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# Remifentanil Anesthesia on the Expression of Apoptosis-Related Proteins Bcl-2 and Bax in Rat Myocardial Cells with Ischemia-Reperfusion Injury

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**ABSTRACT**

Ischemia-reperfusion damage to the myocardium is inevitable. This study mainly explored the effect of remifentanil anesthesia on the expression of apoptosis-related proteins Bcl-2 and Bax in rat myocardial ischemia-reperfusion injury. Select 48 mice (n=6). First, prepare solutions of different concentrations of remifentanil. A model of ischemia-reperfusion cardiomyocytes was established, and 6 slices of tissue were taken from each specimen, and the positive cells were observed with an optical microscope and magnified 100 times. Bcl-2 and Bax were positive in the cytoplasm and yellowish-brown particles in the inner membrane. According to the distribution of positive cells, randomly select 3 clear fields of view from each part, count the number of positive cells in each field, and then take the average of the proportion of positive cells to get Bcl-2 or Bax protein-positive Index (PEI). Comparison of mRNA levels in each group: Compared with the R3 group, the ratio of the M, R1, and R2 groups increased, and the mRNA expression level of the M group increased almost 3 times, $P<0.05$. The results of the study show that remifentanil reduces the mortality of myocardial cells by regulating the appearance of Bcl-2 and Bax proteins, and has a certain protective effect on the rat heart during myocardial ischemia and reperfusion. There is no statistically significant difference in the protective effect of remifentanil on myocardial ischemia-reperfusion.

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## Introduction

Cardiomyocyte apoptosis is an important pathological mechanism of ventricular remodulation and heart failure after myocardial infarction, which can lead to mitochondrial damage. The balance of Bcl-2 and Bax is an important criterion for ischemic cardiomyocyte death. Bax protein, which exists in different forms in the cytoplasm, changes according to the role of apoptosis-inducing factors. As a general anesthetic, remifentanil has a small amount of intravenous stimulating effect and has the characteristics of significant curative effect and fast action. By preventing cell apoptosis, the myocardium of mice can be protected by in vitro ischemia and reperfusion. Inhibiting the appearance of tyrosinase 3 and controlling the reduction of the Bcl-2/Bax ratio showed a protective effect on the myocardial tissue of rats after ischemia and reperfusion, and provided a theoretical basis for clinical drugs.

Remifentanil is an anesthetic. Heusden proposed the design and evaluation of a multi-input single-output (MISO) propofol-remifentanil anesthesia controller, which is guided by the depth of hypnosis (DOH) measure (1). DOH monitors are usually used in clinical practice to guide the administration of anesthetics (2, 3). However, there is no professional person to guide the infusion of remifentanil (analgesia) (4, 5). The variability of DOH measures is related to the insufficient analgesic effect, and the feasibility of closed-loop control of propofol and remifentanil infusion using DOH feedback has been demonstrated (6, 7). However, in the absence of stimulation, the variability of DOH cannot provide analgesia (8, 9). Therefore, a control system that only relies on DOH feedback loses control of the opioid-hypnotic balance (10, 11). His proposed design overcomes this limitation by introducing a second indirect control target (12). His research defines clinical design specifications to achieve

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adequate anesthesia in various clinical cases and proposes modifications to the habitual control framework.

Ischemia-reperfusion has a significant effect on the gene expression of cardiomyocytes. Xiaoling studied the protective effect of metformin (Met) on myocardial ischemia-reperfusion (IR) injury, and whether this mechanism is related to the AMPK/antioxidant enzyme signaling pathway (13, 14). His research used the rat Langendorff test and rat cardiomyocytes (H9c2) treated with $H_2O_2$ (15, 16). Compared with the IR group, Met treatment significantly improved left ventricular (LV) function, reduced infarct size and reduced CK-MB release (17, 18). In the IR + Met group, a decrease in TUNEL staining positive cells was also observed in vitro (19, 20). Compared with the IR group, Met treatment significantly inhibited IR-induced cell death and significantly reduced the generation of reactive oxygen species (ROS) apoptosis in H9c2 cells (21, 22). Compared with the IR group, the expression of phosphorylated LKB1/AMPK/ACC was up-regulated in the IR + Met group, while the expression of apoptotic proteins (Bax and cleaved caspase 3) was down-regulated (23, 24). The results of the study showed that Met significantly up-regulated the expression of antioxidant enzymes (MnSOD and catalase) in the IR program both in vivo and in vitro (25, 26).

In this study, rats were treated with myocardial ischemia and reperfusion. Bcl-2 and Bax were positive in the cytoplasm and yellowish-brown particles in the inner membrane. According to the distribution of positive cells, randomly select 3 clear fields of view from each part, count the number of positive cells in each field, and then take the average of the proportion of positive cells to get Bcl-2 or Bax protein-positive Index (PEI).

## Materials and methods

### Subject

48 mice were selected and randomly divided into 6 groups R1-R6. Experimental animals (anesthesia, surgery, etc.) that died accidentally during drug overdose need to be replaced in time to ensure that each group of experiments can proceed smoothly.

### Specimen Collection

At 20 minutes and 13 hours of reperfusion, 3 ml of blood was extracted from the vein. After standing, the blood was centrifuged, and serum was collected for the measurement of Bcl-2 and Bax. The rats were sacrificed during 13 hours of reperfusion, and the upper tissue (4mmx5mm) of the left heart was quickly collected and stored in liquid nitrogen. In order to determine the biochemical indicators of the tissue, it was moved to the refrigerator to adjust the temperature to -60°. In addition, 0.5m×0.8mm×0.9mm cortical tissue located in the lower part of the left heart was taken and fixed with 5% formalin for 9 hours to prepare a lens specimen. Put 0.5m×0.8mm×0.9mm of the cortical tissue of the lower part of the left heart into a 3.3% glucuronic acid buffer. In order to prepare specimens for the electron microscope, transfer them to a refrigerator at 5°C for more than 3 hours.

### Remifentanil Pretreatment

Prepare remifentanil solution: dissolve 2 mg of remifentanil hydrochloride in 380 ml of normal saline. Using a pipette, absorb 3ml of the solution into other centrifuge tubes, dilute to 20ml with saline, and prepare a remifentanil solution with a concentration of 520mg/ml. From the second remote immersion tube to the third remote immersion tube each absorbs 3ml, and dilute to 8ml with normal saline to prepare a 58mg/ml remifentanil solution. Absorb 2ml from the second remote sinking tube to the third remote sinking tube, dilute to 20ml with normal saline to prepare a remifentanil solution with a concentration of 59mg/ml, and mark each test tube.

### Establishment of an in Vitro Simulated Ischemia-Reperfusion Injury Model

Simulation of the in vitro reperfusion process: take the plate out of the $CO_2$ incubator, replace 50 ml of fresh complete medium with ordinary medium, distribute equally to each group, and incubate in the $CO_2$ incubator for 3 hours. The second plate was taken out of the hypoxic incubator. Under normal conditions (37°C, 5% $CO_2$, the perfusion process simulates the ordinary perfusion process, and the saturation humidity is appropriate), replace the ischemia-reperfusion group, remifentanil pretreatment group, and saline group with no Sugar DMEM medium, each

experimental group retain 60 ml of fresh complete medium.

**Observation Indicators**

(1) ECG, systolic blood pressure (SBP), mean arterial pressure (MBP), HR and systolic heart rate product (RPP).

(2) After feeding for a period of time, remove the rat's heart, remove non-heart tissue, remove water, and measure the weight of the whole heart. After 2 minutes of Langendorf irrigation, the coronary artery was ligated again, and 0.33% Evanbluedye solution was injected from the aorta. Keep the temperature at -70℃ in the refrigerator. In the solution (pH=7.5), 4-5 slices were sliced in parallel, leaving the myocardium with a thickness of 2mm. After placing 0.5% triphenylcresol nitrogen chloride (TTC), the whole heart was frozen from the apex to the base. After staining, it was fixed with 8% formalin. The comparison result is the ischemic part in red, the infarcted area is white, the left and right ventricles are indicated as V and RV, and the ischemia is indicated as AAR. The image analysis software (SigmaScanprogram4) that calculates the area multiplies the area of the infarct area (S) by the volume of the 4mm slice, and finally, the myocardial infarction area is IS/AAR.

**Location Detection of Bcl-2 and Bax Protein Expression**

Take 3μm thick paraffin sections of myocardial specimens and stain them with the SABC method. The specific steps are as follows: remove the endogenous enzymes to ensure the endogenous perkindaze activity, rinse with distilled water, add 0.02molPBS, heat the antigen in 0.3mol/L sodium citrate solution for 10 minutes, 3% BSA repair non-specific sites at room temperature 10 After minutes, increase Bcl-2 or Bax resistance antibody (1:60). After placing the wet box at 4°C overnight, wash it with PBS solution 4 times, add the corresponding tolerance antigen at room temperature for 29 minutes, wash 2 times with PBS, and treat with DAB. When the microscope controls the coloring time, use It is in a dry and transparent sealed device. A PBS solution was used instead of the I antibody as a negative control. The II antibody and the III antibody were Bcl-2 and streptomycin-vitamin complex, respectively. Bcl-2 and Bax were positive, and brown-yellow particles or clumps were observed to appear on the cytoplasm and inner membrane, mainly in the form of cytoplasm.

**Statistical Processing**

All quantitative data are expressed as mean ± standard deviation. SPSS17.0 was used for statistical analysis. The average comparison between groups was performed using a univariate analysis of variance (ANOVA). When the dispersion is equal, the least significant difference T-test is used in the average comparison between groups. In the case of uneven dispersion, the Dunnett-T3 test was used to compare the average between groups, and it was considered that $P<0.05$ was statistically significant.

**Results and discussion**

**RPC on Rat Heart Injury after in Vivo Ischemia**

Comparison of myocardial tissue injury scores in each group: Compared with the S group, M group, R, R2 and R3 group myocardial injury scores were significantly higher, $P<0.01$. With the increase of the dose of remifentanil, the myocardial injury scores of R, R2 and R3 groups were gradually lower than those of the M group, $P<0.01$. Compared with the R1 group, the myocardial injury scores in the R2 and R3 groups were lower, and $P<0.01$. The score of myocardial injury in the R3 group was also lower than that in the R2 group, $P<0.01$. Comparison of W/D values of myocardial tissues in each group: Compared with R3 group, the ratio of M group, R1, R2 group increased, $P<0.05$; compared with M group, the ratio of R, R2, R3 all decreased, $P<0.05$. Compared with the R group, the ratio of R2 and R3 groups both decreased and $P<0.05$. Compared with the R2 group, the ratio of the R3 group decreased, $P<0.05$. The changes of mRNA expression in myocardial tissues of each group are shown in Figure 1. Comparison of mRNA expression in myocardial tissue of each group: Compared with the R3 group, the ratio of M group, R1, R2 group increased, and the M group increased nearly 3 times, $P<0.05$. Compared with the M group, the ratios of R2 and R3 were reduced, $P<0.05$, but the difference in the R1 group was not statistically significant. Compared with the R1 group, the R3 ratio decreased, and $P<0.01$, the R2 group had no statistical significance.

Compared with the R, group, the ratio of the R3 group decreased, P<0.01. Comparison of serum VEGF levels in each group: Compared with the S group, the serum VEGF concentrations of the other groups were significantly increased, and the IR group increased the most, P<0.05, but there was no statistical increase in the serum VEGF concentrations of the R2 and R3 groups Scientific significance, compared with IR group, R1, R2 and R3 group gradually decreased, P<0.05 or 0.001. Compared with the R4 group, the R2 group has no statistical significance. Compared with R2, R3 has no statistical significance.

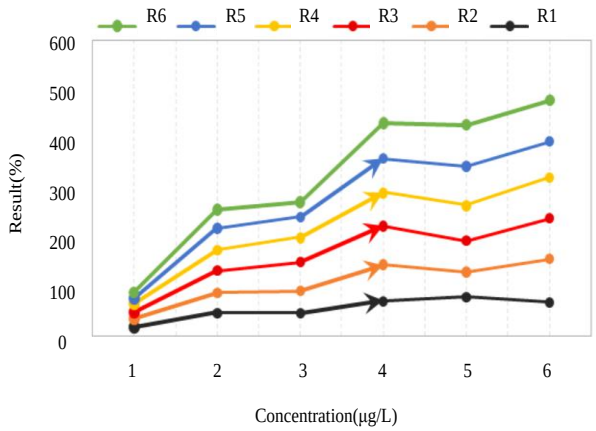


**Figure 1.** Changes in mRNA expression of myocardial tissues in each group

In the sham operation group, there was no significant change before and after ischemia (P>0.05). The statistical results of each group of models are shown in Table 1. In the ischemia-reperfusion group, the low-dose experimental group, and the high-dose experimental group, myocardial Bcl-2 activity increased after 30 minutes of ischemia, continued to rise after reperfusion, reached a peak after 60 minutes of reperfusion, and then maintained a higher activity level. Compared with the sham operation group, the R1 group, hemodynamics: basal value and after 120 min reperfusion, there were no significant differences in HR, MBP, and RPP in each group. Rem was 0.6 μg/L and 2 μg/L RPC at the dose of 30 min after ischemia The MBP of the group was significantly lower than that of the CON group (P<0.05). There were no significant differences in heart weight, LV+RV volume (1.21±0.20-1.28±0.33cm3) and AAR volume (0.384±0.048-0.432±0.057cm3) in each group. Like the IPC group, IS and IS/AAR in each dose group of RPC were significantly lower than those in the CON group. The Rem concentration in the range of 0.6-6 μg/kg/min can make IS/AAR decrease in a dose-dependent manner, with Rem being 6 μg/L. Obviously, IS/AAR decreased from 52.7±5.5% in the CON group to 16.2±6.4%. ED is 2.7μg/L. One side effect of Rem is that it can cause lower blood pressure and bradycardia when used in large doses and in combination with other anesthetics. From this study, a Rem of 0.6 μg/L and a concentration of 50-100 μg/L can significantly reduce the HR and MAP or LVDP of rats, but after 120 minutes of reperfusion, the hemodynamic parameters between the treatment groups and the CON group did not see a significant difference. Due to clinical myocardial ischemia, these parameters will undergo the same changes. Therefore, the RPC in this study may not produce significant blood pressure reduction, bradycardia and other reactions in the clinic. Of course, this needs to be confirmed by future clinical studies.

**Table 1**. Statistical results of each group of models; statistics (S)

| Index Content | n | Treatment | | | S | P |
|---|---|---|---|---|---|---|
| | | HR | MBP | RPP | | |
| CON | 6 | 435±54 | 82±16 | 47±10 | 0.76 | 0.34 |
| IPC | 8 | 428±70 | 82±14 | 44±9 | 0.78 | 0.45 |
| RPC (0.2μg/kg/min) | 10 | 393±61 | 77±12 | 38±1 6 | 0.77 | 0.66 |
| RPC (0.6ug/kg/min) | 9 | 439±64 | 77±17 | 46±17 | 0.79 | 0.57 |
| RPC (2ug/kg/min) | 5 | 446±66 | 73±14 | 45±9 | 0.62 | 0.66 |
| RPC (6μg/kg/min ) | 5 | 445±18 | 70±13 | 40±5 | 0.54 | 0.55 |
| RPC(20μg/kg/min) | 5 | 440±23 | 80±11 | 47±9 | 0.59 | 0.44 |

**Relationship between Cardiomyocyte Apoptosis and Bax and Bcl-2 Protein Expression**

The protein expression levels of Bax and Bcl-2 in each group are shown in Figure 2. After blocking the anterior descending coronary artery (LAD) for 30 minutes and reperfusion for 60 minutes, the cardiomyocyte apoptosis index (AI) of each experimental group was significantly positively correlated with the positive expression of Bax protein in cardiomyocytes (r=0.66, P< 0.01). There was a significant negative correlation with the positive

expression of Bc1-2 protein (I=-0.77, P<0.01). The number of positive cells between Bax protein expression and Bcl-2 protein expression in different groups of cardiomyocytes was significantly negatively correlated (r=-0.88, P<0.01). The serum SOD, SP-D and NE of patients in R1 and R2 groups were different between T1 and T2 and T3 time points (P<0.05), and there were differences between T2 and T4 time points (P<0.05). Serum Bax and Bcl-2 of the R1 group and R2 group were different between groups at T4 and T5 time points (P<0.05), and there was no difference between groups at the T2 time point (P>0.05). There was a difference in serum SOD between the R1 group and R2 group at the T3 time point (P<0.05), and there was no difference between the T5 and T6 time points (P>0.05). The Bcl-2 positive signal is mainly located in the cytoplasm. The Bcl-2 positive rate in the R2 group (sevoflurane-remifentanil group) is 40%, and the Bcl-2 positive signal in the P group (propofol-remifentanil group) The positive rate is 20%. The positive signal of Bax is mainly located in the nucleus. The positive rate of Bax in the R2 group is 30%, and that in the R3 group is 50%. The expression of Bcl-2 protein in the myocardial tissue of the R2 group was higher than that of the P group, which was statistically significant (P<0.05). Comparison between group S and group P: There was no significant difference in the content of Bax protein. The relationship between Bcl-2 and Bax protein expression was negatively correlated. The correlation coefficient of the R4 group was r=-0.66 (P<0.05). The correlation coefficient of the R5 group was r=-0.464 (P<0.05).

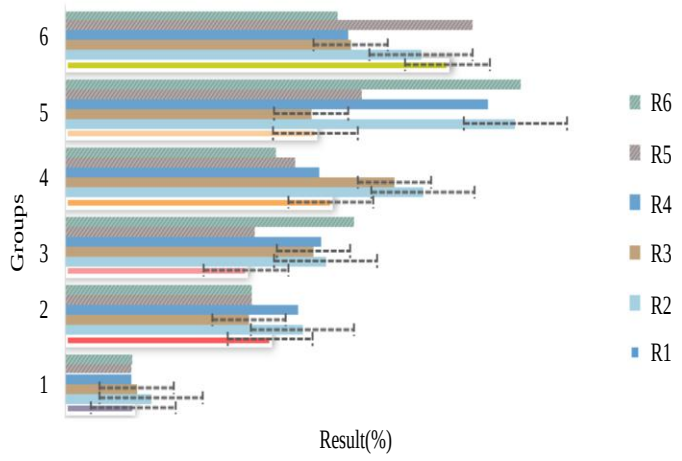


**Figure 2.** Bax and Bcl-2 protein expression in each group

**Pretreatment with Different Concentrations of Remifentanil on Cell Viability**

The effect of pretreatment with different concentrations of remifentanil on cell viability is shown in Figure 3. Observed under a light microscope, Bcl-2 immunopositive cells are mainly expressed in cardiomyocytes. In the R1 group, part of the myocardial cytoplasm showed a light brown-yellow homogeneous coloration, that is, Bcl-2 protein-positive cells. The number of Bcl-2 protein-positive cells in the R2 group and the two drug groups were larger and the staining was deeper, and the OD value of Bcl-2 protein in myocardial tissue increased (P<0.05). And the increase in the two-drug groups was more obvious (P<0.05); but there was no significant difference in the OD value of the two drug groups (P>0.05). There was no significant difference in the OD values of R2, R3, and R4 in each phase (P>0.05). The cell viability of the ischemia-reperfusion group was significantly lower than that of the normal culture group (P<0.05), but there was no statistically significant difference from the normal saline group. In the pretreatment group with different concentrations of remifentanil, compared with the ischemia-reperfusion group and the normal saline group, the cell viability of the R1-R5 group increased (P<0.05), while the cell viability of the R6 group did not increase significantly (P >0.05). The cell viability increased with the increase of the pretreatment concentration of remifentanil, reaching a peak when the concentration of remifentanil was 5ng/ml, and then the increase of the pretreatment concentration of remifentanil gradually decreased. When the concentration of Nylon pretreatment reached 30ng/ml, there was no significant difference in cell viability with the ischemia-reperfusion group. The above results indicate that the balance state of Bcl-2 and Bax is the central link in the occurrence of ischemic cardiomyocyte apoptosis, and also reveals that IPO can reduce rat ischemia-reperfusion cardiomyocyte apoptosis. The mechanism may be mainly through the upregulation of BcI-2mRNA. Express, down-regulate the ratio of Bax/BcI-2 to achieve. It can be seen that remifentanil can reduce the expression of Bax mRNA and the ratio of Bax/Bcl-2, and remifentanil can mobilize cell resistance by inducing the expression of Bcl-2 mRNA and reducing the expression of Bax mRNA and reducing the ratio of Bax/Bcl-2. The self-

protection mechanism of injury reverses the process of apoptosis and necrosis initiated by myocardial IR, thereby protecting myocardial cells.

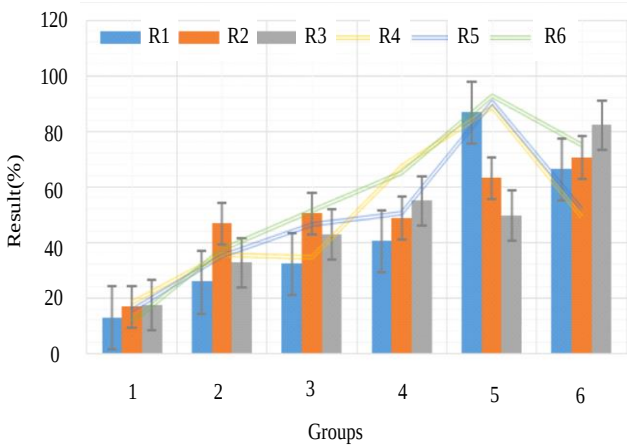


**Figure 3.** Effect of pretreatment with different concentrations of remifentanil on cell viability

**Different Doses of Remifentanil on Myocardial Ischemia-Reperfusion Injury in Rats**

Baximmunopositive cells are mainly expressed in cardiomyocytes. In the Sham group, some of the myocardial cytoplasms showed light brown-yellow granular staining, namely Bax protein-positive cells. In the IR group and the two drug groups, the number of Bax protein-positive expression cells was larger and the staining was deeper, and the OD value of the myocardial tissue Bax protein increased ($P<0.05$); and the increase in the R2 group was more obvious ($P<0.05$); the OD of the dual-drug group The value difference was not significant ($P>0.05$). There was no significant difference in the OD values of the R1, R2, and R3 groups in each phase ($P>0.05$). Myocardial tissue Bcl-2 and Bax activity: Compared with the R4 group, Bax content in M, R1, R2 and R3 groups all increased, and Bcl-2 activity decreased ($P<0.01$). The degree of myocardial ischemia-reperfusion injury in rats is shown in Figure 4. Compared with the M group, the Bax content of R1, R2 and R3 groups all decreased, and the Bcl-2 activity increased ($P<0.05$ or 0.01). Compared with the R1 group, the Bax content of the R2 and R3 groups both decreased ($P<0.01$), and the Bcl-2 activity increased ($P<0.05$ or 0.01). Compared with the R2 group, the Bax content in the R3 group decreased, and the Bcl-2 activity increased ($P<0.01$). In this experiment, different doses of remifentanil were infused intravenously. The results showed that the myocardial function of the high-dose remifentanil group was significantly better than that of the low-dose group, and the myocardial pathological changes were significantly reduced. Among the three doses of remifentanil used in this study, the greater the infusion dose, the more significant the protective effect on the myocardium. The reason may be related to the protective effect of remifentanil by acting on opioid receptors. Low-dose remifentanil can only bind to a small number of opioid receptors, but cannot achieve the best protective effect. With the increase of the dose of remifentanil, the number of bound opioid receptors gradually increased, and its protective effect also increased significantly. Therefore, among the three doses used in this experiment, the infusion rate is 1.0μg/kg/min, but the exact optimal infusion dose and its mechanism to achieve the maximum protective effect need to be further studied.

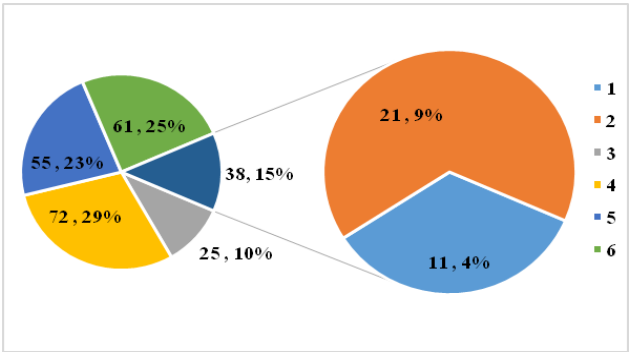


**Figure 4.** The degree of myocardial ischemia-reperfusion injury in rats

After the calcium ion concentration is reduced, RPC can reduce the effect of 5-HD before and after the blockage of MIA and reperfusion-KATP channel of single ventricular cells, but sacr-KATP has no such effect and cannot eliminate the KATP channel of the myocardial ischemia-reperfusion signal transmission factor. The Sacr-katp channel does not run these functions.

The effect of drugs on the body is achieved by affecting the original physiological and biochemical functions of body tissues and cells. However, most drugs have specific toxicity, and excessive use can cause toxic reactions. Therefore, preconditioning with an appropriate concentration of remifentanil has important clinical significance to prevent ischemia-reperfusion disorders. Cell necrosis and apoptosis are important parts of the etiology of reperfusion

disorders. In recent years, in the molecular biology of cells, the emergence of genetic factors regulated in order to prevent cell apoptosis and reduce reperfusion disorders has attracted many people's attention. Studies have shown that the production of apoptosis is controlled by genetic factors. At the same time, they formed a very complex interaction network to regulate cell proliferation.

This study is suitable for establishing a cardiac perfusion model in vitro, applying it to cell receptors, using remifentanil to simulate the heart rate and myocardial enzymes in the adaptation process after ischemia, and then observing the changes in myocardial cells and mitochondria. Remifentanil explores the effects and mechanisms after myocardial ischemia and studies the possibility of clinical application. Further studies on reperfusion show that timely reperfusion (within a few minutes) is closely related to subsequent reperfusion disorders, including cardiomyocyte function, contractile dysfunction, and increased necrosis. Studies have shown that Bcl-2 receptors have the effect of protecting the myocardium. As the starting point for receptor activation and as the main intracellular information transmission, remifentanil has become the subject of intracellular protein gene research after adaptation.

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**Interest conflict**

The authors declare no conflict of interest.

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