

## Original Research Article

# Sufentanil reduces myocardial apoptosis in rats with myocardial ischemia-reperfusion injury

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Sent for review: 20 March 2023

Revised accepted: 29 July 2023

### Abstract

**Purpose:** To determine the effect of sufentanil on myocardial apoptosis in rats with myocardial ischemia-reperfusion injury (MIRI).

**Methods:** Fifty Sprague Dawley rats were randomly assigned to five groups: sham, model, low-dose, moderate-dose, and high-dose. The groups, except sham, underwent ligation of the left anterior descending coronary artery to establish the MIRI model. The low, moderate, and high-dose groups received intraperitoneal injections of sufentanil at different concentrations. Cardiac function, serum LDH, creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), superoxide dismutase (SOD) and malondialdehyde (MDA) content were evaluated. The mRNA expression levels of apoptosis genes and protein levels of p38 and p-p38 were assessed in myocardial tissues using various methods while apoptosis was assessed by TUNEL assay.

**Results:** Compared to sham group, the model group exhibited significant decrease in fractional shortening (FS) and ejection fraction (EF), increase in CK activity, LDH, and MDA contents, lower SOD activity. Model group also showed increase in mRNA levels of B-cell lymphoma-2 (Bcl-2) and caspase-3, higher apoptosis, significant increase in protein levels of p38 and p-p38, and higher level of myocardial apoptosis ( $p < 0.05$ ). High-dose group demonstrated significant increase in FS and EF, decrease in LDH content and CK activity, lower MDA content, higher SOD activity, decrease in mRNA levels of Bcl-2 and caspase-3, lower apoptosis, decrease in protein levels of p38 and p-p38, and lower level of myocardial apoptosis ( $p < 0.05$ ), when compared with model group.

**Conclusion:** High-dose sufentanil reduces myocardial apoptosis and improves cardiac function, and thus can potentially be developed as a cardioprotective agent.

**Keywords:** Myocardial ischemia-reperfusion, Cardioprotective, Apoptosis, Sufentanil

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## INTRODUCTION

Myocardial ischemia-reperfusion injury (MIRI) is a kind of disease caused by recovery of blood perfusion after a certain period of interruption of myocardial blood supply. Reperfusion is

accompanied by coronary artery recanalization, which causes severe myocardial damage, and expansion of the infarction area, and leads to arrhythmia, thereby resulting in injury and cardiac dysfunction in the ischemic region [1]. MIRI may lead to cardiac dysfunction and myocardial injury,

increasing the risk of myocardial infarction with arrhythmia, and serious effect on the prognosis of disease [2,3]. Moreover, coronary thrombolytic therapy, interventional therapy, and bypass surgery are limited by MIRI [4]. Approximately 2 million patients experience MIRI during instances of acute myocardial infarction or sudden cardiac arrest annually [5], and its potential mechanisms include oxidative stress, calcium overload, apoptosis, and leukocyte accumulation [6]. Studies have demonstrated that apoptosis induced by MIRI increases myocardial injury, which is associated with myocardial cell death [6]. Reducing myocardial apoptosis during IRI is crucial for mitigating irreversible harm to cardiac function. Therefore, it holds significant importance in enhancing the effectiveness of treatments for cardiovascular diseases.

According to studies, opioid receptor agonists enhance myocardial tolerance during IRI, and have a beneficial effect when used for preventive and acute treatment prior to reperfusion. It has been confirmed that opioids are able to effectively prevent ischemic arrhythmia [7]. Several studies have demonstrated the protective effect of intravenous administration of sufentanil, a selective opioid receptor agonist, against IRI [8,9]. Sufentanil has been shown to enhance cell viability and decrease apoptosis in IR cells. These effects are accompanied by a reduction in inflammatory factors such as HIF-1 $\alpha$ , TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 [10]. The present study aims to explore the effects of sufentanil at different concentrations on myocardial apoptosis and cardiac function in MIRI rats.

## EXPERIMENTAL

### Animals and grouping of rats

A total of 50 specific pathogen-free Sprague Dawley (SD) rats weighing  $280 \pm 20$  g were divided into sham group, model group, low dose group, moderate dose group and high dose group using a random number table. Each group had 10 rats. The rats were fed adaptively in cages (5 rats/cage) for 1 week, followed by modeling. This study was approved by the Ethics Committee of Jinan Municipal Hospital of Traditional Chinese Medicine (approval no. JN-TCM-001) and all procedures were conducted in accordance with the 'Animal Research: Reporting *In vivo* Experiments, Guidelines 2.0' [11].

### Animal modeling

The MIRI model was established *via* ligation of left anterior descending coronary artery. After

preoperative fasting for 12 h, the rats were anesthetized *via* intraperitoneal injection of 3.0 % pentobarbital, and fixed on the surgical plate, and the trachea was separated, cut open and connected to a small animal ventilator. The thoracic cavity was cut open between the left 3<sup>rd</sup> and 4<sup>th</sup> intercostal space, exposing the heart. Then the left anterior descending coronary artery was ligated, and the blood flow was restored after ischemia for 30 min, thus constructing the MIRI model. In addition, various concentrations of sufentanil were administered intraperitoneally to different groups: a low-dose group (0.5 nmol/L), a moderate-dose group (5 nmol/L), and a high-dose group (50 nmol/L). In sham group, the heart was exposed only without vascular ligation, and an equal volume of normal saline was injected.

### Major reagents and equipment

The primary antibodies used were p38, phosphorylated (p)-p38, B-cell lymphoma-2 (Bcl-2), and Caspase-3, which were obtained from CST (Danvers, MA, USA). The microscope used for analysis was the Leica DM4000B LED microscope from Leica (Wetzlar, Germany). The TUNEL assay kit was sourced from Shanghai Beyotime Biotechnology (Shanghai, China). The protein quantification was performed using the bicinchoninic acid (BCA) protein quantification kit also from Shanghai Beyotime Biotechnology (Shanghai, China). The HiScript II Q RT SuperMix for quantitative polymerase chain reaction (qPCR) (+ gDNA wiper) kit was obtained from Vazyme (Nanjing, China). The fluorescence qPCR instrument used was the ABI 7500 from Applied Biosystems (Foster City, CA, USA). The image analysis system employed was the Image-Lab system from Bio-Rad (Hercules, CA, USA).

### Determination of cardiac function

The cardiac function of rats was determined at the time of modeling. After the rats were anesthetized and fixed, the fractional shortening (FS) and ejection fraction (EF) were determined using the Doppler ultrasound diagnostic system (electronic phased array probe), and the cardiac systolic and diastolic functions were also determined.

### Determination of activity of serum myocardial enzymes

The blood was drawn from the abdominal aorta and centrifuged to obtain the serum. Then the activities of creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), superoxide dismutase (SOD), and malondialdehyde (MDA) were

determined using the kits. The absorbance was determined *via* colorimetry using a spectrophotometer, based on which the content of CK and LDH was calculated.

### Determination of mRNA expressions of Bcl-2 and caspase-3

Total RNA was extracted using TRIzol® reagent (Invitrogen; Carlsbad, CA, USA) from the myocardial tissues in each group and reverse-transcribed into complementary deoxyribose nucleic acid (cDNA) using the reverse transcription kit. Primer sequences are as follows (Table 1). Then amplification was performed using 20 µL of reaction system under the following conditions: pre-denaturation at 94 °C for 10 min, denaturation at 94 °C for 15 s, and annealing at 60 °C for 15 s, for a total of 40 cycles. The relative mRNA expression levels in myocardial tissues in each group were calculated. the relative expression level of mRNA was calculated by  $2^{-\Delta\Delta Cq}$  method.

**Table 1:** Primer sequences used RT-PCR

Gene		Primer sequence
Bcl-2	Forward	5'- CCCAGCATGCGGCC TCTGTTT-3'
	Reverse	5'- GGGGGGCAGGAGG GGCUCA-3'
Caspase-3	Forward	5'- GACCCGGTGCCTCA GGATGC-3'
	Reverse	5'- GTGGCATGAGCTCT TGATAATG-3'
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	Forward	5'- CCCATCACCATCTTC CAGGA-3'
	Reverse	5'- CATCGCCCCACTTG ATTTTG-3'

### Determination of myocardial apoptosis

After the rats in each group were anesthetized *via* intraperitoneal injection of 5 % pentobarbital sodium, the heart was perfused and fixed with 4 % paraformaldehyde, and soaked overnight. The specimens were routinely embedded in paraffin and sliced into 5 µm-thick coronal sections. After deparaffinization, the sections underwent dehydration using a gradient of alcohol and antigen retrieval with citrate. The working solution was then added dropwise for incubation at room temperature for 20 min. Subsequently, protease was added dropwise, and the sections were washed three times with phosphate-buffered saline (PBS) Then, the sections were

added dropwise with TUNEL assay solution for incubation in an oven in the dark at 37 °C for 60 min, and washed with PBS 3 times. Finally, the sections were sealed with an anti-fluorescence quenching blocking buffer and examined under a fluorescence microscope.

### Determination of the content of myocardial proteins

After the fresh myocardial tissues were taken, they were homogenized with RIPA lysate and centrifuged at 5000 rpm and 4 °C for 10 min using a centrifuge, and total protein extraction. The protein concentration was determined using the bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA), and the loading volume was calculated. The extracted proteins were separated using a 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and subsequently transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA), sealed with blocking buffer containing 5 % skimmed milk powder for 2 h, and incubated with p38 and p-p38 primary antibodies (1: 1000) at 4 °C overnight. After the membrane was washed with tris-buffered saline-tween (TBST), the membrane was incubated again with secondary antibodies at room temperature for 1 h, and the membrane was washed again with TBST, followed by image development using electrochemiluminescence (ECL). With GAPDH as an internal reference, the expressions of myocardial proteins were analyzed by Western blotting. The gray value was analyzed using ImageJ software.

### Statistical analysis

Statistic Package for Social Sciences (SPSS) 24.0 software (IBM, Armonk, NY, USA) was used for the data analysis. Data in each group were expressed as mean ± standard deviation (SD). One-way analysis of variance was used to analyze the data, and a non-parametric test was chosen for the data that did not follow a normal distribution.  $P < 0.05$  indicated that differences were statistically significant.

## RESULTS

### Cardiac function

The results of the cardiac function determination showed a significant decrease in both FS and EF in the model group compared to the sham group, with statistically significant differences ( $p < 0.05$ ). However, these values increased in the low, moderate, and high-dose groups compared to the model group ( $p < 0.05$ ), with the highest

improvement observed in the high-dose group (Table 2).

**Table 2:** Comparison of rat cardiac function in each group (mean ± SD, n = 10)

Group	FS	EF
Sham	47.56±5.75	70.51±12.09
Model	20.86±4.13*	43.92±10.18*
Low -dose	28.27±4.08#	54.61±11.12#
Moderate dose	35.34±4.78#	62.24±12.53#
High -dose	40.36±5.22#	67.36±12.84#

**Note:** \**P* < 0.05 vs. sham group, #*p* < 0.05 vs. model group

### Serum myocardial enzymes

The determination results revealed that the model group had increased LDH content and CK activity, higher MDA content, and lower SOD activity when compared with sham group (*p* < 0.05). Compared with the model group, the low, moderate and high dose groups had decreased LDH content and CK activity, lower MDA content, and higher SOD activities (*p* < 0.05), among which the improvement effects were obvious in the high do; se group (*p* < 0.05; Table 3).

### mRNA expressions of apoptosis genes up

According to the results of qRT-PCR showed that mRNA expressions of Bcl-2 and Caspase-3 were increased in model group compared with those in sham group (*p* < 0.05). Compared with those in model group, mRNA expressions of Bcl-2 and Caspase-3 reduced in MIRI rats after sufentanil treatment (*p* < 0.05), among which they were decreased evidently in high-dose group, displaying statistically significant differences (Figure 1).

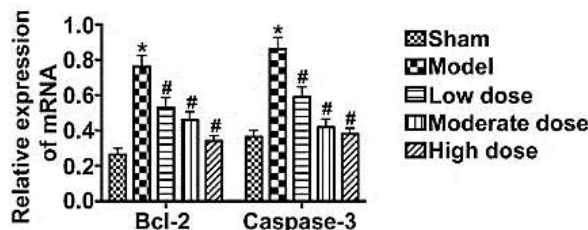
### Apoptosis rate of myocardial tissues

It was observed *via* TUNEL staining that the apoptosis rate was higher in model group than that in sham group (*p* < 0.05), while it was lower in low, moderate and high dose groups than that in model group (*p* < 0.05), among which it was decreased remarkably in high dose group, and there was a statistically significant difference (Figure 2).

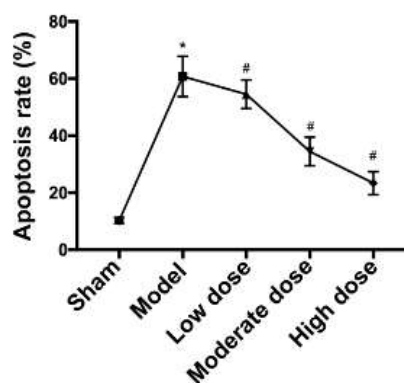
**Table 3:** Comparison of activity of serum myocardial enzymes in among the group (mean ± SD, n = 10)

Group	LDH (μmol/L)	CK (μ/mL)	MDA (nmol/mg)	SOD (μ/mg)
Sham	347.26±15.35	973.49±26.53	4.52±0.34	146.36±21.41
Model	720.86±24.79*	1843.42±56.45*	16.65±1.56*	67.53±15.21*
Low dose	618.82±21.14#	1454.42±46.19#	11.56±1.01#	79.53±16.39#
Moderate dose	565.79±18.54#	1362.27±42.15#	9.34±0.98#	89.92±20.36#
High dose	420.84±16.27#	1167.64±32.46#	6.43±0.62#	125.46±22.64#

**Note:** \**p* < 0.05 vs. sham group, #*p* < 0.05 vs. model group



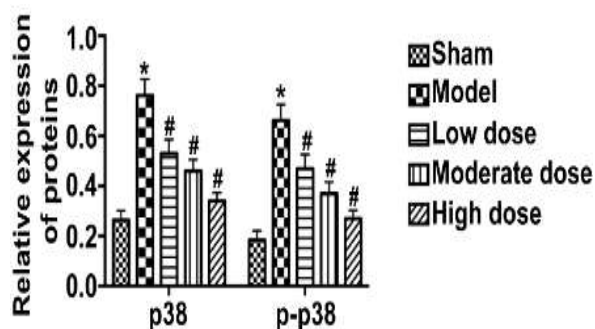
**Figure 1:** Comparison of mRNA expression levels of apoptosis genes among the groups. Note: \**p* < 0.05 vs. sham group, #*p* < 0.05 vs. model group



**Figure 2:** Apoptosis of myocardial tissues. Comparison of apoptosis rate of myocardial tissues in each group. Note: \**p* < 0.05 vs. sham group, #*p* < 0.05 vs. model group

### Expression levels of myocardial apoptosis proteins in each group

The results of Western blotting manifested that compared with the sham group, the protein expression levels of p38 and p-p38 remarkably rose (*p* < 0.05), and the level of myocardial apoptosis was increased in model group. Compared with those in model group, the protein expression levels of p38 and p-p38 reduced (*p* < 0.05), and the level of myocardial apoptosis was decreased in low, moderate and high dose groups, among which they reduced more remarkably in high dose group, and there were statistically significant differences (Figure 3).



**Figure 3:** Expressions of myocardial apoptosis proteins in each group. Comparison of myocardial apoptosis proteins in each group. Note: \* $p < 0.05$  vs. sham group, # $p < 0.05$  vs. model group

## DISCUSSION

Cardiovascular disease is a class of cardiac or vascular diseases, and it is a major cause of death around the world. MIRI is a major factor in morbidity and mortality in patients with ischemic heart disease or after cardiac surgery [12,13]. Various molecular and cellular mechanisms involved in MIR have been identified in other studies [14]. Among these, myocardial apoptosis and cell death are key factors for MIRI. Therefore, developing effective intervention measures and strategies to prevent MIRI is of important clinical significance. Sufentanil, a lipophilic opioid, is widely used as an anesthetic drug in clinical settings. It exhibits a high selectivity for the  $\mu_1$  receptor while displaying low affinity for the  $\delta$  receptor [15]. Due to strong fat solubility and high adherence rate to human plasma proteins, sufentanil possesses a potent analgesic effect, which is 5-10 times higher than that of fentanyl [16]. In this study, the protective effects of sufentanil at different concentrations during MIR were explored in terms of cardiac function and apoptosis using the animal model.

The changes in hemodynamic parameters indicate the degree of IRI in the heart [17]. Creatinine kinase, LDH and oxidative stress indices are commonly used in the diagnosis and monitoring of cardiac function in clinical laboratories. In this study, model group had increased serum LDH content and CK activity, and a higher MDA content and lower SOD activity when compared with the sham group. After treatment with high-dose sufentanil, there were decreases in LDH content and CK activity, lower MDA content and higher SOD activity, suggesting that high-dose sufentanil has a protective effect on the heart. The results of cardiac function also showed that the improvement effects were obvious in the high-

dose group, indicating that high-dose sufentanil improved cardiac function in MIRI rats.

Cellular signal transduction is critical for the ability of cells to respond to the environment, which integrates external responses into the intracellular media and effectors [18,19]. Activated mitogen-activated protein kinase (MAPK) constitutes intracellular signal transduction, and p38, a protein in the MAPK family, is a converter for the response to inflammation and environmental stress [20]. Phosphorylation of threonine and tyrosine residues in p38 leads to conformational changes, thereby increasing the accessibility of MAPK active sites and enhancing catalysis [21]. Studies have also demonstrated that MAPK is involved in the proliferation of myocardial cells [22]. In this study, the protein expression levels of p38 and p-p38 remarkably increased, and the level of myocardial apoptosis was increased in model group. After treatment with high-dose sufentanil, the protein expression levels of p38 and p-p38 were reduced, and the level of myocardial apoptosis was decreased in MIRI rats. Moreover, the myocardial apoptosis rate reduced, and the mRNA expressions of Bcl-2 and Caspase-3 also reduced in high-dose group, demonstrating that sufentanil suppresses myocardial apoptosis and protects MIRI rats myocardial tissues.

## CONCLUSION

High-dose sufentanil significantly reduces myocardial apoptosis and improves cardiac function, thereby exerting a cardioprotective effect in MIRI rats. The findings suggest that sufentanil has the potential to be developed for the treatment of myocardial damage.

## DECLARATIONS

### Acknowledgements

None provided.

### Funding

None provided.

### Ethical approval

None provided.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Conflict of Interest

No conflict of interest associated with this work.

## Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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