

# The effect of butorphanol postconditioning on myocardial ischaemia reperfusion injury in rats

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Received 8 August 2013; received in revised form 8 November 2013; accepted 18 November 2013

## Abstract

**OBJECTIVES:** Butorphanol tartrate is a synthetic opioid partial agonist analgesic. Butorphanol targets the heart, mainly via  $\kappa$ -opioid receptor ( $\kappa$ -OR) activation. The purpose of this study was to determine the effect and mechanism underlying butorphanol postconditioning (B-Post) on myocardial ischaemia reperfusion injury in rats.

**METHODS:** Seventy-five male Sprague–Dawley rats were randomly divided into five groups of 15 each: Group sham; Group I/R (ischaemia/reperfusion); Group B (butorphanol postconditioning); Group B/N (butorphanol postconditioning + antagonist of  $\kappa$ -OR nor-binaltorphimine [Nor-BNI]); Group B/G (butorphanol postconditioning + nonselective ATP-sensitive potassium ( $K_{ATP}$ ) channel blocker glibenclamide [GLI]). The left coronary anterior descending artery (LAD) was occluded for 30 min, followed by a 120-min reperfusion. Blood samples were obtained at the end of reperfusion for determination of serum tumour necrosis factor (TNF)- $\alpha$  and interleukin (IL)-6 concentrations. The hearts were then excised for determination of myocardial infarct size by triphenyltetrazolium chloride staining. The myocardial tissues were used for determination of the expression of myocardial superoxide dismutase (SOD), malondialdehyde (MDA) and myeloperoxidase (MPO).

**RESULTS:** Myocardial infarct size was significantly reduced in B ( $26.4 \pm 1.83\%$ ), B/N ( $34.5 \pm 1.56\%$ ) and B/G ( $31.5 \pm 1.27\%$ ) Groups compared with Group I/R ( $46.8 \pm 1.41\%$ ) (all  $P < 0.001$ ). The serum TNF- $\alpha$  and IL-6 concentrations and the MDA and MPO activities in the ischaemic area in B, B/N and B/G Groups were significantly lower than those in the I/R Group (all  $P < 0.001$ ). In addition, myocardial infarct size, TNF- $\alpha$  and IL-6 concentrations and the MDA and MPO activities in B/N and B/G Groups were higher than those in the B Group (all  $P < 0.001$ ). In contrast, SOD activity was significantly increased in B, B/N and B/G Groups, and SOD activity in B/N and B/G Groups was less than in the B Group (all  $P < 0.001$ ).

**CONCLUSIONS:** These results suggest that postconditioning of butorphanol tartrate can provide a potent cardioprotective effect against myocardial ischaemic and reperfusion injury. Both the  $\kappa$ -OR and the  $K_{ATP}$  channels were involved in this effect.

**Keywords:** Butorphanol • Postconditioning • Ischaemia reperfusion injury •  $K_{ATP}$  channel •  $\kappa$ -OR

## INTRODUCTION

Acute coronary syndrome is a medical emergency and has high morbidity and mortality rates. Restoration of blood vessel integrity in a timely fashion can significantly decrease mortality. A potential risk in reperfusion injury is myocardial cell necrosis, apoptosis and no reperfusion. Thus, the prognosis cannot be promised. We must pay attention to prevention of reperfusion injury, such as ischaemic preconditioning, pharmacological protection and post-conditioning (postC) [1, 2]. Apart from mechanical or pharmacological interventions that open the occluded artery, the heart has endogenous mechanisms of protection called ischaemic pre- and postconditioning.

Data demonstrated that opioid-induced preconditioning or postconditioning provided a powerful cardioprotective effect, which seemed to be similar to that of ischaemic preconditioning or postC [3]. All  $\mu$ -,  $\delta$ - and  $\kappa$ -opioid receptors (OPRs) play a crucial role in opioid-induced cardioprotection [4–6]. Meanwhile, the

ATP-sensitive potassium ( $K_{ATP}$ ) channel is considered to play an important role in modulating infarct size [7, 8].

Butorphanol tartrate exerts activity at  $\mu$ -,  $\delta$ - and  $\kappa$ -opioid receptors in rats and monkeys and  $\mu$ -like effects in humans [4]. Butorphanol can be given via intramuscular, intravenous or nasal spray. Recently, it has been demonstrated that the addition of butorphanol provided better analgesia or relaxation and less severe side effects like coughing, gagging, shivering and pruritus [9–11]. However, there were few reports focused on butorphanol-induced post-conditioning (B-Post) in the field of myocardial ischaemia reperfusion injury in rats, although there is one study that found that premedication with butorphanol raised the threshold for ischaemic preconditioning in adult open-chest mongrel dogs [12]. This difference may be due to different experimental methods and different animals. Thus, the purpose of this investigation was to evaluate the effects of B-Post on myocardial ischaemia reperfusion injury and potential mechanisms in rats.

## MATERIALS AND METHODS

### Materials

Butorphanol tartrate was supplied by Hengrui Medicine Company (NO 080425; Jiangsu province, China). Glibenclamide (GLI), norbinaltorphimine (Nor-BNI), tumour necrosis factor (TNF)- $\alpha$  and interleukin (IL)-6 detection kits, Evans Blue and triphenyltetrazolium chloride were provided by Alfa Aesar (Tianjin, China) and Sigma (St. Louis, MO, USA). Myeloperoxidase (MPO), superoxide dismutase (SOD) and malondialdehyde (MDA) detection kits were provided by Jiancheng Bioengineering Research Agent (Nanjing province, China). Male adult Sprague–Dawley rats (250–350 g) were purchased from Tongji Medical College of Huazhong University of Science and Technology (HUST; China). All the animals received humane treatment in accordance with *The Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Publication no. 85–23, revised 1996)*. The experimental procedures were reviewed and approved by the *Animal Experiment Committee of Wuhan University (China)*. The animals were housed under standard laboratory conditions at  $25 \pm 1^\circ\text{C}$ , relative humidity of  $55 \pm 5\%$  and 12 h dark and 12 h light. The animals were allowed free access to food and water.

### Rat myocardium ischaemia/reperfusion model preparation

Each rat was anesthetized with 2% pentobarbital sodium ( $50 \text{ mg kg}^{-1}$  intraperitoneal), intubated through a tracheotomy and ventilated with 100% oxygen with a DW 2000 animal breathing machine ( $V_T = 2\text{--}3 \text{ ml}/100 \text{ g}$ ,  $\text{RR} = 60 \text{ bpm}$ ,  $\text{I:E} = 1:2$ ). Electrocardiograph probes were applied to three legs to produce an ECG image. The left femoral artery was cannulated to monitor arterial blood pressure. To administer chemicals, a catheter filled with saline was placed in the right iliac vein. A thoracotomy was performed between the third and the fourth costal bones. An eyelid bracer was used to expose the thoracic cavity and a 5–0 silk suture was placed around the left anterior descending coronary artery. The ends of the suture were passed through a small piece of soft vinyl tubing to form a snare. Then, the snare was pulled and fixed by clamping the tubing with a small haemostat.

### Experimental protocols

Seventy-five rats were randomly divided into five groups ( $n = 15$  in each). Except for the sham Group, all the hearts were subjected to 30 min of ischaemia and 120 min of reperfusion. In the I/R Group, sodium chloride was administered intravenously at the onset of reperfusion. In the B, B/N and B/G Groups, butorphanol ( $50 \mu\text{g kg}^{-1}$ ) was administered intravenously at the time of reperfusion. In the B/N Group, Nor-BNI ( $2 \text{ mg kg}^{-1}$ ) was administered intravenously 2 min after butorphanol. In the B/G Group, GLI ( $1 \text{ mg kg}^{-1}$ ) was administered intravenously 2 min after butorphanol.

### Determination of myocardial infarct size

At the completion of reperfusion, the suture was tied and 2 ml of 4% Evans blue dye (Sigma) was injected anterograde via the aortic root to delineate the area-at-risk. Hearts were excised and cut into transverse slices of equal thickness (2 mm) after freezing. The

slices were incubated in 1% triphenyltetrazolium chloride (Sigma) in 0.1 mol/l phosphate buffer (pH 7.4) for 30 min at  $37^\circ\text{C}$ . After fixation with 4% paraformaldehyde overnight at room temperature, infarcted myocardium within the area-at-risk was carefully separated from the non-infarcted myocardium within the area-at-risk and weighed. The infarct size is expressed as a percentage of the area-at-risk.

### Determination of myocardial superoxide dismutase, malondialdehyde and myeloperoxidase activities

The activities of the myocardial enzymes (SOD, MDA and MPO) were determined based on the corresponding detection kit. Myocardial tissue was obtained inferior to the site of deligation. Then, the tissue samples were weighed accurately and homogenated.

A spectrophotometer was used to detect optical density at 550 nm of ultraviolet light for SOD, 532 nm for MDA and 460 nm for MPO. All optical density values were transferred to the final concentration.

### Determination of serum tumour necrosis factor- $\alpha$ and interleukin-6 concentrations

Serum TNF- $\alpha$  and IL-6 concentrations were detected using an enzyme-labelled immunosorbent assay. After reperfusion for 120 min, 2 ml of blood was collected, centrifuged at  $4^\circ\text{C}$  (3000 r/min for 10 min) and transferred to a 1.5-ml EP tube. The next steps were performed according to the kit instructions. A coating plate was then placed in the special equipment (the length of the ultraviolet light wave was 450 nm). All optical density values were transferred to the final concentration.

### Statistical analysis

Calculation of sample size was based on the results of an earlier research study [13] about butorphanol pretreatment on myocardial ischaemia reperfusion injury in rats by using  $G^*$ power analysis. For the results to be of statistical significance with  $\alpha = 0.05$  and  $\beta = 0.90$ , a minimum sample size of 3.8 cases per group was required. To increase the reliability of the study, we determined a sample size of 15 animals for each group.

All data are expressed as means  $\pm$  standard deviations (SD). One-way analysis of variance with the SPSS 19.0 statistical software was used to evaluate differences between different experimental groups, and a value of  $P < 0.05$  was considered to be statistically significant.

## RESULTS

### Effect on myocardial infarct size

Treatment with butorphanol (B, B/N and B/G Groups) significantly reduced the infarct size in contrast to the I/R Group (all  $P < 0.001$ ), but treatment with Nor-BIN or GLI (B/N and B/G Groups) increased the infarct size compared with Group B (both  $P < 0.001$ ). In addition, the infarct size in Group B/N was higher than Group B/G ( $P < 0.001$ ) as shown in Table 1.

## Effect on myocardial enzymes

The MDA and MPO activities of the ischaemic area in B, B/N and B/G Groups were significantly less than the I/R Group (all  $P < 0.001$ ), while the MDA and MPO activities in the B/N and B/G Groups were higher than the B Group (all  $P < 0.001$ ). However, There were no significant differences between the B/N and B/G Groups ( $P = 0.071$ ,  $P = 0.721$ , respectively) as shown in Table 2.

The SOD activity in B, B/N and B/G Groups was higher than in the I/R Group (all  $P < 0.001$ ), while the SOD activity in the B/N and B/G Groups was less than in the B Group (both  $P < 0.001$ ). In addition, the SOD activity in Group B/N was lower than in Group B/G ( $P < 0.001$ ; Table 2).

## Effect on serum cytokines

At the end of the experiment, the serum TNF- $\alpha$  and IL-6 concentrations in B, B/N and B/G Groups were less than in the I/R Group (all  $P < 0.001$ ). The TNF- $\alpha$  and IL-6 concentrations in B/N and B/G Groups were higher than in the B Group (all  $P < 0.001$ ). In addition, The TNF- $\alpha$  and IL-6 concentrations in Group B/N were lower than in Group B/G (both  $P < 0.001$ ; Table 3).

## DISCUSSION

Cardiovascular disease has recently become the leading cause of death in humans. Moreover, ischaemic heart disease is a major

health hazard. Reperfusion therapy is an important intervention in patients with acute coronary syndrome. Reperfusion therapy is associated with side effects, such as reperfusion injury, reperfusion arrhythmia, contraction and relaxation dysfunction of muscles and metabolic abnormalities. PostC effectively attenuates those responses [14].

MDA is a marker that oxidizes mediators of membrane phospholipids and SOD catalyses the dismutation of superoxide anion. MPO is released when neutrophilic leucocytes are stimulated, and catalyses a series of reactions involving oxygen at sites of inflammation. Thus, SOD represents the degree of neutrophil infiltration [15, 16]. Generally, the activity of SOD, MDA and MPO has a close relationship to cardiac muscle injury.

In the current study, we showed that MDA and MPO activities of the ischaemic area in all groups with butorphanol (Group B, B/N and B/G) were significantly less than those in the I/R Group, and SOD activities in butorphanol-treatment groups were higher than in Group I/R.

In addition, when ischaemia-reperfusion injury occurs, TNF- $\alpha$  expression level increased, leading to myocardial injury [17]. Cheng et al. [18] concluded that ischaemia during post-treatment inhibited an increase in TNF- $\alpha$ , and further inhibited an increase in IL-1 and IL-6, thereby inhibiting the interaction between pre-inflammatory cytokines, and further prevented the 'inflammatory cascade' from occurring, so as to achieve a cardioprotective effect. The results of this study showed that the concentrations of TNF- $\alpha$  and IL-6 in butorphanol-treatment groups were significantly lower than those in the I/R Group.

**Table 1:** The level of ischaemia and infarct in different groups

Group	Ischaemia degree (%)	Infarct degree (%)
I/R	47.21 $\pm$ 1.21	46.8 $\pm$ 1.41
B	42.54 $\pm$ 1.33	26.4 $\pm$ 1.83*
B/N	44.51 $\pm$ 1.23	34.5 $\pm$ 1.56***
B/G	46.37 $\pm$ 0.96	31.5 $\pm$ 1.27***

Values are means  $\pm$  standard deviations.

\*Significant difference from I/R at the level of  $P < 0.001$ .

\*\*Significant difference from B at the level of  $P < 0.001$ .

\*\*\*Significant difference from B/N at the level of  $P < 0.001$ .

**Table 3:** Concentration of plasma TNF- $\alpha$  and IL-6 in different groups

Group	TNF- $\alpha$ (ng/l)	IL-6 (ng/l)
Sham	40.45 $\pm$ 2.48	51.15 $\pm$ 2.27
I/R	137.67 $\pm$ 7.18*	176.98 $\pm$ 3.70*
B	88.21 $\pm$ 3.64***	125.50 $\pm$ 1.80***
B/N	102.60 $\pm$ 6.67***	137.48 $\pm$ 4.35***
B/G	114.09 $\pm$ 3.67***	147.00 $\pm$ 5.35***

Values are means  $\pm$  standard deviations.

\*Significant difference from the sham Group at the level of  $P < 0.001$ .

\*\*Significant difference from I/R at the level of  $P < 0.001$ .

\*\*\*Significant difference from B at the level of  $P < 0.001$ .

\*\*\*\*Significant difference from B/N at the level of  $P < 0.001$ .

**Table 2:** Myocardial SOD, MDA and MPO activities in different groups

Group	SOD (U/mg prot)	MDA (nmol/mg prot)	MPO (U/g prot)
Sham	77.935 $\pm$ 6.240	0.949 $\pm$ 0.044	2.487 $\pm$ 0.159
I/R	47.646 $\pm$ 1.321*	5.301 $\pm$ 0.161*	5.974 $\pm$ 0.711*
B	126.709 $\pm$ 3.174***	2.674 $\pm$ 0.123**	3.080 $\pm$ 0.262***
B/N	97.845 $\pm$ 1.502***	3.644 $\pm$ 0.150***	4.149 $\pm$ 0.250***
B/G	106.270 $\pm$ 2.695***	3.791 $\pm$ 0.418***	4.101 $\pm$ 0.096***

Values are means  $\pm$  standard deviations.

\*Significant difference from the sham Group at the level of  $P < 0.001$ .

\*\*Significant difference from I/R at the level of  $P < 0.001$ .

\*\*\*Significant difference from B at the level of  $P < 0.001$ .

\*\*\*\*Significant difference from B/N at the level of  $P < 0.001$ .

SOD: superoxide dismutase; MDA: malondialdehyde; MPO: myeloperoxidase

Taken together, our data showed that B-Post limited myocardial infarction reduced myocardial injury and inflammatory response. It strongly suggested that B-Post has a protective effect on MIRI.

It has been well known that OPRs play an important role in the cardioprotection. A large number of evidence reveal that the  $\mu$ -,  $\delta$ - and  $\kappa$ -OPR mediate and regulate cardiovascular system function [3]. Zetta *et al.* [19] demonstrated that the cardioprotective effect of I-Post appeared to involve endogenously activated  $\mu$ -OPRs because the infarct sparing-effect by I-Post was abrogated by the potent  $\mu$ -OPR antagonist CTAP administered at reperfusion in rat hearts. Kim *et al.* [5] reported that morphine-induced post conditioning (M-Post) reduced myocardial infarct size in isolated rat hearts. However, 7-benzylidenenaltrexone,  $\delta_1$ -OR antagonist, aborted totally the effect by M-Post. Meanwhile, Peart *et al.* [6] found that  $\kappa$ -OPR activation by U50,488H prior to reperfusion affords cardioprotection in both rat and mouse hearts. The infarct-reducing effect was abolished by Nor-BNI.

Down-stream signalling effectors of opioid-induced cardioprotection include mito- $K_{ATP}$  channels, protein kinase C, glycogen synthase kinase-3 $\beta$ , extracellular signal regulated kinase1/2 and mitochondrial permeability transition pore and so on [3, 5, 8, 20, 21]. Recently,  $K_{ATP}$  channels in myocytes have attracted much attention [18, 22, 23]. Zhang *et al.* [24] first reported that remifentanyl-induced preconditioning (R-Pre) reduced myocardial infarct size in rat hearts. The infarct limiting effect was mediated via  $\delta$ - and  $\kappa$ -OPRs. This group also demonstrated that  $K_{ATP}$  channels play an important role in R-Pre [25]. This finding is also in agreement with the finding of the current study. Compared with the B Group, myocardial damages of the group combined with  $\kappa$ -OPRs antagonist Nor-BNI (Group B/N) or the  $K_{ATP}$  blocking agent GLI (Group B/G) were more serious. It suggested that  $\kappa$ -OPRs antagonist partly abrogated the beneficial cardioprotective effect of B-Post. In addition, the cardioprotective effect by M-Post was also partly blocked by the  $K_{ATP}$  blocking agent, which suggests the involvement of  $K_{ATP}$  channels in cardioprotection by B-Post.

This study confirmed the protective effect of butorphanol postC on ischaemic myocardium in reperfusion injury. We used  $50 \mu\text{g kg}^{-1}$  of butorphanol based on our previous report that myocardial infarct size could be reduced by  $25 \mu\text{g kg}^{-1}$  butorphanol pretreatment [13]. In addition, our pre-experiment suggested that higher concentrations ( $50 \mu\text{g kg}^{-1}$ ) of butorphanol may be required in B-Post. The time after ischaemia and before reperfusion is a good therapeutic target for acute coronary syndrome patients. The clinical value cannot be ignored. Using drugs at the right time may be a good method to prevent myocardial infarction. However, this study only observed the effect of butorphanol on acute myocardial ischaemia-reperfusion. There is no research on the long-term effects of butorphanol on chronic ischaemic injury. In addition, the optimum dose of butorphanol in postC needs to be confirmed, which will guide clinical practice.

In conclusion, butorphanol post-conditioning provide cardioprotection against myocardial ischaemic and reperfusion injury via  $\kappa$ -OR and KATP channel.

## ACKNOWLEDGEMENTS

Final editing and language help of this work were done by International Science Editing.

## Funding

This work was supported by the National Natural Science Foundation of China (No. 81201499) and the Hubei Research Foundation of Nature Sciences (Wuhan, Hubei, China) (No. 2010CDB00403).

**Conflict of interest:** none declared

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