinical treatment of myocardial damage after burn.

Materials and Methods

Cell Culture

Myocardial cells were isolated, digested with trypsin and purified *via* differential adhesion and bromodeoxyuridine (Brdu) chemical inhibition. The specific operation procedure was as follows: the heart was collected from 1-2-day-old Wistar rats, washed with 0.01 M phosphate-buffered saline (PBS) to remove the residual blood, cut into 1 mm^3 pieces using ophthalmic scissors, and digested with 0.125% trypsin under constant temperature magnetic stirring at 37°C for 10 min. Subsequently, the digestive solution containing a large number of blood cells was discarded, and trypsin was added into the tissue block for digestion under constant temperature magnetic stirring at 37°C for 7 min. The digestive solution was collected, and the activity of residual trypsin was inactivated in time by the medium containing 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA). The above digestion and collection processes were repeated 7-8 times until the tissue block was completely digested. The collected digestive solution was filtered with a 200-mesh steel filter, followed by centrifugation at 1000 rpm for 5 min at room temperature. Then the cell precipitate was washed with PBS once. Finally, the obtained cells were re-suspended in Dulbecco's Modified Eagle' Medium (DMEM)-F12 (Gibco, Grand Island, NY, USA) containing 10% FBS and 0.01 mM Brdu, and cultured in 5% CO_2 at 37°C for 60 min until fibroblasts adhered. The cell suspension was transferred and counted, and the cell density was adjusted to 1×10/mL. Finally, the cells were inoculated into a CO_2 incubator, and the solution was replaced every other day.This study was approved by the Animal Ethics Committee of Qingdao University.

Cell Hypoxia

The original medium was replaced with an equal volume of sugar-free DMEM, and the cells were immediately cultured in a hypoxic incubator containing 94% N_2, 5% CO_2, and 1% O_2 at 37°C for 6 h. Then, the model of hypoxia-ischemia of myocardial cells was established *in vitro*[13]. The cells in the blank group were still cultured in a normal incubator.

Flow Cytometry

BGC823 cells were first inoculated into 6-well plates (5×10^5/mL) overnight. Silybin solution at a final concentration of 0 μM, 50 μM, 100 μM and 200 μM was added, respectively, followed by cul-

ture in a 5% CO_2 and saturated humidity incubator at 37°C for 24 h. Then the cells were digested with trypsin, collected and centrifuged at 1000 rpm and 4°C for 4 min. Subsequently, the cells were collected, and the medium was discarded. After centrifugation, the cells were washed with cold PBS twice and suspended in 200 μL binding buffer at a concentration of about 1×10^6/mL. 10 μL Annexin V-fluorescein isothiocyanate (FITC) was added into the cell suspension and mixed evenly, followed by incubation at room temperature for 15 min in the dark. 5 μL propidium iodide (PI) was added, and the mixture was detected by flow cytometry within 1 h. Experimental results were obtained and analyzed using Cellquest professional software. Each experiment was repeated 3 times.

Cell Counting Kit-8 (CCK-8)

The cells in logarithmic growth phase were digested, collected and adjusted into cell suspension at a concentration of 1×10^5/mL. Then the cells were inoculated into 96-well plates with 100 μL cell suspension per well. Three replicates were set in the experiment, and the blank control wells were also set. After inoculation overnight, the adherence of cells was confirmed *via* microscopic observation. After grouping and treatment, 20 μL methyl thiazolyl tetrazolium (MTT; Sigma-Aldrich, St. Louis, MO, USA) was added to each well, followed by culture at 37°C for another 4 h. Then, the supernatant was carefully discarded, and 150 μL dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) was added into each well and mixed evenly. Optical density (OD) value of each well at the wavelength of 570 nm was detected using a microplate reader. The experiment was repeated 3 times.

Lactate Dehydrogenase (LDH)

Cells in the logarithmic growth phase were digested, collected and adjusted into cell suspension at a concentration of 1×10^5/mL. Then, the cells were inoculated into 96-well plates with 100 μL cell suspension per well. Three replicates were set in the experiment, and the blank control wells were also set. After cell adherence, the culture plate was placed in an incubator with 5% CO_2 for incubation at 37°C for 24 h. After grouping, 20 μL supernatant was collected from each well. The corresponding reagent was added according to the instructions of the kit, mixed evenly and placed at room temperature for 3 min. Zero setting was performed with 440 nm double distilled water using the 1 cm-light-path cuvette. OD value of each tube was detected by a microplate reader. After action between 1000 mL culture solution and substrate at 37°C for 15 min, 1 gmoL pyruvic acid produced in the reaction system was used as 1 unit. LDH content in the medium was calculated according to the formula.

Mitochondrial Membrane Potential

After treatment, the cells were collected and re-suspended in 0.5 mL cell culture medium containing serum. 0.5 mL JC-1 staining working solution was added, inverted several times and mixed evenly. Then the cells were incubated in a 37°C incubator for 30 min in the dark, followed by centrifugation at 600 rpm and 4°C. The supernatant was discarded, and the cells were washed with 1×JC-1 staining buffer twice. Then 1×JC-1 staining buffer was added for cell resuspension, and the cells were centrifuged at 600 rpm and 4°C. The supernatant was then discarded. The above washing step was repeated once. Fluorescence intensity of cells was detected by flow cytometry.

ATP

Cell lysate extracted using PBS was rewarmed to 4°C and vibrated for 3.5 s. 110 μL lysate was collected and added into an Eppendorf (EP) tube. Subsequently, 110 μL $HClO_4$ was added into each tube, vibrated and mixed evenly, followed by centrifugation at 16,000 rpm and 4°C for 20 min. Meanwhile, 150 μL supernatant was collected into another EP tube, and added with 60 μL 2.5 M K_2CO_3 solution. The pH value was adjusted to 7.0, followed by centrifugation at 16,000 rpm and 4°C for 20 min. Then the supernatant was used as an ATP sample. OD value at 560 nm was detected by a multifunctional microplate reader in accordance with the instructions of kit. Finally, the concentration was calculated.

NAD

NAD^+ and $NADP^+$ in cells were detected according to the instructions of NAD^+/NADH and $NADP^+$/NADPH kits. Cell lysate extracted with PBS was rewarmed to 4°C and vibrated for 3.5 s. 110 μL lysate was collected into an EP tube. Subsequently, 100 μL NAD^+ or $NADP^+$ extraction solution was added into each tube, homogenized and preheated for 5 min. Then 20 μL substrate buffer and 100 μL NAD^+ or $NADP^+$ extraction solution were added and vibrated simply, followed by centrifugation at 14,000 rpm for 5

min. The supernatant was used as the detection sample. The standard curve was plotted in accordance with the instructions of the kit. OD value was detected by a multifunctional microplate reader, and the release amount was calculated.

Polymerase Chain Reaction (PCR)

After treatment, the cells were collected. Total ribonucleic acid (RNA) in cells was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The concentration of extracted RNA was measured. Then the reverse transcription reaction was performed based on the extracted RNA. The first 40 cycles were used for complementary deoxyribonucleic acid (cDNA) synthesis, and reverse transcription reaction conditions were set for PCR amplification. After each cycle, Real-Time fluorescence signal was collected, and the amplification and dissociation curves were recorded. Primer sequences used in this study were as follows: caspase3, F: 5'-CCCTTACTCAGGAACCCCCT-3', R: 5'-TGTGTCCTAGGGACGAAGGA-3'; AMPK, F: 5'-GTCCTCGAGGGTGTAAACACTG-3', R: 5'-CGTGTCGATTGTGGAGTCG-3'; GAPDH: F: 5'-CGCTCTCTGCTCCTCCTGTTC-3', R: 5'-ATCCGTTGACTCCGACCTTCAC-3'.

Western Blotting

The cells in each group were collected and washed twice with D-Hank's solution. D-Hank's solution was then sucked dry using absorbent paper. 150 μL pre-cooled lysis buffer was added to each group, followed by cell lysis on ice for 30 min. The protein in each group was collected into an EP tube by cell scraper and centrifuged at 12,000 rpm and 4°C. The supernatant was collected and transferred into a new EP tube. The concentration of the extracted protein was determined according to the instructions of bicinchoninic acid (BCA) (Pierce, Rockford, IL, USA). Subsequently, 5 × loading buffer was added and mixed evenly, and the protein was heated at 100°C for 6 min. A total of 30 μL proteins were added into the loading wells of prepared separation gel and spacer gel, followed by electrophoresis in electrophoresis buffer under appropriate voltage. After that, the gel adhered closely to polyvinylidene difluoride (PVDF) membranes (Roche, Basel, Switzerland), and proteins were transferred onto the membranes under a constant voltage of 100 V in transfer buffer at 0°C for 60 min. After sealing with 5% skim milk powder at room temperature for 1 h, the PVDF membranes were cut according to the molecular weight and incubated with primary antibody at 4°C overnight. On the next day, the PVDF membranes were washed with Tris-Buffered Saline and Tween 20 (TBST), and incubated with corresponding secondary antibody IgG (1:5000) at room temperature for 1 h. After that, the membranes were washed again with TBST. Finally, image development was performed using the Tannon 5200 fluorescence immune image development system, and the gray level was detected.

Statistical Analysis

Data were expressed as mean ± standard deviation. Statistical Product and Service Solutions (SPSS) 13.0 software (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. One-way analysis of variance (ANOVA) was used to compare the differences among different groups, followed by Post-Hoc Test (Least Significant Difference). Independent-samples *t*-test was applied to compare the difference between the two groups. $p < 0.05$ was considered statistically significant. Data were presented as the percentage of the control group.

Results

Effect of NAM on Survival of Myocardial Cells

After treated with different doses (5, 10 and 20 mM) of NAM for 16 h, the viability, toxicity and apoptotic ratio of myocardial cells under normoxia were detected. The results of CCK-8 revealed that the viability of myocardial cells increased in a dose-dependent manner compared with that of the blank group after NAM treatment. There was a statistically significant difference between 10 mM group and 5 mM group ($p < 0.05$). However, there was no statistically significant difference between 20 mM group and 10 mM group (Figure 1A). Cell toxicity was then detected *via* LDH. The results showed that the release of LDH in cells was decreased in a dose-dependent manner after NAM treatment. There was a statistically significant difference between 10 mM group and the blank group as well as 5 mM group ($p < 0.05$). However, no statistically significant difference was found between 20 mM group and 10 mM group (Figure 1B). The proportion of early apoptosis was detected *via* flow cytometry. It was found that NAM significantly inhibited early

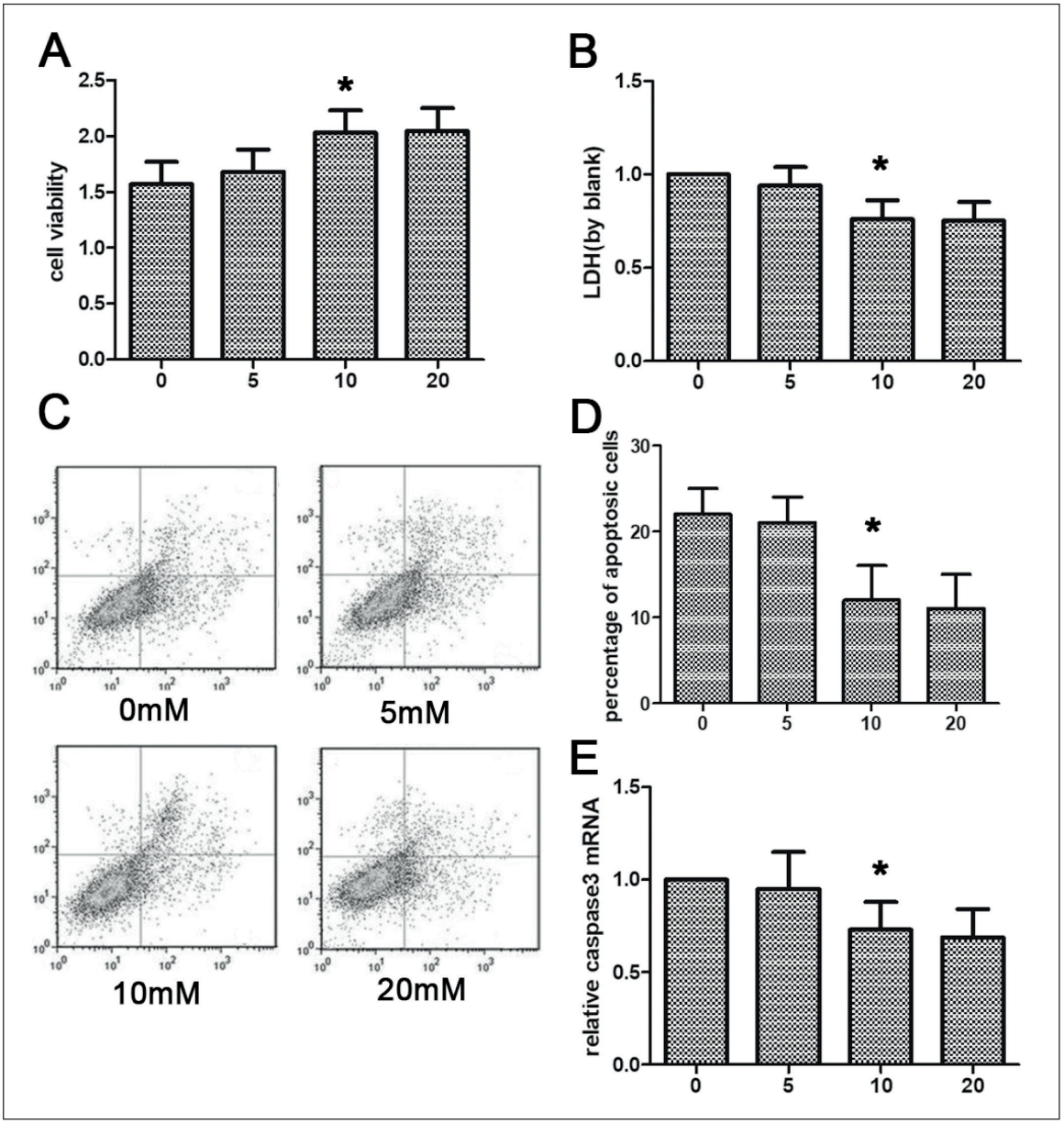

Figure 1. Effect of NAM on myocardial cells. ***A,*** Viability of myocardial cells in NAM groups at different doses (0, 5, 10 and 20 mM) was detected *via* CCK-8 assay. ***B,*** Toxicity of myocardial cells in NAM groups at different doses (0, 5, 10 and 20 mM) was measured *via* LDH. ***C,*** The proportion of myocardial apoptosis in NAM groups at different doses (0, 5, 10 and 20 mM) was determined *via* flow cytometry. ***D,*** Statistical analysis of apoptosis ratio in each group. ***E,*** The mRNA expression of caspase3 in NAM groups at different doses (0, 5, 10 and 20 mM) was detected *via* PCR. *$p < 0.05$ compared with blank.

apoptosis. The proportion of early apoptosis in 10 mM group was remarkably lower than that of 5 mM group ($p < 0.05$). However, there was no significant difference between 20 mM group and 10 mM group (Figure 1C, 1D). Meanwhile, the expression of caspase3 in each group was detected *via* PCR. The results demonstrated that the mRNA expression of caspase3 in cells of the 10 mM group was remarkably decreased compared with the 5 mM group ($p < 0.05$). No significant difference was found between the 20 mM group and the 10 mM group ($p < 0.05$, Figure 1E).

Protective Effect of NAM on Hypoxic Myocardial Cells

Myocardial cells were first treated with NAM (10 mM) for 16 h. Then the cells were treated with hypoxia (94% N_2, 5% CO_2 and 1% O_2) for

6 h together with the control group. The level of apoptotic pathway in each group was detected *via* PCR and Western blotting. The expression of caspase3 in each group was detected *via* PCR, and the results indicated that the mRNA expression of caspase3 in cells after hypoxia was markedly increased when compared with that of the blank group ($p < 0.05$). However, it was significantly declined after NAM treatment when compared with that of the control group ($p < 0.05$, Figure 2A). The protein expressions of B-cell lymphoma-2 (Bcl-2) and Bcl-2 associated X protein (Bax) were detected *via* Western blotting. It was found that after hypoxia, the protein expression of Bcl-2 in cells was significantly decreased, while Bax expression was remarkably increased ($p < 0.05$, Figure 2B, 2C). Meanwhile, the Bax/Bcl-2 ratio was also markedly increased (Figure 2D). After NAM treatment, the expression of Bcl-2 was significantly increased. However, Bax expression was inhibited, and the Bax/Bcl-2 ratio was remarkably lower than that of the control group. The above results proved that NAM could protect hypoxic myocardial cells from apoptosis.

NAM Induced Energy Production in Hypoxic Myocardial Cells

ATP content and AMPK pathway activation were detected to evaluate the cellular energy level in the blank group, control group and NAM group (10 mM), respectively. The results manifested that ATP content in cells after hypoxia was remarkably decreased when compared with that of the blank group ($p < 0.05$, Figure 3A). However, it was significantly increased in the NAM group than that of the control group ($p < 0.05$, Figure 3A). The mRNA level of AMPK in each

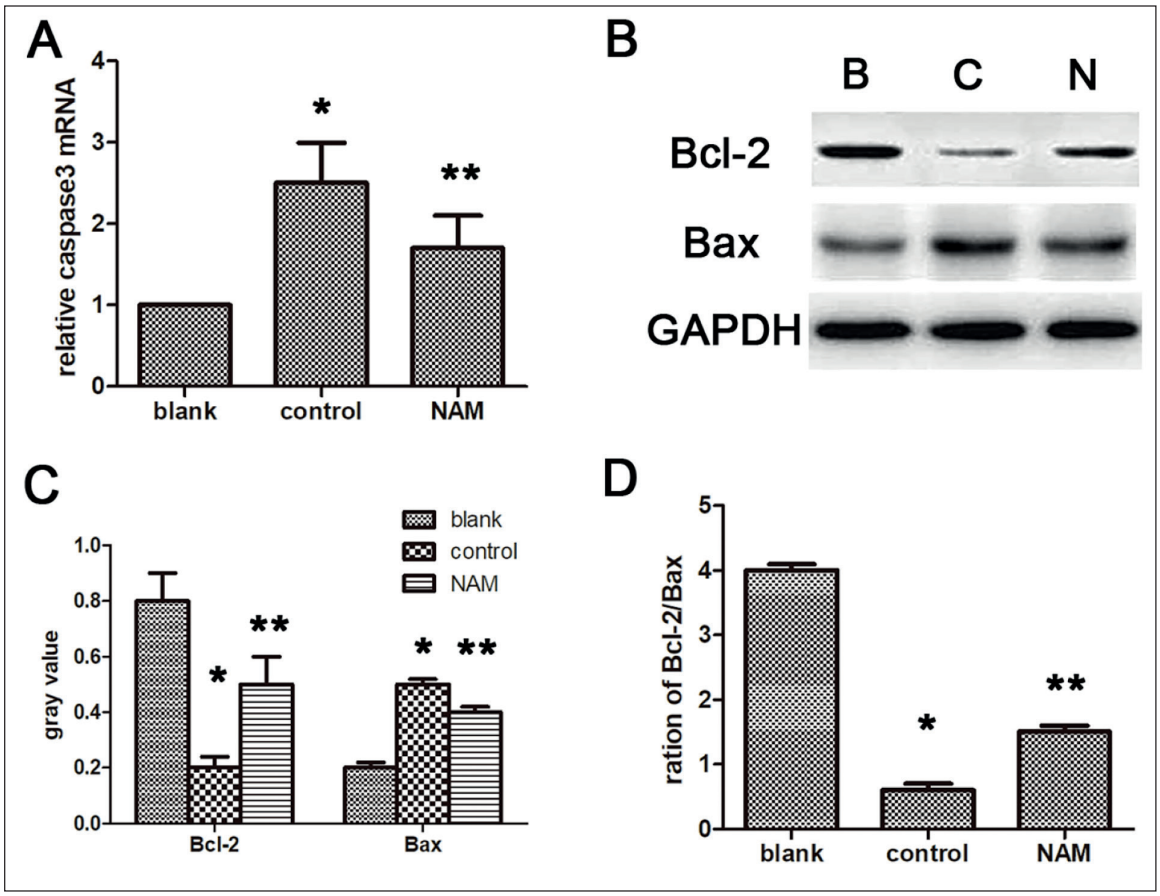

Figure 2. Effect of NAM on apoptosis of hypoxic myocardial cells. ***A,*** The mRNA expression of caspase3 in blank group, control group and NAM group was detected *via* PCR. ***B,*** The protein expression of apoptotic proteins (Bcl-2 and Bax) in blank group, control group and NAM group was measured *via* Western blotting. ***C,*** Gray analysis of Bcl-2 and Bax protein concentration in each group. ***D,*** Gray analysis of Bax/Bcl-2 ratio in each group. *$p < 0.05$ compared with blank. **$p < 0.05$ compared with control.

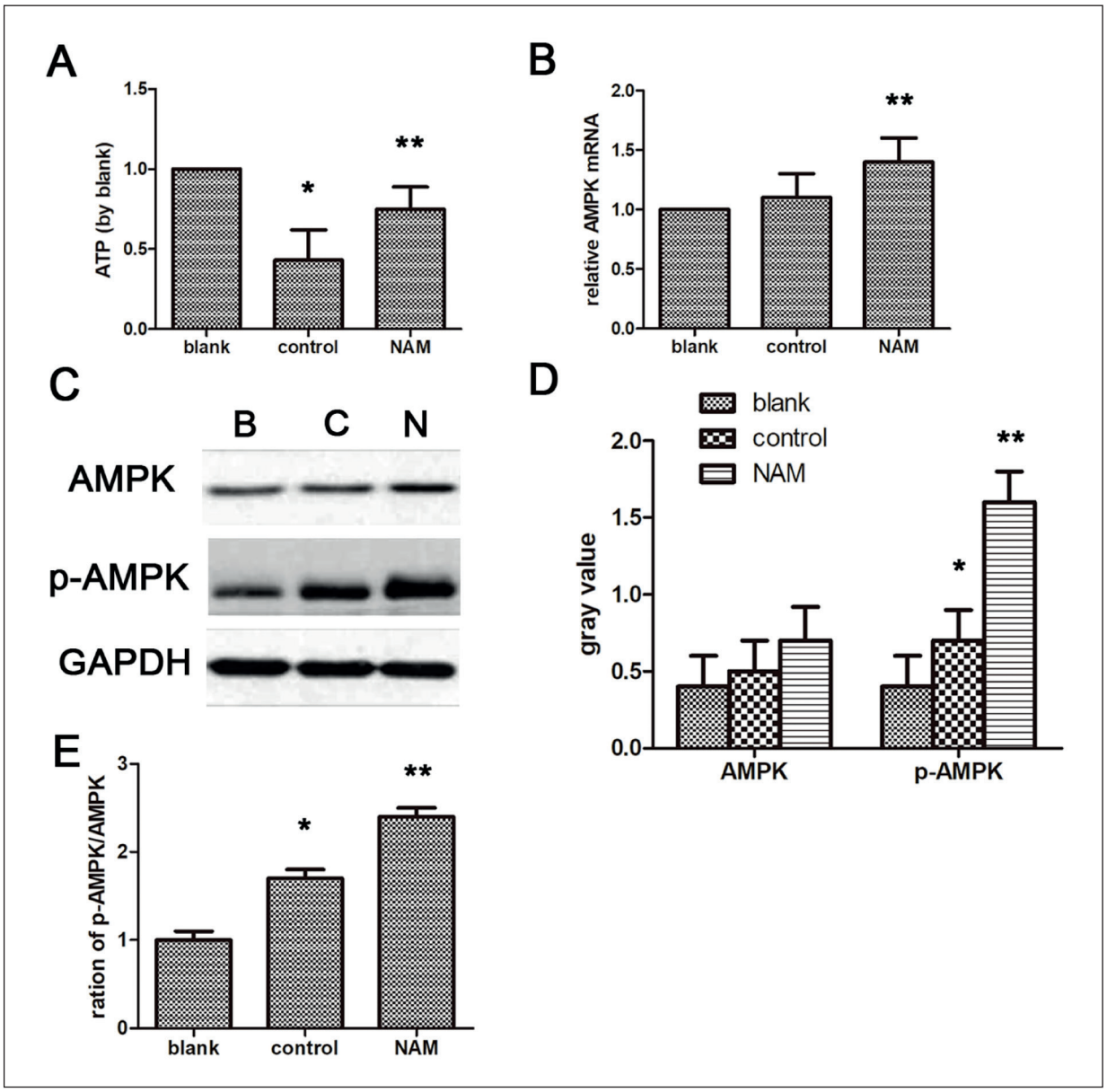

Figure 3. Effect of NAM on energy metabolism of hypoxic myocardial cells. ***A,*** Intracellular energy level in blank group, control group and NAM (10 mM) group was detected *via* ATP. ***B,*** The mRNA expression of AMPK in cells of blank group, control group and NAM (10 mM) group was measured *via* PCR. ***C,*** The protein expressions of AMPK and p-AMPK in cells of blank group, control group and NAM (10 mM) group were determined *via* Western blotting. ***D,*** Gray analysis of AMPK and p-AMPK concentrations in each group. ***E,*** Gray analysis of p-AMPK/AMPK ratio in each group. *$p < 0.05$ compared with blank. **$p < 0.05$ compared with control.

group was detected *via* PCR. The results found that the activation of AMPK in cells after hypoxia was slightly increased compared with that of the blank group, showing no statistically significant difference. Besides, the AMPK activation in the NAM group was remarkably higher than that of the blank and control groups ($p < 0.05$, Figure 3B). The protein expressions of AMPK and p-AMPK were detected *via* Western blotting. The findings showed that p-AMPK in cells after hypoxia was significantly higher than that of the blank group. However, it was markedly increased in the NAM group compared with that of the control group. Meanwhile, the p-AMPK/AMPK ratio was also significantly increased ($p < 0.05$, Figure 3C, 3D, 3E). The above results proved that NAM could effectively improve energy supply in cells under hypoxia.

Protective Effect of NAM on Mitochondria of Hypoxic Myocardial Cells

The content of ROS, mitochondrial membrane potential, as well as NAD^+ and $NADP^+$ levels, were detected to evaluate the mitochondrial function in the blank group, control group and NAM group. The results revealed that the content of ROS in the control group was significantly increased when compared with that of the blank group. Meanwhile, the increase of ROS induced by hypoxia was significantly inhibited after NAM was added ($p < 0.05$, Figure 4A). The mitochondrial membrane potential was detected *via* JC-1 staining. It was found that the mitochondrial membrane potential in the control group was damaged when compared with that of the blank group ($p < 0.05$). However, it was significantly increased in the NAM group compared with that of the control group ($p < 0.05$, Figure 4B, 4C). Moreover, the NAD^+ and $NADP^+$ levels in the control group were remarkably lower than those of the blank group ($p < 0.05$). The NAD^+ and $NADP^+$ levels were remarkably increased after NAM treatment compared with those of the control group ($p < 0.05$, Figure 4D). These findings proved that NAM could improve the mitochondrial function in hypoxic myocardial cells.

Discussion

After ischemia-hypoxia, the normal metabolism of cells is significantly inhibited, such as biological processes of DNA transcription and translation. Protein assembly and transport cannot proceed normally and cell viability declines. At the same time, insufficient production of mitochondrial energy, poor stability of inner and

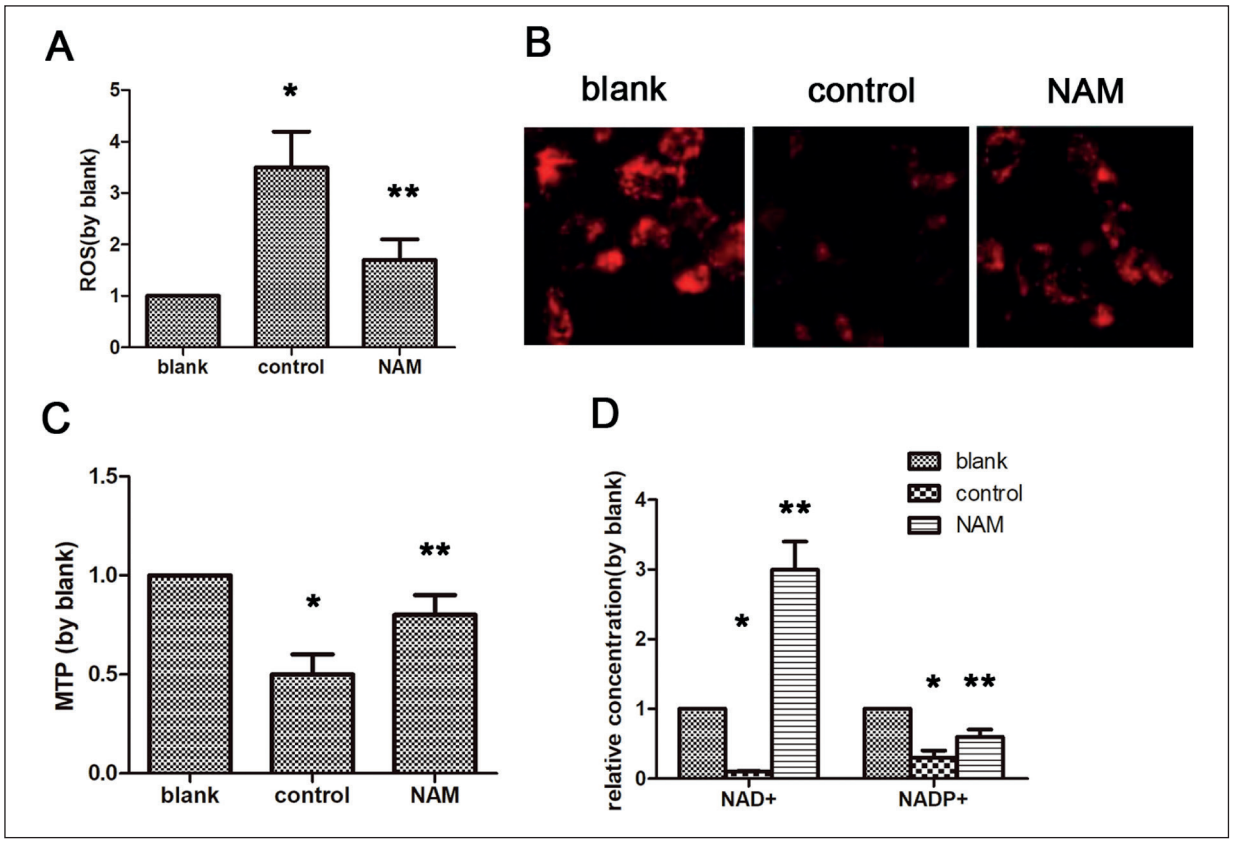

Figure 4. Effect of NAM on mitochondrial function of hypoxic myocardial cells. ***A,*** ROS level in cells of blank group, control group and NAM (10 mM) group. ***B,*** Mitochondrial membrane potential in blank group, control group and NAM (10 mM) group was detected *via* JC-1 staining. ***C,*** Data analysis of mitochondrial membrane potential in each group. ***D,*** NAD^+ and $NADH^+$ levels in cells of blank group, control group and NAM (10 mM) group. *$p < 0.05$ compared with blank. **$p < 0.05$ compared with control.

outer membranes, and increased permeability may induce apoptosis and necrosis. The mitochondrial respiratory chain complex fails to work normally. Meanwhile, a large number of oxygen radicals are produced with calcium overload, further impeding the synthesis of ATP[12]. Therefore, the disorder of cellular energy metabolism is an important cause of hypoxia-ischemia injury. Nowadays, finding a way to promote energy metabolism and reduce ATP loss from drugs is a key issue in clinical treatment. Currently, conventional therapies mainly focus on how to improve hemodynamic changes, thereby increasing myocardial oxygen supply and maintaining cardiac pump power. However, this will lead to certain side effects. The simple supplement of ATP cannot fundamentally alter cellular metabolism[8]. Therefore, the exploration of drugs that can promote mitochondrial energy production and reduce apoptosis or necrosis may bring a broader therapeutic perspective.

NAM, also known as niacinamide, is an amide compound of the B-vitamin niacin. B-vitamin niacin is a component of coenzyme I and coenzyme II, involved in cell respiration and energy synthesis. NAM possesses pharmacological effects, such as anti-inflammation, anti-aging and promotion of metabolism in the body. However, it has no adverse effects on the digestive system, including the gastrointestinal tract, liver and biliary tract[13]. Clinically, NAM is mainly used in the treatment of cardiac conduction block and viral myocarditis, obtaining an evident curative effect. AMPK, an "energy sensor" of cells, plays an important role in maintaining cellular energy metabolism balance and homeostasis[14]. The activation of AMPK can increase glucose uptake, enhance fatty acid oxidation and inhibit protein biosynthesis, thus increasing energy reserve in cells[15]. Moreover, activating AMPK can not only increase ATP synthesis and reduce ATP consumption, but also regulate mitochondrial function and its biosynthesis[16]. In the present study, our findings revealed that NAM had a protective effect on hypoxic primary myocardial cells. The ATP level and AMPK expression in cells were detected. Moreover, it was found that NAM could significantly induce the AMPK activation and promote ATP energy synthesis in cells.

Ischemia-hypoxia leads to ROS production and lipid peroxidation in the mitochondrial membrane. This may further damage the stability of mitochondria and even the whole cell. The resulting increase in mitochondrial membrane permeability will promote the release of cyt C, activate mitochondrial necrotic and apoptotic pathways, and increase the production of ribosome-inactivating protein and caspase3. This may ultimately lead to cell apoptosis and necrosis[17]. In this study, our results demonstrated that NAM could significantly improve mitochondrial stress after hypoxia, protect mitochondrial membrane potential and reduce the oxidative stress response. The possible reason might be that NAM induced the increase of ATP and improved mitochondrial energy metabolism in cells.

Conclusions

We observed that NAM improves mitochondrial oxidative stress by activating the AMPK pathway. Furthermore, such a protective effect may be related to the improvement of the mitochondrial energy metabolism *via* inducing ATP production in hypoxic myocardial cells.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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