European Review for Medical and Pharmacological Sciences 2019; 23: 8658-8664

Effect of ulinastatin on myocardial ischemia-reperfusion injury through JNK and P38 MAPK signaling pathways

Z.-H. YANG[1], Y.-J. LU[1], K.-P. GU[2], Z.-Y. XIANG[2], H.-M. HUANG[3]

[1]Department of Anesthesiology, Sanmen Hospital of Traditional Chinese Medicine, Taizhou, China
[2]Department of Anesthesiology Sanmen People's Hospital, Taizhou, China
[3]Department of Anesthesiology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China

Zhenhua Yang and Yongjian Lu contributed equally to this work

Abstract. – OBJECTIVE: The aim of this study was to investigate the effect of ulinastatin (UTI) on myocardial ischemia-reperfusion injury (MIRI) through the c-Jun N-terminal kinase (JNK) signaling pathway and p38 mitogen-activated protein kinase (MAPK) signaling pathway.

MATERIALS AND METHODS: Healthy adult male Sprague-Dawley (SD) rats were randomly divided into 5 groups, including the sham group (*n*=10), MIRI group (model group, *n*=10), UTI group (*n*=10), UTI + JNK inhibitor SP600125 (UTI+SP600125 group, *n*=10) and UTI + p38 MAPK inhibitor SB203580 (UTI+SB203580 group, *n*=10). Hemodynamics, myocardial infarction and the messenger ribonucleic acid (mRNA) expressions of p38 MAPK, JNK, superoxide dismutase (SOD), malondialdehyde (MDA), nitric oxide (NO), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and high-sensitivity C-reactive protein (hs-CRP) were compared among groups. The protein levels of p38 MAPK and JNK in serum were detected *via* Western blotting. Furthermore, the correlations of serum p38 MAPK and JNK with TNF-α were analyzed.

RESULTS: In the UTI group, the left ventricular systolic pressure (LVSP), the left ventricular end-diastolic pressure (LVEDP) and maximal rate of increase of the left ventricular pressure (+dp/dtmax) were significantly higher than those of the model group. However, the maximal rate of the decrease of the left ventricular pressure (-dp/dtmax), infarction area and ischemia area were significantly smaller than those of the model group. LVSP, LVEDP and +dp/dtmax in UTI+SP600125 group and UTI+SB203580 group were markedly higher than those of the UTI group. Meanwhile, the mRNA expressions of p38 MAPK and JNK in the UTI group were significantly lower than those of the model group. However, the mRNA expression levels of p38 MAPK and JNK in UTI+SP600125 group and UTI+SB203580 group were remarkably higher than the UTI group. Besides, the UTI group showed a markedly higher level of SOD and significantly lower levels of MDA, NO, TNF-α, IL-6 and hs-CRP than the model group. Furthermore, UTI+SP600125 group and UTI+SB203580 group showed significantly higher levels of MDA, NO, TNF-α, IL-6 and hs-CRP than the UTI group. The protein levels p38 MAPK and JNK were remarkably lower in the UTI group than those of the model group. However, they were remarkably higher in UTI+SP600125 group and UTI+SB203580 group than the UTI group. In addition, both p38 MAPK and JNK proteins were positively correlated with TNF-α (*r*=0.983 and 0.892, *p*=0.043 and *p*=0.033).

CONCLUSIONS: UTI may inhibit MIRI caused by p38 MAPK signaling pathway and JNK signaling pathway by down-regulating TNF-α expression, thereby protecting and improving MIR.

Key Words:
Ulinastatin, p38 MAPK, JNK, Myocardial ischemia-reperfusion (MIR).

Introduction

Currently, the morbidity rate of myocardial infarction is increasing in the clinic. Myocardial injury after infarction has always been a major concern worldwide. To adopt more effective therapeutic measures, the pathogenesis of myocardial injury should be studied thoroughly. However, it is believed that the pathological conduction pathway of myocardial injury is complex. Meanwhile, a few researches have elucidated the pathogenesis of myocardial injury. Therefore, its treatment is still in the exploration stage[1,2]. Previous studies[3,4] have indicated that the pathological mechanism of ischemia-reperfusion is mainly related to free radicals and inflammatory response. Therefore, the drugs used are mostly to scavenge free radicals and inhibit inflammation.

Corresponding Author: Haiming Huang, MD; e-mail: heminghw@163.com

Ulinastatin (UTI) is widely applied in the treatment of shock, immunoregulation and tumor chemotherapy. Clinical studies[5-7] have found that UTI possesses a potential inhibitory effect on inflammation and protective effect in cerebral ischemia-reperfusion injury. However, the exact role of UTI in myocardial ischemia-reperfusion injury (MIRI) has not been studied comprehensively.

In the present study, the MIRI model was established to explore the protective mechanism of UTI in MIRI through the c-Jun N-terminal kinase (JNK) signaling pathway and p38 mitogen-activated protein kinase (MAPK) signaling pathway.

Materials and Methods

Materials

Animals: a total of 50 healthy adult male Sprague-Dawley (SD) rats weighing 200-230 g were enrolled in this study. All rats were fed under the temperature of 20-28°C and humidity of 55-70%, with free access to water and food. This study was approved by the Animal Ethics Committee of the Animal Center of Sanmen Hospital of Traditional Chinese Medicine.

Drugs and reagents: UTI (NMPN H19990134, Guangdong Techpool Biochemical Pharmaceutical Co., Ltd., Guangzhou, China), JNK inhibitor SP600125 (Cell Signaling Technology, Danvers, MA, USA), p38 MAPK inhibitor SB203580 (Cell Signaling Technology, Danvers, MA, USA), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), high-sensitivity C-reactive protein (hs-CRP), superoxide dismutase (SOD), and malondialdehyde (MDA) assay kits (Jiancheng, Nanjing, China), and anti-mouse JNK and p38 MAPK immunohistochemistry kits (Sigma-Aldrich, St. Louis, MO, USA).

Animal Grouping and Drug Administration

All rats were randomly divided into 5 groups using a random number table, including the sham group (n=10), MIRI group (model group, n=10), UTI group (n=10), UTI + JNK inhibitor SP600125 (UTI+SP600125 group, n=10) and UTI + p38 MAPK inhibitor SB203580 (UTI+SB203580 group, n=10). The rats in the UTI group were treated with UTI injection (10000 U/kg). The rats in the sham group and model group were treated with an equal amount of normal saline. Meanwhile, the rats in the other two groups were intravenously injected with UTI+SP600125 (0.2 mg/kg) and UTI+SB203580 (0.2 mg/kg).

Modeling

The MIR model was established at 1 h after drug administration. The rats were first anesthetized with urethane (1 g/kg, 20%), and the trachea was cut open and connected to a ventilator (respiratory frequency: 90 times/min, tidal volume: 12 mL). Electrocardiogram monitoring was then performed. Subsequently, the skin was cut between the 3rd and 4th intercostal spaces at the left sternal border, and the thoracic cavity was opened to squeeze out the heart. The left anterior descending coronary artery was ligated at the junction of the pulmonary arterial cone and left auricle. After that, the heart was placed back into the thoracic cavity. In the sham group, the thoracic cavity was opened and threaded. However, the left anterior descending coronary artery was not ligated[8].

Observation Indexes

Hemodynamic indexes: cardiac function indexes, such as left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP) and maximal rate of the increase/decrease of left ventricular pressure (±dp/dtmax), were measured at 120 min after reperfusion using a physiological recorder.

Myocardial infarction: coronary artery was blocked at 2 h after reperfusion. 2 mL Evans blue (2%) was injected through the internal carotid artery. Ischemic tissues (no blue) and non-ischemic tissues (blue) were then observed. Subsequently, the heart was taken out, sliced and sucked dry. Ischemic myocardial sections were incubated with TIC phosphate buffer (5%) at 37°C for 15 min, followed by observation of infarction tissues. The mass of necrotic region (gray-white) and non-necrotic region (dark red) was detected: ischemia area = percentage of the mass of ischemic tissues in the mass of left ventricle, and infarction area = percentage of the mass of infarction tissues in the mass of left ventricle[9].

The mRNA levels of JNK and p38 MAPK in myocardium were determined *via* Polymerase Chain Reaction (PCR)[10,11].

Determination of SOD, MDA, nitric oxide (NO), TNF-α, IL-6, and hs-CRP content: at 120 min after reperfusion, 4 mL of blood was collected from the femoral artery, followed by centrifugation at 3000 rpm for 10 min. Meanwhile, the serum was collected to detect TNF-α, IL-6, and hs-CRP *via* enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN, USA). NO, SOD and MDA were determined *via* nitrate reductase method, xanthine oxidase method and thiobarbituric acid method, respectively.

Table I. Effects of UTI on hemodynamics and infarction area in MIRI rats ($\bar{\chi}$±s).

Group	LVSP/ mmHg	LVEDP/ mmHg	+dp/dtmax/ mmHg.s-1	-dp/dtmax/ mmHg.s-1	Infarction area/%	Ischemia area/%
Sham group	145.78±11.09	4.34.45±0.43	4365.69±497.78	-3678.67.78±387.43		
Model group	92.43±9.78	13.09±1.23	2689.65±286.78	-1943.97±209.67	49.89	45.89
UTI group	128.56±10.98*#	7.54±0.98*#	3587.78±398.76*#	-2698.67.89±234.78*#	25.89*#	25.78*#
UTI+SP600125	101.87±10.78	12.09±1.21	2898.53±297.67	-2123.87±209.78	43.78	40.89
UTI+SB203580	100.53±11.09	11.99±1.09	2909.87±308.75	-2232.78±212.78	45.89	39.87

Note: *p<0.05 *vs*. model group, #p<0.05 *vs*. UTI+SP600125 group and UTI+SB203580 group.

The protein expressions of JNK and p38 MAPK in myocardium were determined *via* immunohistochemistry[11].

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 18.0 software (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis. Measurement data were expressed as ($\bar{\chi}$±s). The *t*-test was performed to compare the difference between the two groups. Enumeration data were expressed as n, and chi-square test was performed to compare the differences among different groups. p<0.05 was considered statistically significant.

Results

Effects of UTI on Hemodynamics and Infarction Area in MIRI Rats

In the UTI group, LVSP, LVEDP and +dp/dtmax were significantly higher than those of the model group (p<0.05). However, -dp/dtmax, infarction area and ischemia area were significantly smaller than those of the model group, displaying significant differences (p<0.05). In addition, LVEDP and -dp/dtmax in UTI+SP600125 group and UTI+SB203580 group were remarkably higher than those of the UTI group, and the differences were statistically significant (p<0.05; Table I).

Comparison of p38 MAPK mRNA among Groups

The mRNA level of p38 MAPK was significantly lower in the UTI group than that of the model group (p<0.05). However, it was markedly higher in the UTI+SP600125 group and UTI+SB203580 group when compared with the UTI group (p<0.05; Table II).

Effect of UTI on mRNA of JNK in MIRI Rats

The mRNA level JNK in the UTI group was markedly lower than that of the model group, while it was remarkably higher in the UTI+SP600125 group and UTI+SB203580 group than the UTI group (p<0.05; Table III).

Effects of UTI on SOD, MDA and NO in MIRI Rats

SOD level in the UTI group was remarkably higher than the model group, while it was significantly lower in the UTI+SP600125 group and UTI+SB203580 group than the UTI group (p<0.05). MDA and NO levels were markedly lower in the UTI group than those of the model group (p<0.05). However, they were remarkably higher in the UTI+SP600125 group and UTI+SB203580 group when compared with the UTI group (p<0.05; Table IV).

Table II. Effect of UTI on p38 MAPK mRNA in MIRI rats ($\bar{\chi}$±s).

Group	No.	p38 MAPK mRNA (%)
Sham group	10	0.19±0.05
Model group	10	0.39±0.26
UTI group	10	0.23±0.11*#
UTI+SP600125	10	0.31±0.23
UTI+SB203580	10	0.30±0.11

Note: *p<0.05 *vs.* model group, #p<0.05 *vs.* UTI+SP600125 group and UTI+SB203580 group.

Table III. Effect of UTI on JNK mRNA in MIRI rats ($\bar{\chi}$±s).

Group	No.	JNK mRNA (%)
Sham group	10	1.09±0.05
Model group	10	2.34±0.26
UTI group	10	1.26±0.11**#
UTI+SP600125	10	2.00±0.23
UTI+SB203580	10	1.98±0.11

Note: *p<0.05 *vs.* model group, #p<0.05 *vs.* UTI+SP600125 group and UTI+SB203580 group.

Table IV. Effects of UTI on SOD, MDA and NO in MIRI rats ($\bar{\chi}$±s).

	SOD (U/mL)	MDA (nmol/mL)	NO (μmol/mL)
Sham group	338.78±31.09	3.53±0.45	119.34±10.34
Model group	72.23±6.89	7.93±0.77	156.23±16.45
UTI group	298.65±30.86**#	4.32±0.27*##	123.54±11.09**##
UTI+SP600125	121.56±10.21	7.09±0.87	153.12±12.76
UTI+SB203580	119.78±11.33	7.21±0.79	150.98±11.09

Note: **p<0.01 *vs.* model group, *p<0.05 *vs.* model group. #p<0.05 *vs.* UTI+SP600125 group and UTI+SB203580 group. ##p<0.01 *vs.* UTI+SP600125 group and UTI+SB203580 group.

Table V. Effects of UTI on inflammatory factors in MIRI rats ($\bar{\chi}$±s).

	TNF-α (pg/mL)	IL-6 (pg/mL)	hs-CRP (μg/mL)
Sham group	8.23±31.09	243.43±21.45	3.41±10.34
Model group	20.98±6.89	843.23±81.77	9.89±16.45
UTI group	10.98±30.86**##	323.21±30.27*##	4.01±11.09*##
UTI+SP600125	17.34±10.21	678.76±32.87	7.67±12.76
UTI+SB203580	17.32±11.33	706.76±0.79	7.88±11.09

Note: **p<0.01 *vs.* model group, *p<0.05 *vs.* model group. ##p<0.01 *vs.* UTI+SP600125 group and UTI+SB203580 group.

Effects of UTI on Inflammatory Factors in MIRI Rats

The levels of TNF-α, IL-6 and hs-CRP in the UTI group were remarkably lower than those in the model group (p<0.05). However, they were significantly higher in the UTI+SP600125 group and UTI+SB203580 group than those of the UTI group (p<0.05; Table V).

Effects of UTI on the Protein Expressions of p38 MAPK and JNK in MIRI Rats

The protein levels of p38 MAPK and JNK in the UTI group were remarkably lower than those of the model group (p<0.05). However, they were remarkably higher in the UTI+SP600125 group and UTI+SB203580 group than UTI group (p<0.05; Table VI).

Table VI. Effects of UTI on p38 MAPK and JNK proteins in MIRI rats ($\bar{\chi}$±s).

	p38 MAPK protein	JNK protein
Sham group	0.87±0.09	0.45.43±0.04
Model group	11.98±2.09	1.59±0.11
UTI group	3.98±3.32**##	0.57±0.03*#
UTI+SP600125	10.34±1.09	1.03±0.01
UTI+SB203580	12.32±1.12	1.10±0.01

Note: **p<0.01 *vs.* model group, *p<0.05 *vs.* model group. #p<0.05 *vs.* UTI+SP600125 group and UTI+SB203580 group. ##p<0.01 *vs.* UTI+SP600125 group and UTI+SB203580 group.

Correlation Between p38 MAPK Protein and TNF-α

The protein level of p38 MAPK was positively correlated with TNF-α (r=0.983, p=0.043; Figure 1).

Correlation Between JNK Protein and TNF-α

The protein expression of JNK was positively correlated with TNF-α (r=0.892, p=0.033) as well (Figure 2).

Discussion

MIR is a common cardio-cerebrovascular disease in clinical practice. Oxygen and nutrients can be delivered to tissue cells through blood circulation in the human body, thereby ensuring normal metabolism. However, insufficient tissue blood perfusion will lead to ischemic injury. Therefore, it is necessary to restore tissue blood perfusion as soon as possible to avoid ischemic injury in tissue cells[12,13]. It has been found in clinic that metabolic disturbance and structural damage occurs in the body even after blood reperfusion is restored. This may further lead to tissue damage, namely ischemia-reperfusion. The disease is mainly correlated with the expression of oxygen free radicals. In the case of excessive expression of free radicals in the body, lipid peroxidation occurs in unsaturated fatty acids, eventually resulting in myocardial injury[14,15].

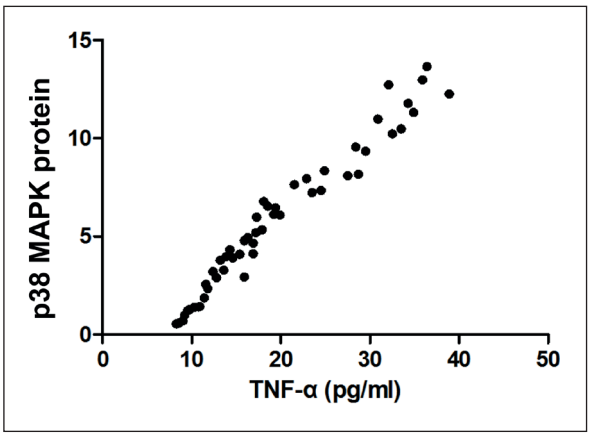

Figure 1. Correlation between p38 MAPK protein and TNF-α.

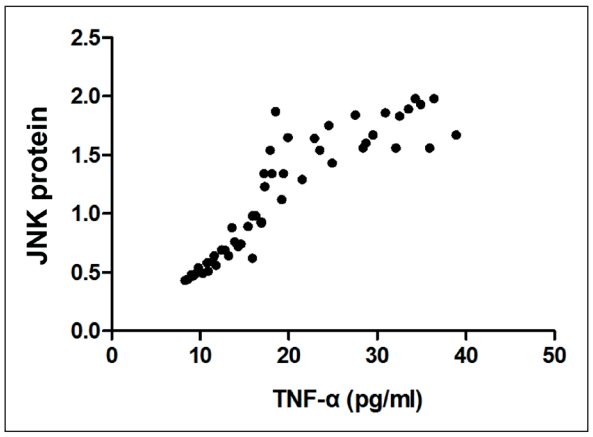

Figure 2. Correlation between JNK protein and TNF-α.

The pathogenesis of MIRI has not been comprehensively studied. Currently, it is generally believed that MIR is correlated with the JNK signaling pathway and p38 MAPK signaling pathway. The JNK signaling pathway is an important component of the MAPK pathway. According to clinical studies, it is mainly associated with inflammation and immunoregulation, which transmits stress response in cells. After the activation of JNK in the cytoplasm, inflammation- and apoptosis-related genes can be promoted. In addition, scholars[16,17] have found that excessive activation of the JNK signaling pathway will also lead to neuronal ischemic injury. P38 MAPK is also an important component of MAPK, which is mainly associated with cellular stress and injury response. It can transmit extracellular stimulus signals to the nucleus, thus playing an important role in clinical phenomena such as free radical damage and apoptosis[18,19]. UTI is a hydrolysis inhibitor in the urine and can inhibit the expression of the myocardial depressant factor *in vivo*. Meanwhile, it facilitates the stability of the cell membrane and organelle membrane, which can promote systemic circulation during shock as well. Clinical studies have also found that UTI is associated with the expression of inflammation- and immunoregulation-related factors, protecting the myocardium through scavenging oxidative free radicals.

Myocardial ischemia can destroy the integrity of the mitochondrial membrane, thus damaging the ultrastructure of myocardial cells. This is manifested as significantly enlargement of ischemic tissues and infarction tissues[20]. In the present research, it was found that the infarction and ischemia areas in the UTI group were markedly smaller than those of the model group. Meanwhile, significant differences were observed between the UTI+SP600125 group and UTI+SB203580 group. These results indicated that UTI could remarkably improve the symptoms of infarction and protect myocardial cells from injury. Both p38 MAPK and JNK stay at low levels in normal myocardial tissues. However, they are significantly up-regulated after MIR. In the present study, the results demonstrated that the mRNA and protein levels of p38 MAPK and JNK in the UTI group were remarkably lower than those of the model group after reperfusion. Meanwhile, they showed significant differences from the UTI+SP600125 group and UTI+SB203580 group. These findings suggested that UTI could also inhibit the degree of myocardial apoptosis and improve cardiac function. Furthermore, it might regulate myocardial injury in MIR through p38 MAPK and JNK pathways. It's worth noting that the effect of UTI is temporary and generally ends after the administration.

MIRI is associated with excessive expression of inflammatory factors and the release of oxidative free radicals. Clinical reports have revealed that the levels of inflammatory factors (such as TNF-α) and oxidative free radicals (such as MDA and SOD) are up-regulated in MIRI, and TNF-α, and IL-6 can induce IRI. Therefore, they have always been used as important indexes for the degree of myocardial injury. In the present work, the levels of inflammatory factors in the UTI group were markedly lower than those of the model group. Meanwhile, they also showed significant differences from UTI+SP600125 group and UTI+SB203580 group. The levels of MDA, SOD and NO in the UTI group were remarkably lower than those of the model group as well. These findings suggested that UTI could significant-

ly reduce the expression levels of inflammatory factors and improve the ability to scavenge free radicals. Moreover, p38 MAPK and JNK were found positively correlated with TNF-α in this study. In other words, a higher level of TNF-α *in vivo* corresponded to higher expressions of p38 MAPK and JNK. All these results indicated that the mechanism of p38 MAPK and JNK signaling pathways might be related to TNF-α.

The results of this work were similar to those of previous clinical tests. For example, Li et al[21] explored the protective mechanism of UTI in cerebral ischemia-reperfusion injury. They have found that UTI can inhibit JNK signal transduction pathway by up-regulating HSP70 expression, thereby protecting against cerebral injury caused by ischemia-reperfusion. Wang et al[22] observed the effects of UTI on inflammatory mediators and myocardial injury in acute myocardial infarction patients complicated with MIRI after direct percutaneous coronary intervention. Their findings have indicated that the application of UTI before and after PCI can inhibit the release of inflammatory mediators and alleviate inflammatory response during MIR.

The deficiency of this study was that the correlations of p38 MAPK and JNK with inflammatory factors IL-5 and hs-CRP were not fully investigated. Therefore, further in-depth studies were still needed.

Conclusions

We found that UTI can inhibit the expressions of inflammatory factors and improve oxygen free radical scavenging capacity by down-regulating the expressions of p38 MAPK and JNK, thereby improving MIRI.

Conflict of Interests

The authors declare that they have no conflict of interest.

References

1) Bolayir HA, Gunes H, Kivrak T, Sahin O, Akaslan D, Kurt R, Bolayir A, Imadoglu O. The role of SCUBE1 in the pathogenesis of no-reflow phenomenon presenting with ST segment elevation myocardial infarction. Anatol J Cardiol 2017; 18: 122-127.
2) Zhang Y, Liu YJ, Liu T, Zhang H, Yang SJ. Plasma microRNA-21 is a potential diagnostic biomarker of acute myocardial infarction. Eur Rev Med Pharmacol Sci 2016; 20: 323-329.
3) Hassan MQ, Akhtar MS, Akhtar M, Ali J, Haque SE, Najmi AK. Edaravone, a potent free radical scavenger and a calcium channel blocker attenuate isoproterenol induced myocardial infarction by suppressing oxidative stress, apoptotic signaling and ultrastructural damage. Ther Adv Cardiovasc Dis 2016; 10: 214-223.
4) Jagadeesh GS, Nagoor Meeran M, Selvaraj P. Protective effects of 7-hydroxycoumarin on dyslipidemia and cardiac hypertrophy in isoproterenol-induced myocardial infarction in rats. J Biochem Mol Toxicol 2016; 30: 120-127.
5) Laing ST. High-intensity statins: guideline expectations and clinical application. JAMA 2017; 317: 2543-2544.
6) Wang S, Cheng ZY, Chen XJ, Xue HZ. Ulinastatin protects rats with myocardial infarction by activating Nrf2/NOS pathway. Eur Rev Med Pharmacol Sci 2018; 22: 8990-8998.
7) He F, Song Y, Ying WJ, Jin XY, Wang ZX, Su JD, Wang GY. Effects of Ulinastatin on myocardial oxidative stress and inflammation in severely burned rats. Eur Rev Med Pharmacol Sci 2018; 22: 5719-5728.
8) Ek RO, Yildiz Y, Cecen S, Yenisey C, Kavak T. Effects of tamoxifen on myocardial ischemia-reperfusion injury model in ovariectomized rats. Mol Cell Biochem 2008; 308: 227-235.
9) Garcia-Prieto J, Villena-Gutierrez R, Gomez M, Bernardo E, Pun-Garcia A, Garcia-Lunar I, Crainiciuc G, Fernandez-Jimenez R, Sreeramkumar V, Bourio-Martinez R, Garcia-Ruiz JM, Del Valle AS, Sanz-Rosa D, Pizarro G, Fernandez-Ortiz A, Hidalgo A, Fuster V, Ibanez B. Neutrophil stunning by metoprolol reduces infarct size. Nat Commun 2017; 8: 14780.
10) Toraih EA, Abdallah HY, Rashed EA, El-Wazir A, Tantawy MA, Fawzy MS. Comprehensive data analysis for development of custom qRT-PCR miRNA assay for glioblastoma: a prevalidation study. Epigenomics 2019; 11: 367-380.
11) Luna C, Mendoza N, Casao A, Perez-Pe R, Cebrian-Perez JA, Muino-Blanco T. c-Jun N-terminal kinase and p38 mitogen-activated protein kinase pathways link capacitation with apoptosis and seminal plasma proteins protect sperm by interfering with both routesdagger. Biol Reprod 2017; 96: 800-815.
12) Liao YH, Xia N, Zhou SF, Tang TT, Yan XX, Lv BJ, Nie SF, Wang J, Iwakura Y, Xiao H, Yuan J, Jevallee H, Wei F, Shi GP, Cheng X. Interleukin-17A contributes to myocardial ischemia/reperfusion injury by regulating cardiomyocyte apoptosis and neutrophil infiltration. J Am Coll Cardiol 2012; 59: 420-429.
13) Hu T, Wei G, Xi M, Yan J, Wu X, Wang Y, Zhu Y, Wang C, Wen A. Synergistic cardioprotective effects of Danshensu and hydroxysafflor yellow A against myocardial ischemia-reperfusion injury are mediated through the Akt/Nrf2/HO-1 pathway. Int J Mol Med 2016; 38: 83-94.
14) Mauler M, Herr N, Schoenichen C, Witsch T, Marchini T, Hardtner C, Koentges C, Kienle K, Ollivier V, Schell M, Dorner L, Wippel C, Stallmann D, Normann C, Bugger H, Walther P, Wolf D, Ahrens I, Lammermann T, Ho-Tin-Noe B, Ley K, Bode C, Hilgendorf I, Duerschmied D. Platelet serotonin aggravates myocardial ischemia/reperfusion injury via neutrophil degranulation. Circulation 2019; 139: 918-931.

15) Chen O, Ye Z, Cao Z, Manaenko A, Ning K, Zhai X, Zhang R, Zhang T, Chen X, Liu W, Sun X. Methane attenuates myocardial ischemia injury in rats through anti-oxidative, anti-apoptotic and anti-inflammatory actions. Free Radic Biol Med 2016; 90: 1-11.
16) Jin Q, Li R, Hu N, Xin T, Zhu P, Hu S, Ma S, Zhu H, Ren J, Zhou H. DUSP1 alleviates cardiac ischemia/reperfusion injury by suppressing the Mff-required mitochondrial fission and Bnip3-related mitophagy via the JNK pathways. Redox Biol 2018; 14: 576-587.
17) Xu P, Zhang G, Sha L, Hou S. DUSP1 alleviates cerebral ischaemia reperfusion injury via inactivating JNK-Mff pathways and repressing mitochondrial fission. Life Sci 2018; 210: 251-262.
18) Zhen Y, Ding C, Sun J, Wang Y, Li S, Dong L. Activation of the calcium-sensing receptor promotes apoptosis by modulating the JNK/p38 MAPK pathway in focal cerebral ischemia-reperfusion in mice. Am J Transl Res 2016; 8: 911-921.
19) Prompunt E, Sanit J, Barrere-Lemaire S, Nargeot J, Noordali H, Madhani M, Kumphune S. The cardioprotective effects of secretory leukocyte protease inhibitor against myocardial ischemia/reperfusion injury. Exp Ther Med 2018; 15: 5231-5242.
20) Yu H, Guan Q, Guo L, Zhang H, Pang X, Cheng Y, Zhang X, Sun Y. Gypenosides alleviate myocardial ischemia-reperfusion injury via attenuation of oxidative stress and preservation of mitochondrial function in rat heart. Cell Stress Chaperones 2016; 21: 429-437.
21) Li XF, Zhang XJ, Zhang C, Wang LN, Li YR, Zhang Y, He TT, Zhu XY, Cui LL, Gao BL. Ulinastatin protects brain against cerebral ischemia/reperfusion injury through inhibiting MMP-9 and alleviating loss of ZO-1 and occludin proteins in mice. Exp Neurol 2018; 302: 68-74.
22) Wang P, Li W, Yang JL, Wang Y, Liu Q, Wang WZ, Qi HN, Li J. [Effect of ulinastatin on myocardial injury in patients with acute severe carbon monoxide poisoning]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi 2018; 36: 154-156.