

# Influences of remifentanil on myocardial ischemia-reperfusion injury and the expressions of Bax and Bcl-2 in rats

J. LI<sup>1</sup>, H.-P. HU<sup>2</sup>, Y. LI<sup>1</sup>, W. SHAO<sup>1</sup>, J.-Z. ZHANG<sup>1</sup>, L.-M. WANG<sup>3</sup>

<sup>1</sup>Department of Anesthesiology, Yantai Yantaishan Hospital, Yantai, China

<sup>2</sup>Department of Anesthesiology, Liaocheng Third People's Hospital, Liaocheng, China

<sup>3</sup>Department of Anesthesiology, Affiliated Hospital of Jining Medical University, Jining, China

**Abstract.** – **OBJECTIVE:** To investigate the influences of remifentanil on myocardial ischemia-reperfusion injury in rats and the expressions of b-cell lymphoma 2 (Bcl-2), Bcl-2-associated X protein (Bax) and other apoptosis-related proteins.

**MATERIALS AND METHODS:** A total of 60 Sprague-Dawley (SD) rats were randomly divided into sham operation (S) group, model (M) group, low-dose remifentanil (L) group and high-dose remifentanil (H) group, with 15 rats in each group. The rat model of myocardial ischemia-reperfusion injury was established by the ligation of the left anterior descending branch (LAD). After ischemia for 30 min and reperfusion for 24 h, the cardiac function of rats in each group was measured by an ultrasonic instrument. Triphenyl tetrazolium chloride (TTC) staining was used to detect the myocardial infarction area of rats in each group. The activity of myocardial enzymes in the serum of rats in each group was detected. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining was adopted to examine the apoptosis level of rat cardiomyocytes in each group. Quantitative polymerase chain reaction (qPCR) and Western blotting were applied to detect the expression levels of apoptosis-related proteins and messenger ribonucleic acids (mRNAs) in rat cardiomyocytes in each group.

**RESULTS:** Compared with those in S group, left ventricular internal dimension in systole (LVIDs) and left ventricular internal dimension in diastole (LVIDd) were markedly increased ( $p<0.01$ ), while left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS) were notably decreased in M group ( $p<0.01$ ). LVIDs and LVIDd in L group and H group were lower than those in M group ( $p<0.05$ ,  $p<0.01$ ), whereas LVEF and LVFS were higher than those in M group ( $p<0.05$ ,  $p<0.01$ ). The myocardial infarction area in M group was significantly larger than that in S group ( $p<0.01$ ), and those in L group and H group were remarkably smaller than that in M group ( $p<0.05$ ,  $p<0.01$ ). The activities of aspartate aminotransferase (AST), lactate dehydrogenase (LDH)

and creatine kinase-muscle/brain (CK-MB) in the serum of rats in M group were evidently higher than those in S group ( $p<0.01$ ), and compared with those in M group, those in L group and H group were significantly decreased ( $p<0.05$ ,  $p<0.01$ ). The apoptosis level of myocardial cells in M group was significantly higher than that in S group ( $p<0.01$ ), while those in L group and H group were markedly lower than that in M group ( $p<0.05$ ,  $p<0.01$ ). Compared with those in S group, the expression levels of cleaved caspase-3 and its mRNAs in myocardial cells in M group were remarkably increased ( $p<0.01$ ), while those of Bcl-2/Bax and its mRNAs were significantly decreased ( $p<0.01$ ). The expression levels of cleaved caspase-3 and its mRNAs in myocardial cells in L group and H group were significantly lower than those in M group ( $p<0.05$ ,  $p<0.01$ ), but those of Bcl-2/Bax and its mRNAs were significantly higher than those in M group ( $p<0.05$ ,  $p<0.01$ ).

**CONCLUSIONS:** Remifentanil can effectively reduce myocardial cell injury caused by myocardial ischemia-reperfusion in rats, improve cardiac function, reduce the myocardial infarction area, decrease cleaved caspase-3 in myocardial cells, and increase Bcl-2/Bax.

Key Words

Remifentanil, Myocardial ischemia-reperfusion injury, Apoptosis.

## Introduction

At present, ischemic heart disease is an important cause of death in the global population. The survey and research of the World Health Organization reveal that patients with ischemic heart disease are getting younger and younger under the influences of the increasing pressure of life and other factors<sup>1</sup>. Removing the ischemic factors and ensuring the blood supply to the heart in time are important measures for the

treatment of ischemic heart disease. Thrombolytic therapy in internal medicine and coronary artery bypass grafting in surgery are currently the most commonly used methods<sup>2,3</sup>. In recent years, a large number of literatures have reported that myocardial ischemia-reperfusion will aggravate myocardial cell injury and markedly increase the level of apoptosis of myocardial cells and the area of myocardial infarction, thus leading to heart failure and seriously affecting the prognosis of patients<sup>4,5</sup>. Research<sup>6</sup> evidence has indicated that apoptosis and oxidative stress are important causes of myocardial ischemia-reperfusion injury. After ischemia-reperfusion, adenosine triphosphate (ATP) production and active oxygen content will increase significantly, thereby leading to serious injury of myocardial cells and dysfunction of the myocardium. Szobi et al<sup>7</sup> manifested that the expression levels of apoptosis-related proteins, cleaved caspase-3 and b-cell lymphoma 2 (Bcl-2)-associated X protein (Bax), in myocardial cells of rats with ischemia-reperfusion injury, are significantly increased, and the expression level of anti-apoptosis protein, Bcl-2, is significantly decreased. Genade et al<sup>8</sup> demonstrated that antioxidants can effectively protect myocardial cells from injury during ischemia-reperfusion. Researchers<sup>9</sup> have denoted that opioids can protect the heart by activating opioid receptors in heart tissues. According to Sharma et al<sup>10</sup>, low-dose morphine can effectively reduce the infarction area after heart ischemia, decrease the expression of apoptosis-related proteins and protect the heart. Remifentanyl, as a new short-acting opioid receptor agonist, is widely distributed outside blood vessels and has powerful sedative, analgesic and anesthetic effects. Remifentanyl is an ideal intravenous anesthetic drug<sup>11</sup> because it can effectively excite the  $\mu$  receptor and has the characteristics of short acting time and no accumulation. Jeongwara et al<sup>12</sup> found that remifentanyl pretreatment can effectively reduce the infarction area in brain tissues of the intraluminal middle cerebral artery occlusion (MCAO) rat model and lower the level of apoptosis in hippocampal tissues. However, there is still little research on remifentanyl's effect on myocardial ischemia-reperfusion injury. This experiment is intended to evaluate the protective effect of remifentanyl on myocardial cells and analyze its possible mechanism by establishing a rat model of myocardial ischemia-reperfusion injury, so as to provide a theoretical basis for developing remifentanyl into a protective drug for myocardial ischemia-reperfusion injury.

## Materials and Methods

### *Animals and Grouping*

A total of 60 male Sprague-Dawley (SD) rats, weighing 220-240 g, were purchased from the Laboratory Animal Center of Jining Medical University (Laboratory Animal Production License No.: SCXK2015-0006). The feeding environment for rats: specific-pathogen-free (SPF) animal feeding room with a day and night rule of 12-h illumination and a humidity of (45±2)% at (23±2)°C. The rats could eat and drink freely, adapt to the environment for 1 week, and were subjected to solid but not liquid fasting before the experiment. The rats were randomly divided into sham operation (S) group, model (M) group, low-dose remifentanyl (L) group and high-dose remifentanyl (H) group, with 15 rats in each group. The rats in L group were infused with 0.6  $\mu\text{g}/\text{kg}/\text{min}$  remifentanyl through a micropump at 30 min before ischemia, received the ligation of the left anterior descending branch (LAD) for 30 min, and re-perfused for 24 h. The rats in H group were infused with 1.8  $\mu\text{g}/\text{kg}/\text{min}$  remifentanyl through the micropump at 30 min before ischemia, underwent the ligation of the LAD for 30 min, and re-perfused for 24 h. The rats in M group was infused with normal saline using the micropump at 30 min before ischemia, underwent the ligation of the LAD for 30 min, and re-perfused for 24 h. In S group, only the roots of the LAD of rats were threaded, but not ligated. All animal operations and programs were in accordance with the Guidelines for the Breeding and Use of Laboratory Animals issued by the U.S. National Institutes of Health, and the experimental programs were reviewed and approved by the Ethics Committee of Jining Medical University Animal Center.

### *Establishment of the Animal Model*

The myocardial ischemia-reperfusion model was established according to the experimental method of Yogaratnam et al<sup>13</sup>: 10% chloral hydrate was prepared at a dose of 3 mL/kg to anesthetize rats. After anesthesia and fixation of the rats, the ventilator cannula was inserted into the trachea of the rats (tidal volume: 2 mL/100 g, breathing frequency: 70 times/min and breathing ratio: 3:2), and an oblique incision was cut between the 3<sup>rd</sup> and 4<sup>th</sup> ribs to expose the heart. The left coronary artery was pink-white, running down the left anterior wall from the middle of the left atrial appendage and passing through

the posterior end of the LAD with the No. 6-0 silk thread. Finally, the ligation was successful when the LAD was ligated until the apex became white. After 30 min of ischemia, the ligature was untied, and the ischemic area turning red was used as a sign of coronary artery recanalization. After suturing and closing of the chest, 400,000 U penicillins were injected into the muscles to prevent infection. After the rats woke up, the endotracheal cannula was pulled out, and the rats were transferred to a dry clean squirrel cage. The electric blanket was used to keep warm.

#### **Determination of Cardiac Function**

After 24 h of reperfusion, rats were anesthetized with 2% isoflurane and fixed on a constant temperature heating plate. The echocardiogram of each group of rats was detected via a high-resolution small animal ultrasound imaging system (Vevo2100) with a probe frequency of 250 MHz. The probe was placed in front of the sternum of the rats, with the long and short parasternal axes as the observation fields of view. With the short axis of the left ventricle as the section, M-mode ultrasound was applied to record the movement curve of the left ventricle, left ventricular internal dimension in systole (LVIS), left ventricular internal dimension in diastole (LVISD), left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS) of each group of rats.

#### **Detection of the Myocardial Infarction Area Via Triphenyl Tetrazolium Chloride (TTC) Staining**

After 24 h of reperfusion, rats were killed. The heart was removed, washed with normal saline and drained with filter paper. The left ventricle was cut into 1.5-mm slices, which were weighed, placed in a freshly prepared preheated 1% TTC solution, incubated at 37°C for 15 min in the dark, and fixed with 4% paraformaldehyde for 30 min. The red part of the slices was the area without infarction, and the white part was the infarction area. Image software was adopted to calculate the infarction area and the myocardial infarction area (%).

#### **Determination of Myocardial Enzymes**

After 24 h of reperfusion, the peripheral blood of rats in each group was taken. After serum separation, the levels of the aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatine kinase-muscle/brain (CK-MB) in the

serum of rats in each group were detected by a full-automatic biochemical analyzer.

#### **Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL)**

Staining Detection of the Level of Apoptosis

After 24 h of reperfusion, rats in each group were killed, and paraffin sections of heart tissues were prepared. The TUNEL apoptosis kit [Booster (Wuhan, China) Biological Technology Co., Ltd.] was used to detect the level of apoptosis in hippocampal tissues. Procedures were operated in strict accordance with the instructions of the kit, followed by photographing under a confocal fluorescence microscope (Nikon, Tokyo, Japan) after the staining. If green fluorescence could be observed in the field of view, the cells would be TUNEL positive. TUNEL positive cells in each group of rat heart tissues were counted.

#### **Detection of the Messenger Ribonucleic Acid (mRNA) Expression Level via Quantitative Polymerase Chain Reaction (qPCR)**

After 24 h of reperfusion, rats in each group were killed, and the left ventricle was separated, added with TRIzol (Invitrogen, Carlsbad, CA, USA) lysed tissues at 100 mg: 1 mL and centrifuged at 4°C and 12000 rpm for 10 min. After the supernatant was taken, chloroform was added, followed by vigorous shaking for 30 s and standing for 5 min. Subsequently, centrifugation was conducted at 4°C and 12000 rpm for 10 min. After the supernatant was taken, an equal volume of isopropanol was added, followed by violent shaking and centrifugation at 4°C and 10000 rpm for 5 min. Ultimately, the supernatant was discarded, and 75% freshly prepared ethanol was added, followed by violent shaking and centrifugation at 4°C and 10000 rpm for 10 min. 50  $\mu$ L diethyl pyrocarbonate water were added for dissolution to obtain the total RNA. The purity of the total RNA was determined, and 20  $\mu$ L RT systems were prepared with it as a template for reverse transcription at 37°C for 15 min and 85°C for 5 s. The qPCR system was prepared. Amplification conditions set: pre-denaturation at 95°C for 30 s, 95°C for 5 s and 60°C for 35 s for a total of 40 cycles. Primers were synthesized by Invitrogen (Carlsbad, CA, USA) with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal parameter. The sequences are shown in Table I. Based on the Ct value of the ampli-

fication results, the relative expression level was calculated according to  $2^{-\Delta\Delta Ct}$ .

**Western Blotting Detection of the Protein Expression Level**

After 24 h of myocardial ischemia-reperfusion, rats in each group were killed, and the left ventricle was separated. Radioimmunoprecipitation assay lysis buffer was added according to the mass/volume ratio of 100 mg/1 mL. After the homogenate with an ultrasonic homogenizer at 4°C, centrifugation was performed at 12000 rpm for 10 min, and the supernatant was the total protein. The bicinchoninic acid assay protein determination kit (Pierce, Rockford, IL, USA) was applied to determine the concentration of the protein in each group, and a loading system at the equal concentration was prepared for standby application after boiling and extinguishment. The protein was loaded after sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel was prepared and electrophoresed to the bottom at the constant voltage of 80 V. Polyvinylidene fluoride membrane (5 cm× 8 cm) (IPVH00010, Millipore, Billerica, MA, USA) was prepared, and the protein was transferred onto the membrane after activation at 90 V for 100 min. Freshly prepared 5% skimmed milk powder was used to seal the protein band for 2 h. After washing with tris-buffered saline with Tween 20 (TBST), cleaved caspase-3, Bcl-2, Bax and GAPDH (diluted at 1:1000) (Millipore, Billerica, MA, USA) were incubated at 4°C overnight, then incubated with horseradish peroxidase-conjugated secondary antibodies (Fude Biological Technology Co., Ltd., Dalian, China) at room temperature for 1 h and washed with TBST for 3 times, with 5 min each time. After that, enhanced chemiluminescence solution was prepared, followed by developing and fixation in a dark room, washing and airing. After scanning, the quantitative analysis was carried out with ImageJ.

**Table I.** Primer sequences

Gene		
Bcl-2	Sense	GGTGGGGTCATGTGTGTGG
	Antisense	CGGTTTCAGGTACTCAGTCATCC
Bax	Sense	CCCGAGAGGTCTTTTCCGAG
	Antisense	CCAGCCCATGATGGTTCTGAT
GAPDH	Sense	TATCGGACGCCTGGTTAC
	Antisense	TTCCATTCTCAGCCTTG

**Statistical Analysis**

Data in this study were expressed as mean ± standard deviation and processed using Statistical Product and Service Solutions 19.0 software (SPSS Inc., Armonk, NY, USA). Count data were analyzed by  $\chi^2$  test, and the analysis of variance was adopted for the comparison among multiple groups, followed by post-hoc test (Least Significant Difference). It was stipulated that  $p < 0.05$  represented a statistically significant difference.

**Results**

**Establishment of the SD Rat Model of Myocardial Ischemia-Reperfusion Injury**

The model of myocardial ischemia-reperfusion injury was established by the LAD ligation. The criterion for a successful ligation was that the local myocardium in rats turned white under the observation of naked eyes, and after the ligature was untied, the ischemia area turning red was taken as a sign of coronary artery recanalization. The success of the model establishment is shown in Table II. The success rate of the establishment of myocardial ischemia-reperfusion injury model in M group was the lowest, while the success rates in L group and H group were improved to some extent.

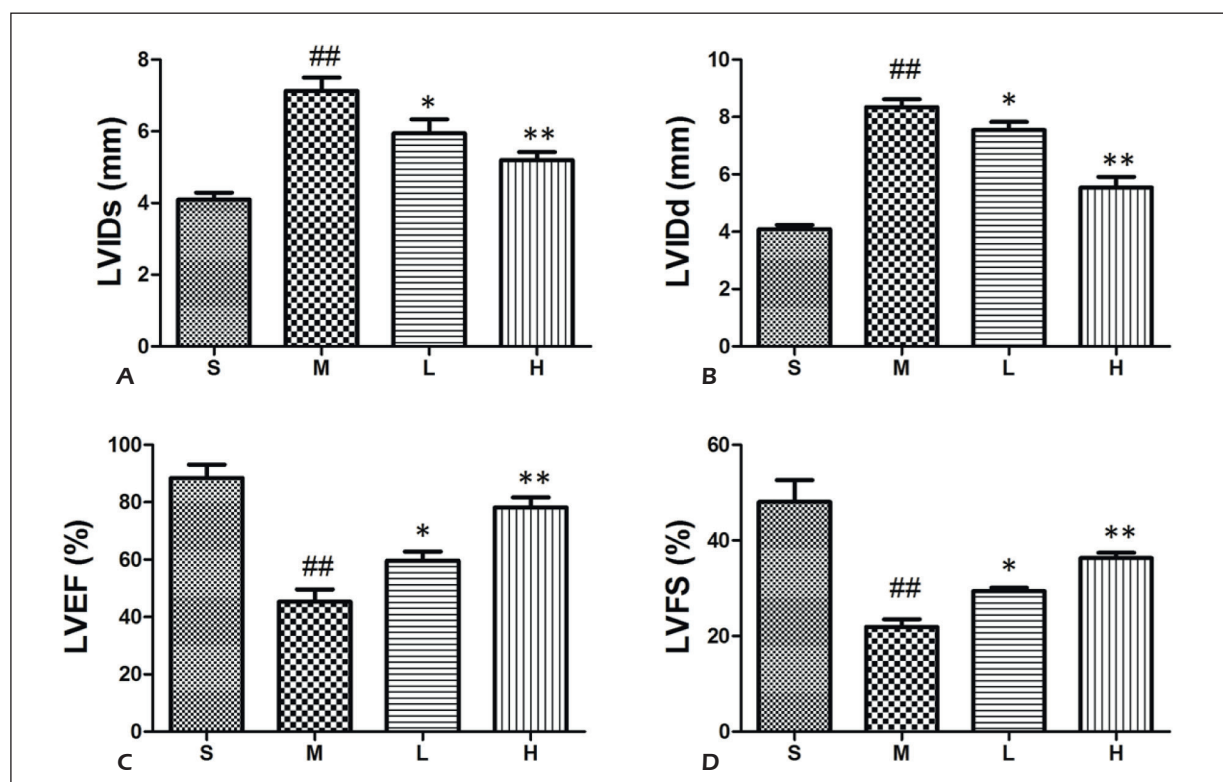
**Changes in Cardiac Function of rats in Each Group**

After 24 h of myocardial ischemia-reperfusion, the changes in the echocardiogram in each group of rats were detected. The results revealed that LVIDs and LVIDd in M group were markedly higher than those in S group ( $p < 0.01$ ). Compared with those in M group, LVIDs and LVIDd in L group and H group were remarkably decreased ( $p < 0.05$ ,  $p < 0.01$ ). LVEF and LVFS in M group were significantly lower than those in S

**Table II.** Success rate of the establishment of the laboratory rat model

	Survived rat with a successfully established model (n)	Success rate of the model establishment (%)
S group	15	100%
M group	10	66.67%
L group	11	73.3%
H group	11	73.3%





**Figure 1.** Changes in cardiac function in each group of rats. **A**, LVIDs. **B**, LVIDd. **C**, LVEF. **D**, LVFS. LVIDs and LVIDd in group M are higher than those in S group, L group and H group, while LVEF and LVFS in M group are lower than those in S group, L group and H group. <sup>##</sup> $p < 0.01$  vs. S group, <sup>\*\*</sup> $p < 0.01$  and <sup>\*</sup> $p < 0.05$  vs. M group.

group ( $p < 0.01$ ). Compared with those in M group, LVEF and LVFS in L group and H group were evidently increased ( $p < 0.05$ ,  $p < 0.01$ ) (Figure 1).

#### Myocardial Infarction Area of Rats in Each Group

At 24 h after reperfusion, the left ventricle was isolated, and the myocardial infarction area of rats in each group was detected by TTC staining. According to the results, compared with that in S group, the myocardial infarction area of rats in M group was significantly increased ( $p < 0.01$ ). After remifentanyl pretreatment, the left ventricular myocardial infarction areas of rats in L group and H group were significantly decreased ( $p < 0.05$ ,  $p < 0.01$ ) (Figure 2).

#### Changes in Myocardial Enzymes in Each Group of Rats

The serum of rats in each group was separated, and the levels of LDH and CK-MB in the serum of rats in each group were detected using a full-automatic biochemical analyzer. The results manifested that the levels of the AST, LDH and CK-MB in the serum of rats in M group were

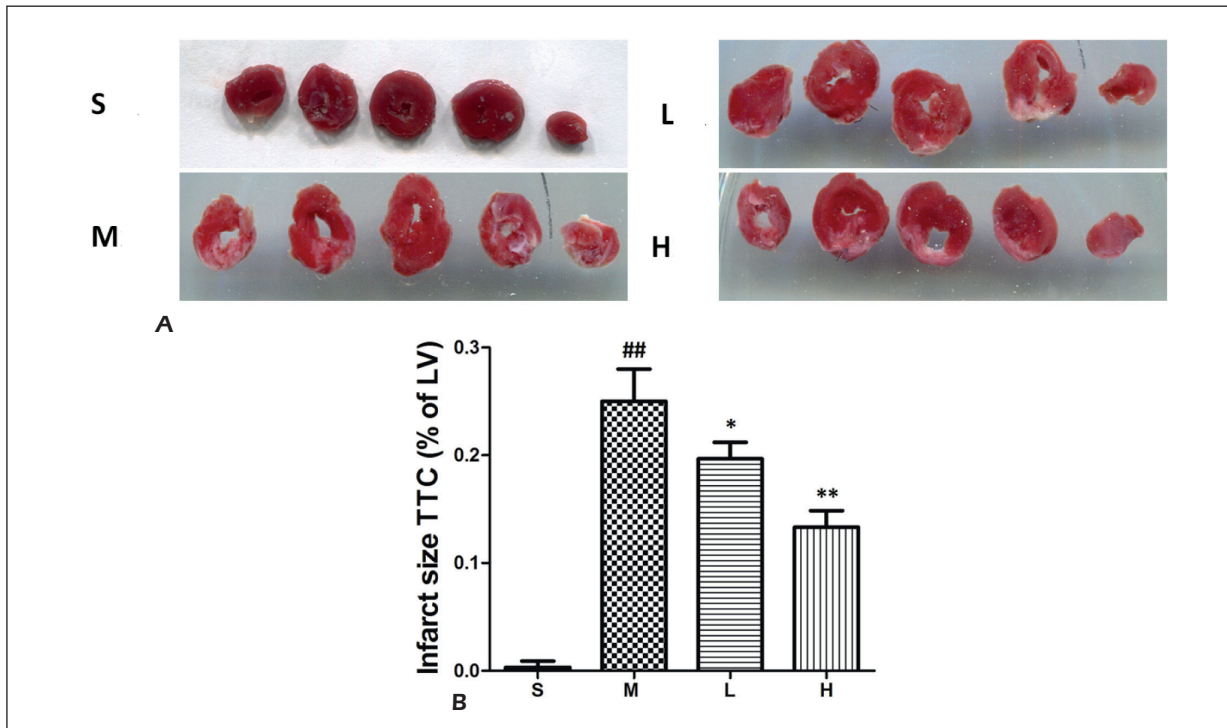
notably higher than those in S group ( $p < 0.01$ ). After remifentanyl pretreatment, the levels of AST, LDH and CK-MB in the serum of rats in L group and H group were significantly reduced ( $p < 0.05$ ,  $p < 0.01$ ) (Figure 3).

#### Apoptosis Level of Rat Cardiomyocytes in Each Group

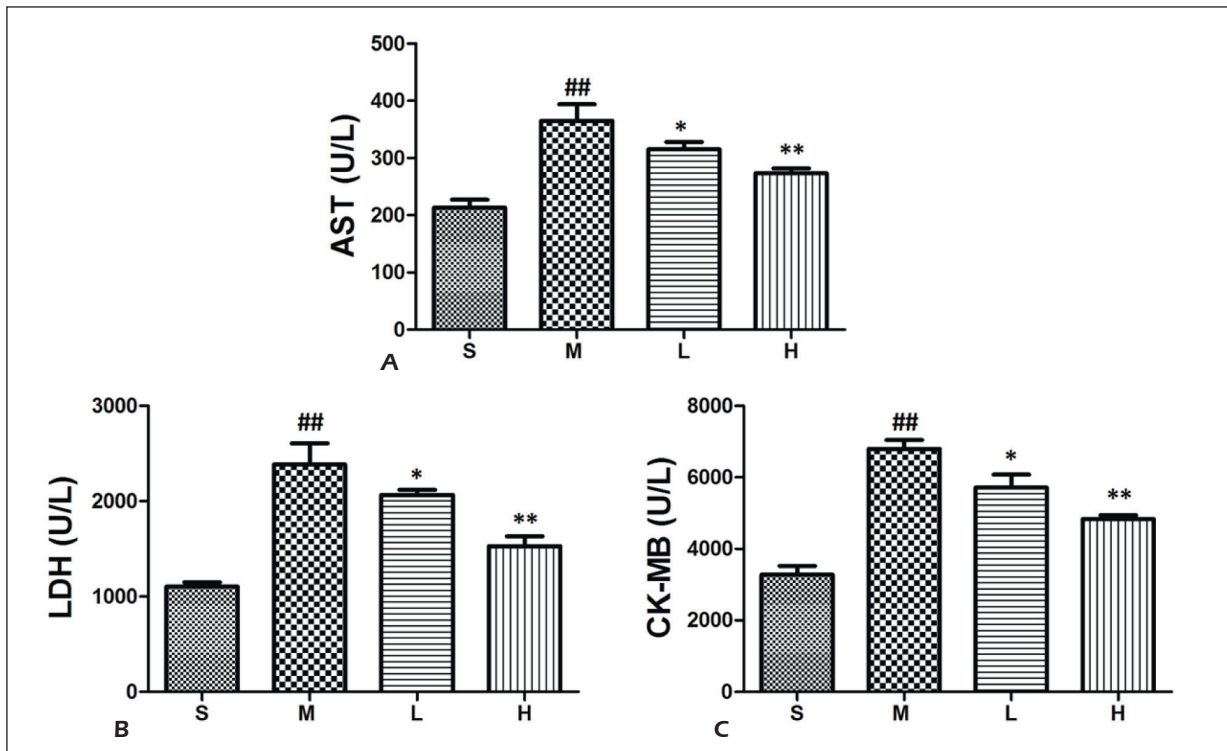
TUNEL staining was used to detect the apoptosis level of rat cardiomyocytes in each group. The results demonstrated that the number of TUNEL positive cells in M group was remarkably higher than that in S group ( $p < 0.01$ ). After remifentanyl treatment, the apoptosis levels of rat cardiomyocytes in L group and H group were significantly reduced ( $p < 0.05$ ,  $p < 0.01$ ) (Figure 4).

#### Expression Level of Apoptosis-Related Genes

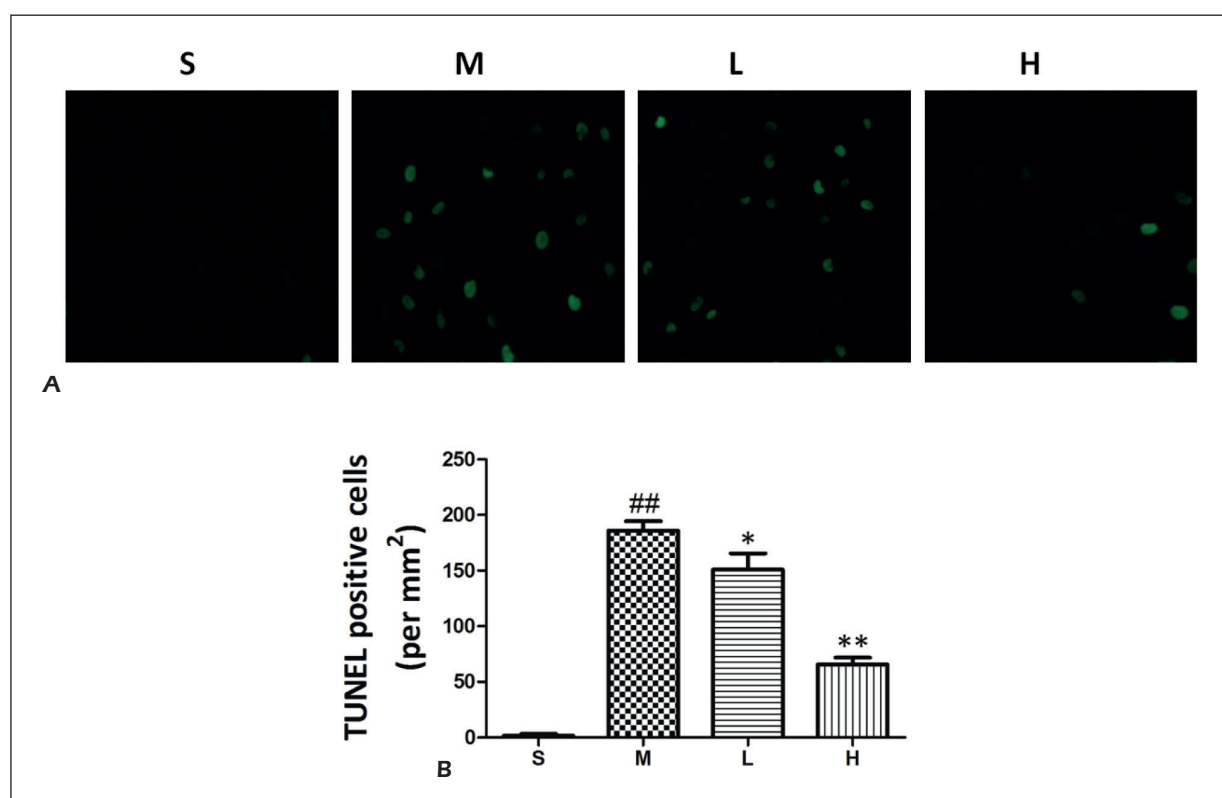
QPCR was carried out to detect the expression level of apoptosis-related genes in left ventricular tissues after 24 h of myocardial ischemia-reperfusion in each group. According to the results, compared with those in S group, the expression level of cleaved caspase-3 mRNAs in M group



**Figure 2.** Myocardial infarction area of rats. **A**, TTC staining graph. **B**, Statistics of infarction area results. The myocardial infarction area in M group is significantly larger than those in S group, L group and H group.  $##p<0.01$  vs. S group,  $**p<0.01$  and  $*p<0.05$  vs. M group.



**Figure 3.** Changes in myocardial enzymes in the serum of rats in each group. **A**, AST level. **B**, LDH level. **C**, CK-MB level. The levels of AST, LDH and CK-MB in M group are evidently lower than those in S group, L group and H group.  $##p<0.01$  vs. S group,  $**p<0.01$  and  $*p<0.05$  vs. M group.



**Figure 4.** TUNEL staining detection of the level of apoptosis of rat cardiomyocytes in each group. **A**, A micrograph. **B**, A statistical graph of TUNEL positive cells. The number of TUNEL positive cells in M group is significantly higher than those in S group, L group and H group. ## $p<0.01$  vs. S group, \*\* $p<0.01$  and \* $p<0.05$  vs. M group.

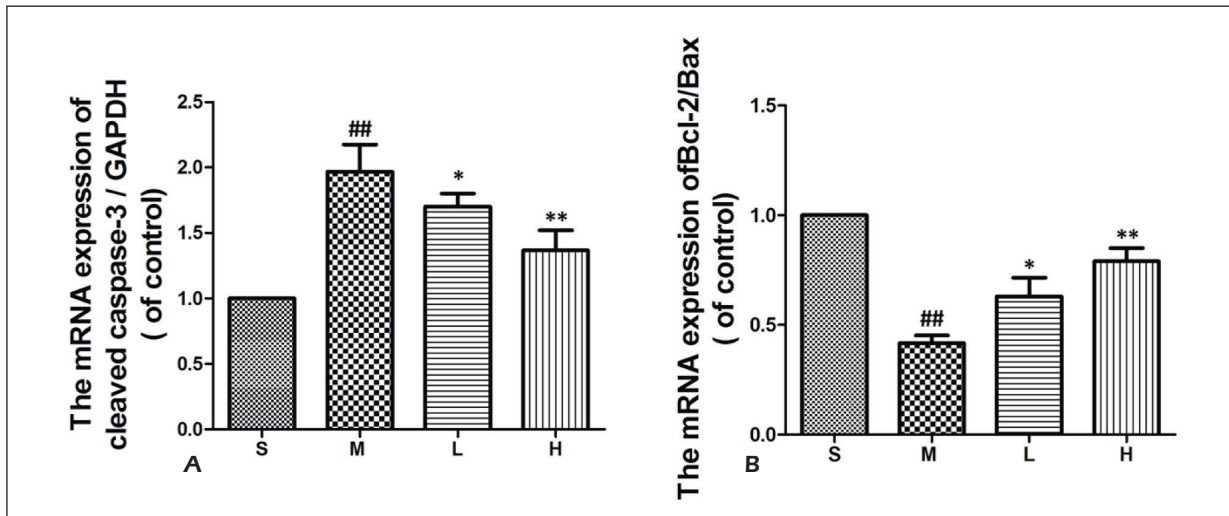
was significantly increased ( $p<0.01$ ), while the expression level of Bcl-2/Bax mRNAs was evidently decreased ( $p<0.01$ ). The expression levels of cleaved caspase-3 mRNAs in L group and H group were lower than that in M group ( $p<0.05$ ,  $p<0.01$ ), but the expression levels of Bcl-2/Bax mRNAs were significantly increased ( $p<0.05$ ,  $p<0.01$ ) (Figure 5).

#### **Expression Level of Apoptosis-Related Proteins**

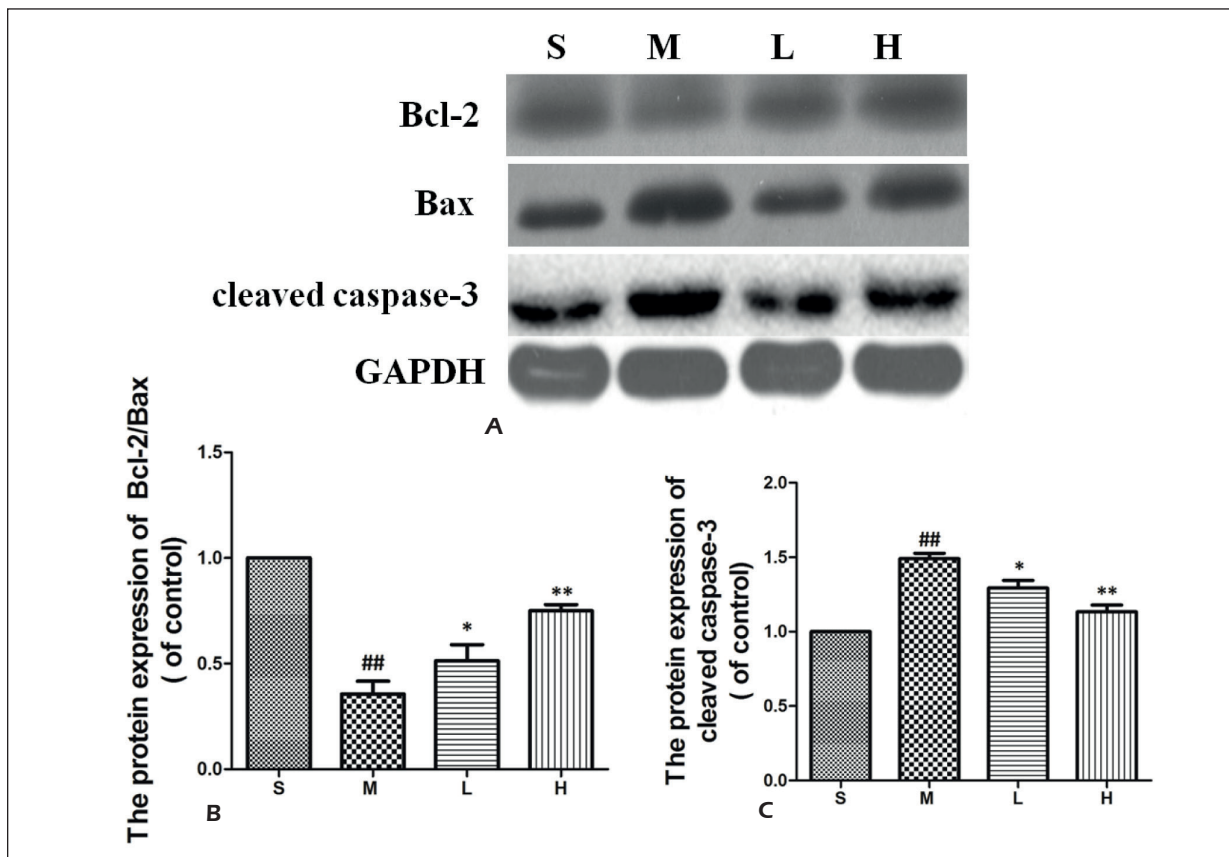
Through Western blotting detection of the expression level of apoptosis-related proteins in left ventricular tissues after 24 h of myocardial ischemia-reperfusion in each group, it was found that compared with those in S group, the expression level of cleaved caspase-3 in M group was significantly increased ( $p<0.01$ ), while the expression level of Bcl-2/Bax was evidently decreased ( $p<0.01$ ). The expression levels of cleaved caspase-3 in L group and H group were lower than that in M group ( $p<0.05$ ,  $p<0.01$ ), but the expression levels of Bcl-2/Bax were significantly increased ( $p<0.05$ ,  $p<0.01$ ) (Figure 6).

## **Discussion**

Myocardial ischemia-reperfusion can effectively reduce the injury of heart tissues caused by ischemia and anoxia, but blood reperfusion can also cause irreversible injury to myocardial cells, resulting in cardiac remodeling and heart failure, so that the mortality rate of myocardial infarction patients after treatment remains high<sup>14</sup>. Rossoni et al<sup>15</sup> used myocardial cells to investigate the injury of hypoxia/reoxygenation to myocardial cells. The results showed that cardiomyocytes are subjected to apoptosis after 2-h hypoxia/2-h reoxygenation. With the prolongation of reoxygenation time, the proportion of apoptotic cells is markedly increased, and the cell activity notably declines. Gao et al<sup>16</sup> manifested that the activities of LDH and CK-MB in the serum of rats with myocardial ischemia-reperfusion injury are significantly increased. Apoptosis is an important form of maintaining homeostasis in cells, and it is an important way for cells to eliminate their own injury. Besides, it also participates in many pathological processes.



**Figure 5.** Detection of the mRNA expression level in rat cardiomyocytes via qPCR. **A**, Expression level of cleaved caspase-3 mRNAs. **B**, Expression level of Bcl-2/Bax mRNAs. Compared with those in S group, the expression level of cleaved caspase-3 mRNAs in M group is significantly increased, but the expression level of Bcl-2/Bax mRNAs is significantly decreased. The expression levels of cleaved caspase-3 mRNAs in L group and H group are lower than that in M group, while the expression levels of Bcl-2/Bax mRNAs are significantly higher than that in M group. ## $p < 0.01$  vs. S group, \*\* $p < 0.01$  and \* $p < 0.05$  vs. M group.



**Figure 6.** Detection of the changes in the expression level of apoptosis-related proteins in each group of rats via Western blotting. **A**, A band graph. **B**, A statistical graph of Bcl-2/Bax. **C**, A statistical graph of cleaved caspase-3. Compared with those in S group, the expression level of Bcl-2/Bax in M group is markedly decreased, while the expression level of cleaved caspase-3 is significantly increased. The expression levels of Bcl-2/Bax in H group and L group are significantly higher than that in M group, while the expression levels of cleaved caspase-3 are significantly lower than that in M group. ## $p < 0.01$  vs. S group, \*\* $p < 0.01$  and \* $p < 0.05$  vs. M group.



Cheng et al<sup>17</sup> found that reperfusion injury is closely related to apoptosis, and ischemia-reperfusion can lead to a significant increase in the expression level of apoptosis-related proteins. In this work, the protective effect of remifentanil on myocardial ischemia-reperfusion injury in rats was evaluated by establishing a model of myocardial ischemia-reperfusion injury. The results showed that remifentanil pretreatment could effectively reduce the myocardial infarction area caused by myocardial ischemia-reperfusion injury, decrease LVIDs and LVIDd and increase LVEF and LVFS. TUNEL staining results also highly denoted that the apoptosis level of myocardial cells after remifentanil pretreatment was significantly reduced. At the same time, we found that remifentanil had a dose-dependent protective effect on cardiomyocytes. 1.8 µg/kg/min remifentanil pretreatment had a significantly better protective effect on cardiomyocytes than 0.6 µg/kg/min remifentanil pretreatment, and the infusion rate of remifentanil was an important factor affecting its effect. Chen et al<sup>18</sup> found that both cerebral ischemia and reperfusion processes in rats can affect the expression of the c-Jun NH(2)-terminal kinase signaling pathway-related proteins, thereby increasing the expression of apoptotic proteins and leading to the apoptosis of cardiomyocytes. Remifentanil can effectively reverse the above effects. Similarly, it was also observed in this study that the expression levels of apoptosis-related proteins, cleaved caspase-3 and Bax, in myocardial tissues were evidently increased, while the expression level of anti-apoptosis protein Bcl-2 was significantly decreased after myocardial ischemia-reperfusion injury. Jang et al<sup>19</sup> showed that myocardial ischemia can lead to significant increases in the synthesis and release of opioid peptides in cardiomyocytes, which can protect cardiomyocytes and reduce myocardial cell injury by acting on opioid receptors to combat the release of high catecholamines. Remifentanil, as an opioid receptor agonist, can also stimulate the opioid receptor by acting on it, which has a protective effect on cardiomyocytes. There are still some deficiencies in this study, and the protective mechanism of remifentanil on myocardial ischemia-reperfusion injury has not been thoroughly studied. Bach et al<sup>20</sup> showed that the activation of p42/p44 extracellular regulated protein kinases (ERKs) after double phosphorylation in the nucleus is an important factor leading to myocardial ischemia-reper-

fusion injury, and ischemia-reperfusion will increase the double phosphorylation of them in the nucleus. Kim et al<sup>21</sup> also revealed that both ischemia and reperfusion can effectively activate p38 mitogen-activated protein kinases (MAPKs) and increase the phosphorylation of them. The protective mechanism of remifentanil on myocardial ischemia-reperfusion injury will be further researched from p38 MAPKs and p42/p44 ERKs.

## Conclusions

We showed that remifentanil can effectively reduce myocardial cell injury caused by myocardial ischemia-reperfusion in rats, improve cardiac function, reduce the myocardial infarction area, decrease cleaved caspase-3 in myocardial cells, and increase Bcl-2/Bax.

## Acknowledgments

This work was supported by the Shenzhen Science and Technology Innovation Funding JCYJ20160429190356200, Shenzhen Health Care System Research Projects Funding No. 201607027.

## Conflict of Interests

The authors declared no conflict of interest.

## References

- 1) ROTH CP, QARMALI M, LITOVSKY SH, BROTT BC. Myocardial rupture after small acute myocardial infarction in the absence of coronary artery disease. *Cardiovasc Pathol* 2018; 37: 26-29.
- 2) WANG YX, LI YQ, CHEN Y, ZHANG CH, DONG Z, WANG Z, ZHAO SN, LI CH, ZHANG PL. Analysis of related factors of orolingual angioedema after rt-PA intravenous thrombolytic therapy. *Eur Rev Med Pharmacol Sci* 2018; 22: 1478-1484.
- 3) GUPTA I, VARSHNEY NK, KHAN S. Emergence of members of TRAF and DUB of ubiquitin proteasome system in the regulation of hypertrophic cardiomyopathy. *Front Genet* 2018; 9: 336.
- 4) ABDELWAHAB AA, ELSAIED AM. Can enhanced external counter pulsation as a non-invasive modality be useful in patients with ischemic cardiomyopathy after coronary artery bypass grafting? *Egypt Heart J* 2018; 70: 119-123.
- 5) LAZAR HL. Stem cell therapy for chronic ischemic cardiomyopathy-Why have these stem cells not taken root? *J Card Surg* 2018; 33: 532-533.

- 6) HUANG WH, LEE JF, WANG D, GOU WH, CHANG CY, WEI J. Postischemia myocardial injury in coronary artery bypass patients (PP6). *Transplant Proc* 2010; 42: 725-728.
- 7) SZOBI A, RAJTIK T, CARNICKA S, RAVINGEROVA T, ADAMOVA A. Mitigation of postischemic cardiac contractile dysfunction by CaMKII inhibition: effects on programmed necrotic and apoptotic cell death. *Mol Cell Biochem* 2014; 388: 269-276.
- 8) GENADE S, GENIS A, YTREHUS K, HUISAMEN B, LOCHNER A. Melatonin receptor-mediated protection against myocardial ischaemia/reperfusion injury: role of its anti-adrenergic actions. *J Pineal Res* 2008; 45: 449-458.
- 9) SURENDRA H, DIAZ RJ, HARVEY K, TROPAK M, CALLAHAN J, HINEK A, HOSSAIN T, REDINGTON A, WILSON GJ. Interaction of delta and kappa opioid receptors with adenosine A1 receptors mediates cardioprotection by remote ischemic preconditioning. *J Mol Cell Cardiol* 2013; 60: 142-150.
- 10) SHARMA NK, MAHADEVAN N, BALAKUMAR P. Adenosine transport blockade restores attenuated cardioprotective effects of adenosine preconditioning in the isolated diabetic rat heart: potential crosstalk with opioid receptors. *Cardiovasc Toxicol* 2013; 13: 22-32.
- 11) DE HOOGD S, VALKENBURG AJ, VAN DONGEN E, DAETER EJ, VAN ROSMALEN J, DAHAN A, TIBBOEL D, KNIBBE C. Short and long-term impact of remifentanil on thermal detection and pain thresholds after cardiac surgery: a randomised controlled trial. *Eur J Anaesthesiol* 2018; 11: 14-21.
- 12) JEONG S, KIM SJ, JEONG C, LEE S, JEONG H, LEE J, YOO KY. Neuroprotective effects of remifentanil against transient focal cerebral ischemia in rats. *J Neurosurg Anesthesiol* 2012; 24: 51-57.
- 13) YOGARATNAM JZ, LADEN G, GUVENDIK L, COWEN M, CALE A, GRIFFIN S. Pharmacological preconditioning with hyperbaric oxygen: can this therapy attenuate myocardial ischemic reperfusion injury and induce myocardial protection via nitric oxide? *J Surg Res* 2008; 149: 155-164.
- 14) WANG Y, SHARBAUGH MS, ALTHOUSE AD, MULUKUTLA S, SABA S. Cardiac resynchronization therapy pacemakers versus defibrillators in older non-ischemic cardiomyopathy patients. *Indian Pacing Electro-physiol J* 2018; 18: 17-23.
- 15) ROSSONI G, GOMARASCHI M, BERTI F, SIRTORI CR, FRANCESCHINI G, CALABRESI L. Synthetic high-density lipoproteins exert cardioprotective effects in myocardial ischemia/reperfusion injury. *J Pharmacol Exp Ther* 2004; 308: 79-84.
- 16) GAO XW, LIU Y, YANG ZC, TAN YZ. [Protective effect of total glycosides of *Ranunculus japonicus* on myocardial ischemic-reperfusion injury in isolated rat hearts]. *Zhong Yao Cai* 2014; 37: 1429-1433.
- 17) CHENG XY, GU XY, GAO Q, ZONG QF, LI XH, ZHANG Y. Effects of dexmedetomidine preconditioning on myocardial ischemia and the role of the PI3K/Akt-dependent signaling pathway in reperfusion injury. *Mol Med Rep* 2016; 14: 797-803.
- 18) CHEN T, ZHU H, WANG Y, ZHAO P, CHEN J, SUN J, ZHANG X, ZHU G. Apoptosis of bone marrow mesenchymal stromal/stem cells via the MAPK and endoplasmic reticulum stress signaling pathways. *Am J Transl Res* 2018; 10: 2555-2566.
- 19) JANG Y, XI J, WANG H, MUELLER RA, NORFLEET EA, XU Z. Postconditioning prevents reperfusion injury by activating delta-opioid receptors. *Anesthesiology* 2008; 108: 243-250.
- 20) BACH DH, KIM SH, HONG JY, PARK HJ, OH DC, LEE SK. Salternamide A suppresses hypoxia-induced accumulation of HIF-1alpha and induces apoptosis in human colorectal cancer cells. *Mar Drugs* 2015; 13: 6962-6976.
- 21) KIM SL, MIN IS, PARK YR, LEE ST, KIM SW. Lipocalin 2 inversely regulates TRAIL sensitivity through p38 MAPK-mediated DR5 regulation in colorectal cancer. *Int J Oncol* 2018; 36: 362-376.