European Review for Medical and Pharmacological Sciences 2019; 23: 10541-10548

Butorphanol protects on myocardial ischemia/reperfusion injury in rats through MAPK signaling pathway

H. WANG, J.-L. WANG, H.-W. REN, W.-F. HE, M. SUN

Department of Anesthesiology, The First Affiliated Hospital, Jinzhou Medical University, Jinzhou, China

Abstract. – OBJECTIVE: To explore the influence of butorphanol on myocardial ischemia/reperfusion (I/R) injury in rats through the mitogen-activated protein kinase (MAPK) signaling pathway.

MATERIALS AND METHODS: The I/R model in Sprague-Dawley rats was established. The rats were randomly divided into normal group (n=20), myocardial I/R model group (model group, n=20), and butorphanol treatment group (treatment group, n=20). Next, the liver function indicators such as alkaline phosphatase (ALP), alanine aminotransferase (ALT), and the myocardial function index creatine kinase (CK) in rats were detected. ELISA was carried out to measure the relative levels of tumor necrosis factor-gamma (TNF-γ), interleukin-6 (IL-6), and IL-1α in serum samples of rats. The cardiac function indexes were examined via magnetic resonance imaging (MRI) and echocardiography (ECG). Besides, the pathological changes of the myocardial tissues were detected through hematoxylin-eosin (HE) staining. The quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) and Western blotting were performed to measure the mRNA and protein expression levels of the relative genes in the MAPK signaling pathway in the rat myocardial tissues.

RESULTS: The serum levels of ALP, ALT, and CK in I/R model group were significantly higher than those in the normal group. In I/R model group, the relative levels of TNF-γ, IL-6, and IL-1α, as well as left ventricular end-diastolic diameter (LVEDd) and left ventricular end-systolic diameter (LVESd), were remarkably higher, while the fractional shortening (FS, %) and the ejection fraction (EF, %) were lower in comparison with those in the normal group. The HE staining results showed that the myocardial tissues in the I/R model group exhibited severe injury. The expression levels of Caspase3, MAPK, and c-Jun N-terminal kinase (JNK) were clearly higher in the I/R model group than those in the treatment group ($p<0.05$), while the expression level of extracellular regulated protein kinase 1 (ERK1) was remarkably lower ($p<0.05$). The protein level of MAPK in the treatment group was overtly reduced compared with that in the I/R model group ($p<0.05$).

CONCLUSIONS: Butorphanol can modulate the recovery of the myocardial injury in the rats after the myocardial I/R by inhibiting the MAPK signaling pathway.

Key Words:
Butorphanol, MAPK signaling pathway, Myocardial ischemia/reperfusion.

Introduction

The myocardial ischemia/reperfusion (I/R) injury is a myocardial injury caused by coronary blood flow recovery after an ischemic attack, and the living myocardial cells are impaired before reperfusion[1]. Currently, the complexity of the myocardium response to ischemic injury has been well explored. The myocardial ischemia leads to characteristic changes in metabolism and ultrastructure, resulting in irreversible injury. The myocardial I/R serves as an important target in the treatment of myocardial infarction and may lead to cell death[2]. The reactive oxygen species production, calcium overload, pro-inflammatory cytokine activation, apoptosis, neutrophil filling, and endothelial cell dysfunction are relevant pathological factors for I/R[3]. The inhibition of oxidative stress and apoptosis may be potential strategies for the treatment of ischemic diseases. In case of myocardial ischemia, the myocardial cell damage occurs via a series of events. In case of focal ischemia, the cell death in the center area rapidly occurs if blood flow disorder is severe, while the damaged cells in the peripheral blood zone with moderate blood circulation may survive through prompt intervention[4]. The I/R injury is mediated by the elements secreted by the damaged myocardial cells and inflammatory cells, as well as apoptosis[5]. Besides, it is found that myo-

Corresponding Author: Hao Wang, MM; e-mail: wh54315@163.com

cardium undergoes apoptosis after transient I/R, whereas the ischemic tissues are not apoptotic in the absence of reperfusion[6]. Therefore, apoptosis suppression may be an effective way to relieve the myocardial injury after the oxidative stress.

In addition, I/R injury-induced reactive oxygen species activate the intracellular signaling pathways such as mitogen-activated protein kinase (MAPK) pathway[7,8]. The subfamilies of MAPK, stress kinases, such as p38, and c-Jun N-terminal kinase (JNK) induce inflammation, apoptosis, and cell death, while the pro-survival kinases regulate the differentiation and proliferation of cells, promote cell survival, and protect tissues[9,10]. Yu et al[11] have manifested that the activated extracellular regulated protein kinase 1/2 (ERK1/2) and the inhibited p38/JNK attenuate oxidative stress and inflammation to maintain the cytoskeletal structure and protect the myocardium against damage. Therefore, the relative activities of these pro-apoptotic and pro-survival kinase pathways determine the survival or death of cells. ERK1/2 modulates various life processes, including myocardial infarction and ventricular remodeling. It is an important signal transduction in myocardial regulation and a powerful mediator in the development and progression of myocardial infarction[12,13].

This study aims to explore the effect of butorphanol on myocardial injury in rats after myocardial I/R and its impact on the MAPK signaling pathway. We first established an *in vivo* I/R model in the rats, and they were intervened by butorphanol application. By assessing the inflammatory response, the cardiac function, and the pathological injury of the myocardium in rats, we analyzed the potential protective effect of butorphanol on I/R injury. In addition, the involvement of the MAPK pathway was determined. Our findings aim to provide the theoretical references for the clinical treatment of myocardial I/R injury using butorphanol.

Materials and Methods

Establishment of Animal Models

A total of 40 male Sprague-Dawley rats were randomly selected, generally anesthetized with sodium pentobarbital (40 mg/kg) and ventilated (50 times/min), followed by performing thoracotomy. Myocardial I/R was conducted by ligation of the left coronary artery. After 50 min of ischemia, the ligature was loosened for 3-h reperfusion. 20 rats in the treatment group were administrated with 25 mg/kg butorphanol. Additionally, 20 rats were selected as normal group and they were subjected to the same surgical procedures except for the ligation of the left coronary artery. This study was approved by the Animal Ethics Committee of Jinzhou Medical University Animal Center.

Examination of Cardiac Function and Liver Function

Since the cardiac function and liver function are abnormal after myocardial I/R, the liver function indexes alanine aminotransferase (ALT), alkaline phosphatase (ALP), and the myocardial function indicator creatine kinase (CK) in rats were detected for assessing the disease severity. The blood samples were routinely collected from femoral veins and centrifuged at 4°C for 10 min to separate and collect serum. Lastly, a biochemical analyzer was employed to determine relevant indexes.

Detection of Cardiac Physiological Function Indexes in Rats

The left ventricular function indicators, including left ventricular end-diastolic diameter (LVEDd), left ventricular end-systolic diameter (LVESd), ejection fraction (EF), and fractional shortening (FS) of all rats was examined using a Philips 7500 ultrasound machine (Philips Medical, Amsterdam, The Netherlands), magnetic resonance imaging (MRI), and echocardiography (ECG) system according to the requirements in the instrument manual. In each group, a rat to be examined was placed in a supine position and underwent an electrocardiographic examination with a probe frequency of 10 MHz.

Determination of Inflammatory Cytokines in Each Group of Rats

The rats were sacrificed to harvest the heart. After washing in normal saline, about 0.5 g of myocardial tissues were collected, homogenated on ice, and centrifuged at 1200×g, at 4°C for 30 min. The supernatant was collected. The serum levels of the inflammatory cytokines were measured using an enzyme-linked immunosorbent assay (ELISA) kit (R&D System, Minneapolis, MN, USA), and a microplate reader was utilized to read the absorbance in each group.

Hematoxylin-Eosin (HE) Staining

The heart tissues were fixed in 10% neutral buffered formalin for 7 d, washed with running water for 24 h, dehydrated with gradient alcohol, and sliced into conventional sections (5 μm in thickness) using a microtome (Leica RM 2125,

Table I. Primer sequences

Target gene	Primer sequence
β-actin	F: 5'-CAGTGCCAGCCTCGTCTCAT-3'
	R: 5'-AGGGCCATCCACAGTCTTC-3'
Caspase3	F: 5'-CTACCGCACCCGGTTACTAT-3'
	R: 5'-TTCCGGTTAACACGAGTGAG-3'
MAPK	F: 5'-CCAGATGCCGAAGATGAACT-3'
	R: 5'-GGGCTGCTGTGATCCTCTTAT-3'
JNK	F: 5'-TTCCATTGTGGGTAGGTGG-3'
	R: 5'-CTTACAGCTTCCGCTTCAG-3'
ERK1	F: 5'-CTTGGCATCCGCACTCTG-3'
	R: 5'-CTGAAGCCTGGCAACCTG-3'

Wetzlar, Germany). Next, the tissue sections were deparaffinized, hydrated with 95%, 90%, 80%, 75%, and 50% ethanol, respectively, permeabilized, and embedded in paraffin. After that, the embedded blocks were prepared into pathological sections. Lastly, the sections were stained with HE and mounted, and a light microscope was employed for histological observation.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

The total ribonucleic acids (RNAs) were extracted from rat myocardial tissues in each group with TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and then, reversely transcribed into complementary deoxyribonucleic acid (cDNA). Thereafter, primer amplification was performed in a 20-μL system (2 μL of cDNA, 10 μL of mix, 2 μL of primer, and 6 μL of ddH_2O), followed by PCR amplification. The qRT-PCR reaction conditions were as follows: 94°C for 30 s, 55°C for 30 s, and 72°C for 90 s, for a total of 40 cycles. The primer sequences (Table I) of the target genes and β-Actin (as internal reference) were designed based on the sequences on GenBank. The expression level of the target genes was measured via quantitative Reverse Transcription (RT)-PCR. The mRNA expression in rat myocardial tissues in each group was calculated through the $2^{-\Delta\Delta Ct}$ method.

Western Blotting

The rat heart tissues were cut into pieces, weighed, and added to radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China) (ratio of 100 mg: 1 mL), and homogenized. Subsequently, the proteins were extracted using lysis buffer, and quantified using a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA). Then, the samples were loaded for electrophoresis and transferred on polyvinylidene difluoride (PVDF) membranes. Thereafter, the membrane was blocked, incubated with primary antibody overnight and secondary antibody for 1 h. The band exposure was conducted using enhanced chemiluminescence (ECL) and analyzed using an Odyssey membrane scanner. The protein levels were normalized to that of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The Western blotting bands were quantified using Image Lab software (Bio-Rad, Hercules, CA, USA).

Statistical Analysis

All experimental data recorded were processed by Statistical Product and Service Solutions (SPSS) 20.0 software (IBM Corp., Armonk, NY, USA). The experimental results obtained were expressed as mean ± standard deviation ($\bar{\chi}$±SD), and $p<0.05$ suggested that the difference was statistically significant. GraphPad Prism 8.0 (La Jolla, CA, USA) was used for the histogram.

Results

Examination Results of Cardiac Function and Liver Function

Levels of ALP, ALT, and CK were significantly higher in the I/R model group than those in the normal group ($p<0.05$). Moreover, they were evidently reduced in the treatment group ($p<0.05$), suggesting that the liver function and myocardial function indexes were significantly impaired due to I/R. Finally, butorphanol greatly improved cardiac and liver function (Figure 1).

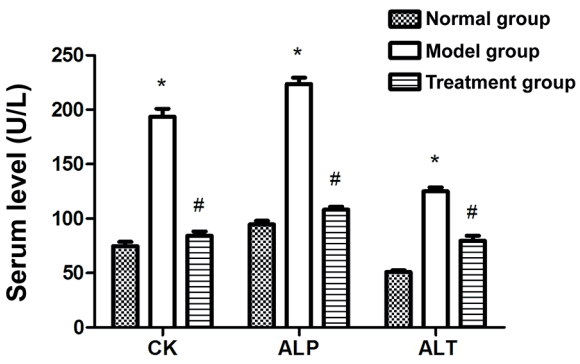

Figure 1. Biochemical results. The ALP, ALT, and CK levels in the treatment group clearly decline, implying that the liver function and myocardial function indicators are abnormal. *$p<0.05$ *vs.* normal group, #$p<0.05$ *vs.* model group.

Table II. Cardiac function indexes in rats determined by MRI and ECG.

Group	LVEDd (mm)	LVESd (mm)	EF (%)	FS (%)
Normal group	4.98±0.86	4.68±0.28	62.2±3.9	55.7±3.1
Model group	9.45±0.15[a]	7.51±0.26[a]	47.6±3.6[a]	39.8±2.8[a]
Treatment group	5.64±0.23[b]	4.99±0.69[b]	59.4±2.9[b]	51.6±1.9[b]

Note: The FS and EF are lower in model group than those in normal group, while the LVEDd and LVESd exhibit contrary tendencies. [a]$p<0.05$ *vs.* normal group, [b]$p<0.05$ *vs.* model group.

Cardiac Function Indexes Determined in Two Groups of Rats

The rats in the I/R model group had notably lowered FS and EF, but markedly elevated LVEDd and LVESd in comparison with the normal group ($p<0.05$), indicating the successful establishment of the I/R model in rats (Table II).

Detection Results of Cytokines in Each Group

The levels of inflammatory factors TNF-γ, interleukin-6 (IL-6), and IL-1β in rats were measured. The results showed that the levels of TNF-γ, IL-6, and IL-1β were remarkably higher in the I/R model group than those in the normal group. These levels in the treatment group were lower compared with those in the I/R group ($p<0.05$) (Figure 2). It is suggested that the massive inflammatory factors were activated after myocardial I/R, which further aggravated myocardial I/R injury (Figure 2).

Results of HE Staining

The results of HE staining revealed the disorderly-arranged myocardial cells and thickened muscle fibers in the I/R model group (Figure 3A), while administration of butorphanol alleviated pathological myocardial injury after I/R (Figure 3B).

Expressions of Caspase3 and JNK Detected via qRT-PCR

QRT-PCR results showed that in the treatment group, the levels of Caspase3 and JNK markedly decreased ($p<0.05$), while the expression levels of MAPK and ERK were overtly upregulated ($p<0.05$) (Figure 4). However, in the model group, these expression levels showed opposite trends, implying that the treatment with butorphanol promoted the proliferation and inhibited the apoptosis of the myocardial ischemic cells.

Expressions of Apoptosis-and Pathway-Related Proteins Determined Through Western Blotting

Western Blotting results revealed that the expression level of MAPK in the treatment group was evidently upregulated ($p<0.05$), which was downregulated in I/R model group, indicating that the treatment with butorphanol facilitated the repair of the I/R injury (Figure 5).

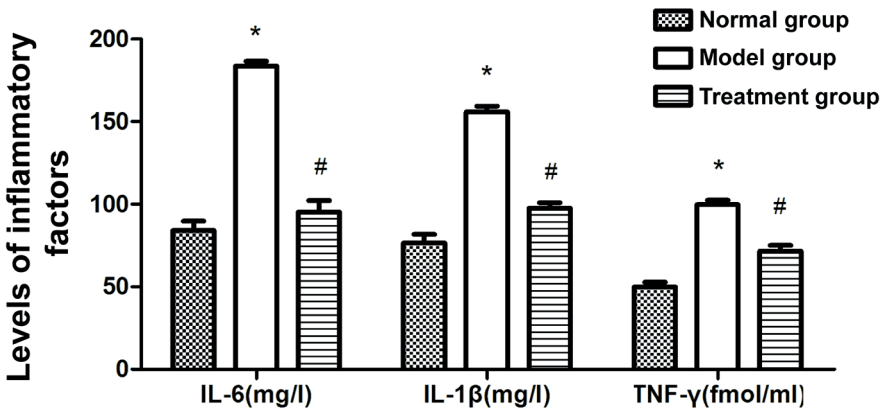

Figure 2. The levels of the inflammatory factors. The levels of TNF-γ, IL-6, and IL-1β are distinctly raised in the model group, but remarkably reduced in the treatment group. *$p<0.05$ *vs.* normal group, #$p<0.05$ *vs.* model group.

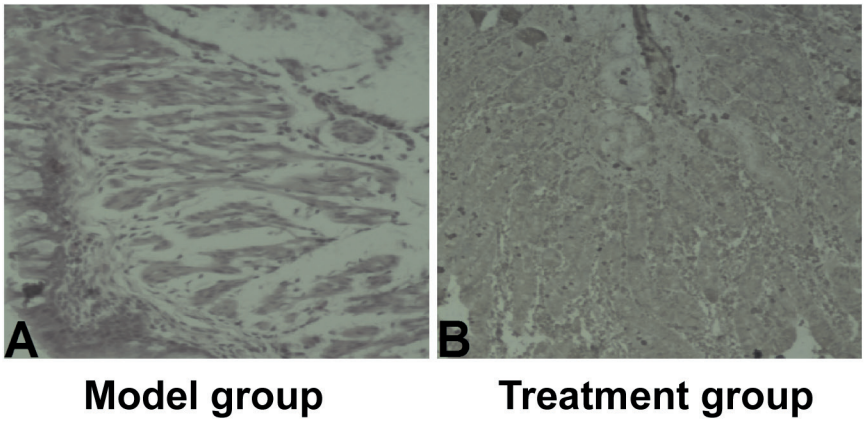

Figure 3. HE staining results of rat heart. **A,** Model group (×10). **B,** Treatment group (×10). Disorderly-arranged myocardial cells and thickened muscle fibers are detected in the model group, while almost no myocardial injury is found in the treatment group.

Discussion

The reperfusion of the ischemic tissues increases the production of the oxygen free radicals, disrupts the balance between oxidants and antioxidants, and leads to uncontrolled myocardial injury[14]. Reperfusion is usually applied for the treatment of myocardial ischemia, which, however, leads to some pathological consequences, namely reperfusion injury. The reperfusion injury is mediated by apoptosis. During myocardial ischemia, the myocardial cell injury occurs through a series of events, and a severe blood flow disorder leads to rapid cell death[15]. The damaged myocardial cells and inflammatory cells secrete elements to mediate the I/R injury. In addition, the inflammatory cells secrete a large number of toxic cytokines[16]. Currently, the studies on the specific mechanisms may help to clarify the pathogenesis and therapeutic mechanism of the disease, providing theoretical support for the treatment of myocardial ischemia-related diseases. The free radicals can cause lipid peroxidation and denaturation of cells, DNAs, and proteins. Inflammation destroys the

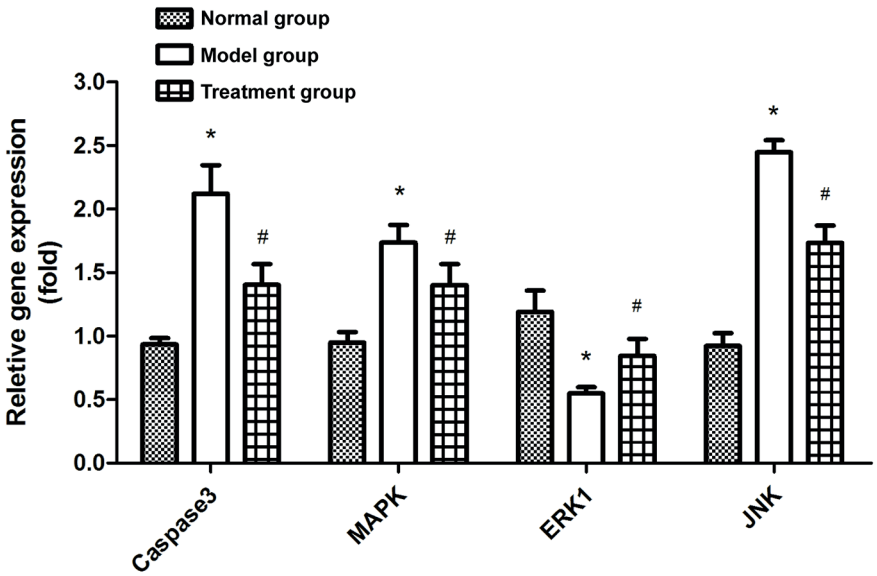

Figure 4. Gene expression levels. The treatment group exhibits remarkably decreased levels of Caspase3 and JNK ($p<0.05$) and notably raised the expression levels of MAPK and ERK ($p<0.05$). $^{*}p<0.05$, $^{\#}p<0.05$.

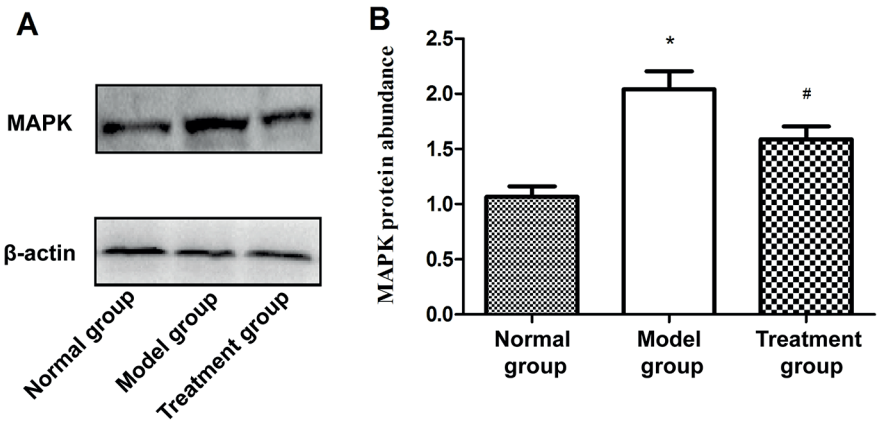

Figure 5. Protein expressions. The MAPK expression level is significanlty elevated in the treatment group ($p<0.05$). $^{*}p<0.05$, $^{\#}p<0.05$.

integrity of the cell membrane, while intracellular LDH, CK-MB, and other myocardial enzymes are released in extracellular fluid[17,18]. In this study, the liver and cardiac functions were assessed. The serum levels of ALP, ALT, and CK were significantly higher in the I/R model group than those in the normal group ($p<0.05$). Besides, the rheological results of the heart revealed that the rats in the I/R model group clearly lowered FS and EF and notably increased LVEDd and LVESd in comparison with the normal group ($p<0.05$). Moreover, pathological morphology in rat myocardium was pronounced in the I/R group. The above pathological indexes were greatly improved in the treatment group.

The pro-inflammatory cytokines TNF-γ and IL-6 are correlated with I/R injury. TNF-γ triggers the inflammatory cascade by up-regulating the expressions of other pro-inflammatory cytokines, including interleukins[19]. In addition, TNF-γ leads to apoptosis of myocardial cells and ventricular remodeling[20,21]. Some works have manifested that I/R is involved in both inflammatory responses and gene activation of cells. In this study, the relative levels of IL-6, IL-1β, and TNF-γ were elevated in the I/R model group, suggesting the aggravated inflammatory response following the I/R injury. The butorphanol treatment significantly decreased these levels, suggesting the anti-inflammatory effect of butorphanol at post-I/R. TNF-γ is an indispensable participant in the progression of inflammation after myocardial I/R, and IL-6 can also trigger the excessive production of other inflammatory mediators[22,23]. It is demonstrated that apoptosis is involved in myocardial I/R injury. Besides, apoptosis can respond to cell invasion. Apoptosis is quickly initiated under different stimuli. In addition, it can serve as an important marker for many relevant clinical diseases, such as tumor and myocardial injury. Consistently with previous reports[24,25], our findings showed upregulation of Caspase3, an apoptosis-related gene, in the I/R model group compared with that in the normal group.

MAPK acts on many biological processes, including cell migration and death. Zhou et al[26] have shown that the drug treatment of I/R can enhance cardiac function, suppress heart damage, down-regulate levels of pro-inflammatory factors, and enhance the antioxidant capacity of myocardial tissues. This effect is associated with the inhibition on the p38 MAPK signaling pathway[27]. Besides, inhibited p38 MAPK protects mitochondria from the I/R invasion and injury and improves cardiac function and ventricular remodeling after I/R[28]. A research has shown that the expression of phosphorylated (p)-p38 MAPK in I/R group is significantly higher than that in the Sham operation group, suggesting that myocardial ischemia leads to the upregulation of p-p38 MAPK. HMGB1 treatment reduces the expression of p-p38 MAPK in a dose-dependent manner. These results imply that the intravenous injection of HMGB1 downregulates p-p38 MAPK in ischemic myocardium[29]. It is known that Bax is activated by MAPK to mediate apoptosis after reperfusion. Moreover, the activation of ERK1/2 limits the damage to the heart after apoptosis. Several authors[18,30,31] have manifested that the targeted inhibition on p38 MAPK is able to mitigate myocardial cell apoptosis and improve myocardial performance after I/R injury. Additionally,

melatonin[32] protects the liver against I/R injury by inhibiting apoptosis that is mediated by inactivation of JNK and p38 MAPK signaling pathways. MAPKs are multifunctional regulators in cardiomyocyte proliferation, survival, apoptosis, actin recombination, cytokine production, and other biological processes. There are four major MAP kinase subfamilies, namely ERK1/2, JNK1, p38 kinases (α, β, γ, and δ), and MAPK[33]. The activation of the ERK1/2 signal[34] is considered to be one of the major components of the risk (reperfusion injury salvage kinase) pathway. In this paper, the expression levels of MAPK and JNK were overtly downregulated in the treatment group, while ERK was remarkably upregulated. These results[35] indicated that the treatment of butorphanol protected apoptosis of myocardial ischemic cells by inactivating the MAPK pathway.

Conclusions

Butorphanol may protect the myocardium against I/R injury by alleviating the infiltration of the inflammatory cells and apoptosis of myocardial cells via inactivating the MAPK pathway. This study provides the theoretical basis for the prevention and treatment of the myocardial I/R injury.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Buja LM. Myocardial ischemia and reperfusion injury. Cardiovasc Pathol 2005; 14: 170-175.
2) Ohno K, Ito M, Ichihara M, Ito M. Molecular hydrogen as an emerging therapeutic medical gas for neurodegenerative and other diseases. Oxid Med Cell Longev 2012; 2012: 353152.
3) Jennings RB. Historical perspective on the pathology of myocardial ischemia/reperfusion injury. Circ Res 2013; 113: 428-438.
4) Baines CP. How and when do myocytes die during ischemia and reperfusion: the late phase. J Cardiovasc Pharmacol Ther 2011; 16: 239-243.
5) Haudek SB, Taffet GE, Schneider MD, Mann DL. TNF provokes cardiomyocyte apoptosis and cardiac remodeling through activation of multiple cell death pathways. J Clin Invest 2007; 117: 2692-2701.
6) Dumont EA, Hofstra L, van Heerde WL, van den Eijnde S, Doevendans PA, DeMuinck E, Daemen MA, Smits JF, Frederik P, Wellens HJ, Daemen MJ, Reutelingsperger CP. Cardiomyocyte death induced by myocardial ischemia and reperfusion: measurement with recombinant human annexin-V in a mouse model. Circulation 2000; 102: 1564-1568.
7) Son Y, Cheong YK, Kim NH, Chung HT, Kang DG, Pae HO. Mitogen-activated protein kinases and reactive oxygen species: how can ROS activate MAPK pathways? J Signal Transduct 2011; 2011: 792639.
8) Gao XF, Zhou Y, Wang DY, Lew KS, Richards AM, Wang P. Urocortin-2 suppression of p38-MAPK signaling as an additional mechanism for ischemic cardioprotection. Mol Cell Biochem 2015; 398: 135-146.
9) Li DY, Tao L, Liu H, Christopher TA, Lopez BL, Ma XL. Role of ERK1/2 in the anti-apoptotic and cardioprotective effects of nitric oxide after myocardial ischemia and reperfusion. Apoptosis 2006; 11: 923-930.
10) Shimada K, Nakamura M, Ishida E, Kishi M, Konishi N. Roles of p38- and c-jun NH2-terminal kinase-mediated pathways in 2-methoxyestradiol-induced p53 induction and apoptosis. Carcinogenesis 2003; 24: 1067-1075.
11) Yu J, Wang L, Akinyi M, Li Y, Duan Z, Zhu Y, Fan G. Danshensu protects isolated heart against ischemia reperfusion injury through activation of Akt/ERK1/2/Nrf2 signaling. Int J Clin Exp Med 2015; 8: 14793-14804.
12) Wang S, Zhang H, Geng B, Xie Q, Li W, Deng Y, Shi W, Pan Y, Kang X, Wang J. 2-arachidonyl glycerol modulates astrocytic glutamine synthetase via p38 and ERK1/2 pathways. J Neuroinflammation 2018; 15: 220.
13) Jiang M, Zhang C, Wang J, Chen J, Xia C, Du D, Zhao N, Cao Y, Shen L, Zhu D. Adenosine A(2A) R modulates cardiovascular function by activating ERK1/2 signal in the rostral ventrolateral medulla of acute myocardial ischemic rats. Life Sci 2011; 89: 182-187.
14) Zhang ZX, Li H, He JS, Chu HJ, Zhang XT, Yin L. Remote ischemic postconditioning alleviates myocardial ischemia/reperfusion injury by up-regulating ALDH2. Eur Rev Med Pharmacol Sci 2018; 22: 6475-6484.
15) Guo YR, Choung SY. Germacrone attenuates hyperlipidemia and improves lipid metabolism in high-fat diet-induced obese C57BL/6J mice. J Med Food 2017; 20: 46-55.
16) Mu F, Duan J, Bian H, Yin Y, Zhu Y, Wei G, Guan Y, Wang Y, Guo C, Wen A, Yang Y, Xi M. Cardioprotective effects and mechanism of Radix Salviae miltiorrhizae and Lignum Dalbergiae odoriferae on rat myocardial ischemia/reperfusion injury. Mol Med Rep 2017; 16: 1759-1770.
17) Malik S, Sharma AK, Bharti S, Nepal S, Bhatia J, Nag TC, Narang R, Arya DS. *In vivo* cardioprotection by pitavastatin from ischemic-reperfusion injury through suppression of IKK/NF-kappaB and up-regulation of pAkt-e-NOS. J Cardiovasc Pharmacol 2011; 58: 199-206.

18) Yu D, Li M, Tian Y, Liu J, Shang J. Luteolin inhibits ROS-activated MAPK pathway in myocardial ischemia/reperfusion injury. Life Sci 2015; 122: 15-25.

19) Naidu BV, Woolley SM, Farivar AS, Thomas R, Fraga CH, Goss CH, Mulligan MS. Early tumor necrosis factor-alpha release from the pulmonary macrophage in lung ischemia-reperfusion injury. J Thorac Cardiovasc Surg 2004; 127: 1502-1508.

20) Zhu J, Liu M, Kennedy RH, Liu SJ. TNF-alpha-induced impairment of mitochondrial integrity and apoptosis mediated by caspase-8 in adult ventricular myocytes. Cytokine 2006; 34: 96-105.

21) Zhou YH, Han QF, Wang LH, Liu T, Meng XY, Wu L, Li T, Jiao YR, Yao HC, Zhang DY. High mobility group box 1 protein attenuates myocardial ischemia reperfusion injury via inhibition of the p38 mitogen-activated protein kinase signaling pathway. Exp Ther Med 2017; 14: 1582-1588.

22) Naka T, Nishimoto N, Kishimoto T. The paradigm of IL-6: from basic science to medicine. Arthritis Res 2002; 4 Suppl 3: S233-S242.

23) Wright JG, Christman JW. The role of nuclear factor kappa B in the pathogenesis of pulmonary diseases: implications for therapy. Am J Respir Med 2003; 2: 211-219.

24) Ravindran S, Kurian GA. The role of secretory phospholipases as therapeutic targets for the treatment of myocardial ischemia reperfusion injury. Biomed Pharmacother 2017; 92: 7-16.

25) Ravindran S, Jahir HS, Boovarahan SR, Kurian GA. Sodium thiosulfate post-conditioning protects rat hearts against ischemia reperfusion injury via reduction of apoptosis and oxidative stress. Chem Biol Interact 2017; 274: 24-34.

26) Adluri RS, Thirunavukkarasu M, Dunna NR, Zhan L, Oriowo B, Takeda K, Sanchez JA, Otani H, Maulik G, Fong GH, Maulik N. Disruption of hypoxia-inducible transcription factor-prolyl hydroxylase domain-1 (PHD-1-/-) attenuates ex vivo myocardial ischemia/reperfusion injury through hypoxia-inducible factor-1alpha transcription factor and its target genes in mice. Antioxid Redox Signal 2011; 15: 1789-1797.

27) Zhou M, Liu L, Wang W, Han J, Ren H, Zheng Q, Wang D. Role of licochalcone C in cardioprotection against ischemia/reperfusion injury of isolated rat heart via antioxidant, anti-inflammatory, and anti-apoptotic activities. Life Sci 2015; 132: 27-33.

28) Kumphune S, Surinkaew S, Chattipakorn SC, Chattipakorn N. Inhibition of p38 MAPK activation protects cardiac mitochondria from ischemia/reperfusion injury. Pharm Biol 2015; 53: 1831-1841.

29) Wang X, Lv H, Gu Y, Wang X, Cao H, Tang Y, Chen H, Huang C. Protective effect of lycopene on cardiac function and myocardial fibrosis after acute myocardial infarction in rats via the modulation of p38 and MMP-9. J Mol Histol 2014; 45: 113-120.

30) Wang H, Zhu QW, Ye P, Li ZB, Li Y, Cao ZL, Shen L. Pioglitazone attenuates myocardial ischemia-reperfusion injury via up-regulation of ERK and COX-2. Biosci Trends 2012; 6: 325-332.

31) Thomas CJ, Ng DC, Patsikatheodorou N, Limengka Y, Lee MW, Darby IA, Woodman OL, May CN. Cardioprotection from ischaemia-reperfusion injury by a novel flavonol that reduces activation of p38 MAPK. Eur J Pharmacol 2011; 658: 160-167.

32) Huang LH, Li J, Gu JP, Qu MX, Yu J, Wang ZY. Butorphanol attenuates myocardial ischemia reperfusion injury through inhibiting mitochondria-mediated apoptosis in mice. Eur Rev Med Pharmacol Sci 2018; 22: 1819-1824.

33) Liu Z, Chen JM, Huang H, Kuznicki M, Zheng S, Sun W, Quan N, Wang L, Yang H, Guo HM, Li J, Zhuang J, Zhu P. The protective effect of trimetazidine on myocardial ischemia/reperfusion injury through activating AMPK and ERK signaling pathway. Metabolism 2016; 65: 122-130.

34) Rose BA, Force T, Wang Y. Mitogen-activated protein kinase signaling in the heart: angels versus demons in a heart-breaking tale. Physiol Rev 2010; 90: 1507-1546.

35) Chen Y, Ba L, Huang W, Liu Y, Pan H, Mingyao E, Shi P, Wang Y, Li S, Qi H, Sun H, Cao Y. Role of carvacrol in cardioprotection against myocardial ischemia/reperfusion injury in rats through activation of MAPK/ERK and Akt/eNOS signaling pathways. Eur J Pharmacol 2017; 796: 90-100.