

## Research Paper

## COX-2 inhibitor nimesulide protects rat heart against oxidative stress by improving endothelial function and enhancing NO production

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**Abstract:** Since a cyclooxygenase 2 (COX-2) inhibitor can reduce infarct size and improve contractility in ischemic myocardium, the aim of the present study was to explore whether COX-2 inhibitor nimesulide could protect myocardial function against oxidative stress injury in rat hearts, and to investigate the underlying mechanisms. The isolated rat hearts perfused by Langendorff method were exposed to 140  $\mu\text{mol/L}$  of  $\text{H}_2\text{O}_2$ , and the cardiac contractility was measured. Then, the responses of coronary arteries, precontracted with U-46619, to the endothelium-dependent vasodilator serotonin (5-HT) and the endothelium-independent vasodilator sodium nitroprusside (SNP) were evaluated. The results were as follows: (1) In hearts exposed to  $\text{H}_2\text{O}_2$  for 20 min, the left ventricular developed pressure [LVDP, (54.8 $\pm$ 4.0)%] and maximal rate of rise/fall of ventricular pressure [ $\pm dp/dt_{\text{max}}$ , (50.8 $\pm$ 3.1)% and (46.2 $\pm$ 2.9)%] were reduced compared with that in the control group (100%). After pretreatment with nimesulide (5  $\mu\text{mol/L}$ ) for 10 min before  $\text{H}_2\text{O}_2$  perfusion, LVDP and  $\pm dp/dt_{\text{max}}$  were enhanced to (79.9 $\pm$ 2.8)%, (80.3 $\pm$ 2.6)% and (81.4 $\pm$ 2.6)%, respectively ( $P < 0.01$ ), and this was partially abolished by the nitric oxide synthase (NOS) inhibitor L-NAME [(60.2 $\pm$ 2.1)%, (63.9 $\pm$ 2.4)% and (63.1 $\pm$ 2.9)%, respectively,  $P < 0.01$ ]. (2) The vasodilatation induced by 5-HT and SNP in  $\text{H}_2\text{O}_2$ -treated group was significantly less than that in the control group. Pretreatment with nimesulide for 10 min antagonized the decrease of endothelium-dependent vasodilatation in  $\text{H}_2\text{O}_2$ -treated group [(-22.2 $\pm$ 4.2)% vs (-6.0 $\pm$ 2.5)%,  $P < 0.01$ ], but had no effect on the decline of endothelium-independent vasodilatation [(-2.0 $\pm$ 1.8)% vs (-7.0 $\pm$ 3.5)%,  $P > 0.05$ ]. (3) Pretreatment with nimesulide for 10 min increased the NO production in  $\text{H}_2\text{O}_2$ -treated hearts [(2.63 $\pm$ 0.40) vs (1.36 $\pm$ 0.23) nmol/g protein,  $P < 0.05$ ], and this was inhibited by L-NAME. (4) Pretreatment with the selective COX-1 inhibitor piroxicam had no effect on LVDP and  $\pm dp/dt_{\text{max}}$  in isolated hearts exposed to  $\text{H}_2\text{O}_2$ , but the left ventricular end diastolic pressure (LVEDP) was much higher than that in the group treated with  $\text{H}_2\text{O}_2$  alone. Piroxicam did not influence the coronary resistance in  $\text{H}_2\text{O}_2$ -treated rat hearts. These data suggest that the COX-2 inhibitor nimesulide improves myocardial function in rat hearts suffering from oxidative stress, and this may be through an improvement in endothelium-dependent arterial relaxation and an enhancement of NO production in rat heart.

**Key words:** oxidative stress; cyclooxygenase 2; myocardium; vascular resistance; nitric oxide synthase

## 环加氧酶-2抑制剂尼美舒利通过改善内皮功能和增加NO含量对抗大鼠心肌氧化应激损伤

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**摘要:** 本文旨在研究冠状动脉内皮和NO在选择性环加氧酶2(cyclooxygenase 2, COX-2)抑制剂尼美舒利(nimesulide)对抗心肌氧化损伤中的作用。离体大鼠心脏行Langendorff灌流, 给予 $\text{H}_2\text{O}_2$  (140  $\mu\text{mol/L}$ )观察心脏收缩功能。用U-46619灌流心脏, 使冠状动脉预收缩后, 观察冠状动脉对内皮依赖性舒张因子5-HT和内皮非依赖性舒张因子硝普钠(sodium nitroprusside, SNP)的反应。结果显示: (1)与空白对照组(100%)相比,  $\text{H}_2\text{O}_2$ 灌流20 min后, 左心室发展压[left ventricular developed pressure, LVDP, (54.8 $\pm$ 4.0)%], 和心室内压最大变化速率[ $\pm dp/dt_{\text{max}}$ , (50.8 $\pm$ 3.1)%和(46.2 $\pm$ 2.9)%]明显降低。 $\text{H}_2\text{O}_2$ 灌流前尼美舒利(5  $\mu\text{mol/L}$ )预

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处理 10 min, 能够显著抑制  $H_2O_2$  引起的 LVDP 和  $\pm dp/dt_{max}$  下降[(79.9±2.8)%, (80.3±2.6)% 和 (81.4±2.6)%,  $P<0.01$ ]。 (2) 与空白对照组相比,  $H_2O_2$  灌流后, 5-HT 和 SNP 引起内皮依赖性和内皮非依赖性血管舒张功能均明显下降; 而尼美舒利预处理 10 min 能明显对抗内皮依赖性血管舒张功能的下降[(-22.2±4.2)% vs  $H_2O_2$  组(-6.0±2.5)%,  $P<0.01$ ], 但对其内皮非依赖性血管舒张功能的下降没有明显作用[(-2.0±1.8)% vs  $H_2O_2$  组(-7.0±3.5)%,  $P>0.05$ ]。 (3) 一氧化氮合酶(nitric oxide synthase, NOS)抑制剂 L-NAME 能够部分取消尼美舒利预处理对  $H_2O_2$  应激心脏功能指标的改善作用[LVDP 和  $\pm dp/dt_{max}$  分别为(60.2±2.1)%, (63.9±2.4)% 和 (63.1±2.9)%,  $P<0.01$ ]。同时尼美舒利预处理 10 min 能使  $H_2O_2$  应激心肌 NO 含量增加[(2.63±0.40) vs (1.36±0.23) nmol/g protein,  $P<0.05$ ], 而 L-NAME 抑制此作用。 (4) 选择性 COX-1 抑制剂吡罗昔康(piroxicam)预处理不能抑制  $H_2O_2$  引起的 LVDP 和  $\pm dp/dt_{max}$  下降, 但促进左心室舒张末压(left ventricular end diastolic pressure, LVEDP)升高; 吡罗昔康对  $H_2O_2$  引起的内皮依赖性和内皮非依赖性血管舒张功能下降无显著作用。以上结果提示, 选择性 COX-2 抑制剂尼美舒利能够对抗大鼠离体心肌氧化应激损伤, 其机制可能是通过改善内皮依赖性血管舒张功能和增加心肌 NO 含量起作用。

**关键词:** 氧化应激; 环加氧酶 -2; 心肌; 血管阻力; 一氧化氮合酶

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely prescribed to control pain and inflammation. The mechanisms of NSAID action include inhibition of cyclooxygenase (COX). COX is the rate-limiting enzyme in the formation of prostanoids by catalyzing the conversion of arachidonic acid to prostaglandin  $H_2$  (PGH<sub>2</sub>). Two forms of COX have been characterized: a constitutive form (COX-1) and a second form (COX-2) inducible by various factors including mitogens, hormones, serum and cytokines<sup>[1,2]</sup>. Although COX-2 is not detectable in the normal heart<sup>[1]</sup>, it is overexpressed in many cardiovascular diseases, such as ischemia/reperfusion, myocardial infarction and congestive heart failure<sup>[3,4]</sup>. *De novo* synthesis of the prostaglandins PGE<sub>2</sub> and PGF<sub>2 $\alpha$</sub>  increases in endocardial and epicardial explants from hearts subjected to transient ischemia<sup>[5]</sup>.

Reactive oxygen species (ROS), measured by electron spin resonance spectroscopy, are significantly enhanced during early reperfusion after ischemia in the heart. Increase in ROS is recognized as the key factor during vascular or cardiac injury associated with various heart diseases such as acute myocardial infarction and coronary heart disease<sup>[6]</sup>. Adderley *et al.* reported that exposure to hydrogen peroxide ( $H_2O_2$ , 50  $\mu$ mol/L) for 10 min induced expression of COX-2, which was prevented by free radical scavengers in cardiac myocytes of rats<sup>[7]</sup>. In different kinds of cells, including cardiomyocytes, many factors (such as LPS and TNF- $\alpha$ ) induce ROS release and significant enhancement of COX-2 expression, which is attenuated by antioxidants<sup>[7,8]</sup>. All of these studies suggest that COX-2 can be induced by many stimuli, but ROS may be the common factor that mediates this process.

Most selective COX inhibitors (such as aspirin and DFU) can reduce the left ventricular end diastolic pressure (LVEDP) and infarct size, and improve contractility in ischemic myocardium<sup>[3,4]</sup>. Whether COX inhibitors can also

provide the protection against ROS is not clear. So the present study was designed to explore the effects of the COX-1 and COX-2 inhibitors piroxicam and nimesulide on myocardial and endothelial function in isolated rat hearts exposed to  $H_2O_2$ , and to investigate the underlying mechanisms.

## 1 MATERIALS AND METHODS

### 1.1 *Animals and reagents*

Male Sprague-Dawley rats (230-250 g) were supplied by the Animal House of Zhejiang University. All rats were provided free access to food and distilled water. All procedures used in this study were approved by the Zhejiang University Ethics Committee for the Use of Experimental Animals.  $H_2O_2$ , nimesulide, piroxicam, *N*<sup>o</sup>-nitro-*L*-arginine methyl ester hydrochloride (L-NAME), serotonin (5-HT), U-46619 (9,11-dideoxy-11 $\alpha$ ,9 $\alpha$ -epoxymethano-prostaglandin F<sub>2 $\alpha$</sub> ), and sodium nitroprusside (SNP) were obtained from Sigma. Commassie Blue kits and NO kits were from NJBI (Nanjing, China). Nimesulide and piroxicam were dissolved with NaOH and diluted with Krebs-Henseleit (KH) buffer, then pH was adjusted to 7.4 with HCl. U-46619 was dissolved in dimethyl sulfoxide (DMSO) and then diluted with KH buffer to give a final concentration of 0.02%. Modified KH buffer contained (mmol/L): NaCl 118, KCl 4.7, K<sub>2</sub>PO<sub>4</sub> 1.2, MgSO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 25, CaCl<sub>2</sub> 1.25, glucose 10, and pH was adjusted to 7.4.

### 1.2 *Langendorff perfusion of isolated rat heart and measurement of left ventricular function*

The method used in the present study is similar to that described previously<sup>[9]</sup>. Briefly, rats were anesthetized with 1% pentobarbital sodium (40 mg/kg body weight, i.p.). Then rat hearts were removed and retrogradely perfused through the aorta in a Langendorff apparatus with KH

buffer. The buffer was equilibrated with 95% O<sub>2</sub> + 5% CO<sub>2</sub> (pH 7.4, 37 °C) for 30 min before use. Hearts were perfused at a constant pressure of 76 mmHg. A water-filled latex balloon-tipped catheter was inserted into the left ventricle. The distal end of the catheter was connected to a data acquisition system (MedLab, China) for continuous monitoring of LVEDP, left ventricular developed pressure (LVDP), maximal rate of rise of ventricular pressure ( $+dp/dt_{max}$ ) and maximal rate of fall of ventricular pressure ( $-dp/dt_{max}$ ).

### 1.3 Experimental protocols

Sprague-Dawley rats were randomly divided into 8 groups (8-10 in each group). After 20 min of equilibration, the hearts were subjected to different treatments as follows (Fig.1), and left ventricular function was continuously monitored. Control group: perfused with KH buffer for 70 min. H<sub>2</sub>O<sub>2</sub> group: perfused with KH buffer for 30 min, then exposed to H<sub>2</sub>O<sub>2</sub> (140 μmol/L) for 20 min followed by 20 min of KH buffer perfusion. Nimesulide + H<sub>2</sub>O<sub>2</sub> group: perfused with KH buffer for 10 min, then treated with nimesulide (5 or 25 μmol/L) for 20 min, followed by 20 min of H<sub>2</sub>O<sub>2</sub> perfusion and 20 min of KH buffer perfusion. Piroxicam + H<sub>2</sub>O<sub>2</sub> group: perfused with KH buffer for 10 min, and then treated with piroxicam (5 or 25 μmol/L) for 20 min, followed by 20 min of H<sub>2</sub>O<sub>2</sub> perfusion and 20 min of KH buffer perfusion. Pre-nimesulide + H<sub>2</sub>O<sub>2</sub> group: same as Nimesulide + H<sub>2</sub>O<sub>2</sub> group, except the hearts were perfused with nimesulide (5 μmol/L) for only 10 min. L-NAME + nimesulide + H<sub>2</sub>O<sub>2</sub> group: same as pre-nimesulide + H<sub>2</sub>O<sub>2</sub> group, except the hearts were perfused with L-NAME (30 μmol/L) for 10 min before nimesulide treatment.

### 1.4 Determination of coronary resistance

In above-described former 5 groups (6-8 in each group), after different treatments for 90 min (Fig.1), coronary arteries were precontracted with U-46619 (0.1 μmol/L) for 40 min. Ten minutes after the U-46619 infusion, endothelial function was evaluated by the vasodilatation produced by 10 μmol/L of 5-HT, whereas coronary smooth muscle function was evaluated with 3 μmol/L of SNP. These infusions were maintained for 10 min, which was long enough to reach a steady-state. A washout period of 10 min was allowed between 5-HT and SNP infusions. Vasodilatation was evaluated by computing percent changes in coronary resistance (coronary perfusion pressure divided by coronary flow), measured immediately before each drug infusion, and after reaching a new steady-state<sup>[10]</sup>.

### 1.5 Measurement of myocardial water content

In above-described former 5 groups (6 in each group), after different treatments for 90 min (Fig.1), the isolated perfused rat hearts were removed and the ventricles were weighed. Wet weight was measured after both ventricles were incised and the excess fluid was blotted. Dry weight was measured after drying for 48 h at 80 °C. Water content was formulated as follows: (wet weight – dry weight)/wet weight×100%.

### 1.6 Determination of NO

In above-described 6 groups (6-8 in each group), after different treatments for 90 min (Fig.1), hearts were removed from the Langendorff apparatus. NO production (nmol/g protein) in heart homogenates was determined by measuring the total nitrite and nitrate concentration, which was measured spectrophotometrically at 532 nm using a commercial assay kit.

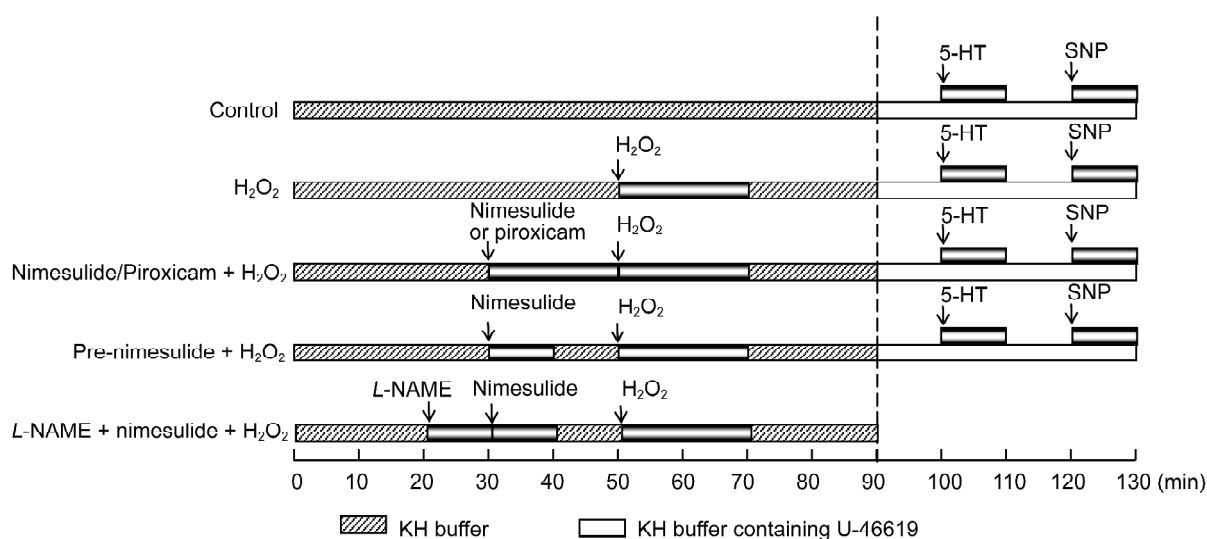


Fig. 1. Experimental protocols.

### 1.7 Statistical analysis

Values were presented as means±SEM. Statistical significance of differences was evaluated by one-way analysis of variance with the Tukey *post hoc* test. A commercially available software program (Prism 4.0) was used.  $P < 0.05$  was considered to be statistically significant.

## 2 RESULTS

### 2.1 Effects of nimesulide and piroxicam on recovery of contractile performance

In hearts exposed to  $H_2O_2$  for 20 min, LVDP and  $\pm dp/dt_{max}$  were reduced, while LVEDP was increased compared with that in the control group (100%). Pretreatment with the selective COX-2 inhibitor nimesulide (5  $\mu\text{mol/L}$ ) for 20 min antagonized  $H_2O_2$ -induced decrease in LVDP [(64.0±3.5)% vs (54.8±4.0)%,  $P < 0.05$ ] and  $+dp/dt_{max}$  [(59.9±3.0)% vs (50.8±3.1)%,  $P < 0.05$ ], but aggravated the rise in LVEDP [(298.5±10.3)% vs (186.3±12.2)%,  $P < 0.01$ ]. Nimesulide at a high concentration (25  $\mu\text{mol/L}$ ) had no effect on LVDP and  $\pm dp/dt_{max}$  in isolated hearts exposed to  $H_2O_2$ , while LVEDP was further raised [(403.1±29.3)% vs (186.3±12.2)%,  $P < 0.01$ ] (Fig.2A). Pretreatment with the selective COX-1 inhibitor piroxicam (5 or 25  $\mu\text{mol/L}$ ) for 20 min had no effect on LVDP and  $\pm dp/dt_{max}$  in isolated hearts exposed to  $H_2O_2$ , but LVEDP was much higher than that in the group treated with  $H_2O_2$  alone [(398.9±25.0)% and (457.5±28.1)%, respectively vs (186.3±12.2)% in  $H_2O_2$  group,  $P < 0.01$ ] (Fig.2B). In isolated hearts pre-perfused with nimesulide (5  $\mu\text{mol/L}$ ) for 10 min (pre-nimesulide +  $H_2O_2$  group), LVDP and  $\pm dp/dt_{max}$  [(79.9±2.8)%, (80.3±2.6)% and (81.4±2.6)%] were enhanced compared with that in  $H_2O_2$  group [(54.8±4.0)%, (50.8±3.1)% and (46.2±2.9)%,  $P < 0.01$ ], and the effect was partially abolished by *L*-NAME [(60.2±2.1)%, (63.9±2.4)% and (63.1±2.9)%,  $P < 0.01$ ]. LVEDP between  $H_2O_2$  group and pre-nimesulide +  $H_2O_2$  group was not significantly different (Fig.2C).

### 2.2 Effects of nimesulide and piroxicam on vascular function

Infusion of U-46619 induced a significant vasoconstriction in the arteries [coronary resistances of (10.84±0.81) and (9.37±2.41) mmHg/mL per minute in the control and  $H_2O_2$  groups, respectively,  $P > 0.05$ ]. Perfusion with 5-HT or SNP induced a diminution in coronary resistance [(-74.1±9.1)% and (-45.28±11.8)%, respectively] in the control group. The vasodilatation induced by 5-HT [(-6.0±2.5)%] and SNP [(-7.0±3.5)%] in  $H_2O_2$  group was significantly less than that in the control group ( $P < 0.01$ ). Pre-

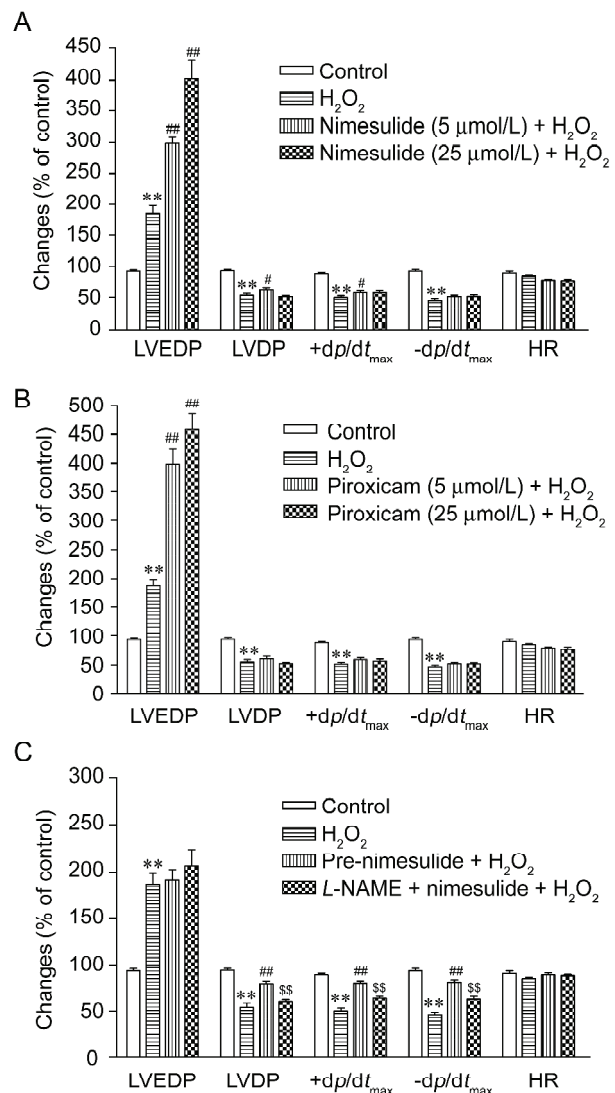


Fig. 2. Changes of inotropic characteristics (LVEDP, LVDP,  $\pm dp/dt_{max}$ ) and heart rate (HR) at the end of  $H_2O_2$  perfusion in isolated rat hearts by nimesulide (A), piroxicam (B), and nimesulide + *L*-NAME (C). means±SEM,  $n=8-10$ . \*\* $P < 0.01$  vs control; # $P < 0.05$ , ## $P < 0.01$  vs  $H_2O_2$ ;  $^{ss}P < 0.01$  vs pre-nimesulide +  $H_2O_2$ .

treatment with nimesulide (5  $\mu\text{mol/L}$ ) for 10 or 20 min antagonized the decrease in endothelium-dependent vasodilatation induced by 5-HT [(-22.2±4.2)% and (-19.4±3.2)%, respectively,  $P < 0.01$ ,  $P < 0.05$ ], but had no effect on the decline in endothelium-independent vasodilatation induced by SNP [(-2.0±1.8)% and (-2.0±2.8)%,  $P > 0.05$ ]. Piroxicam did not influence the coronary resistance in  $H_2O_2$ -treated rat hearts (Fig.3 and 4).

### 2.3 Effects of nimesulide and piroxicam on myocardial water content

Myocardial water content in the isolated rat heart treated with or without  $H_2O_2$  was not significantly different [(81.0±

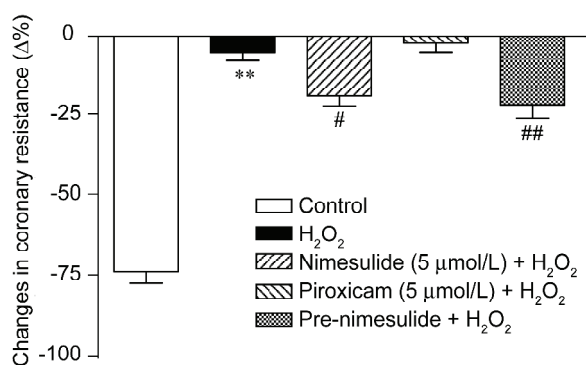


Fig. 3. Changes in coronary resistance ( $\Delta\%$ ) induced by 10  $\mu\text{mol/L}$  of 5-HT. means $\pm$ SEM,  $n=6-8$ . \*\* $P<0.01$  vs control; # $P<0.05$ , ## $P<0.01$  vs  $\text{H}_2\text{O}_2$  group.

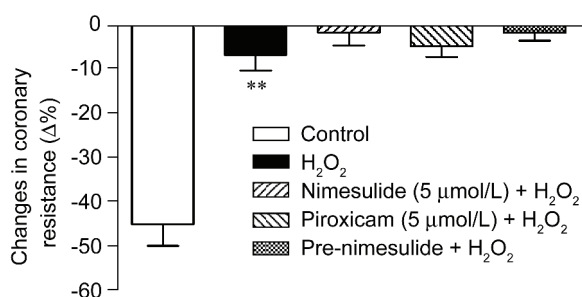


Fig. 4. Changes in coronary resistance ( $\Delta\%$ ) induced by 3  $\mu\text{mol/L}$  of SNP. means $\pm$ SEM,  $n=6-8$ . \*\* $P<0.01$  vs control.

1.2)% vs (83.0 $\pm$ 2.0)%,  $P>0.05$ ]. Pretreatment with nimesulide and piroxicam had no effect on this parameter (Fig.5).

#### 2.4 Effects of nimesulide and piroxicam on NO content

After exposure to  $\text{H}_2\text{O}_2$  followed by 20 min of KH buffer

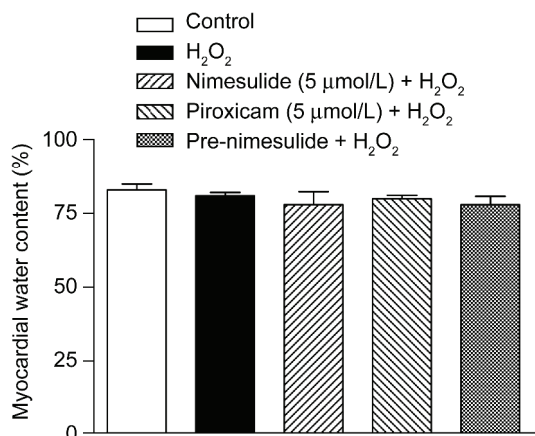


Fig. 5. Myocardial water content in the isolated rat hearts. means $\pm$ SEM,  $n=6$ .

perfusion, the nitrite/nitrate concentration (NO content) was measured in isolated rat hearts. Pretreatment with nimesulide (5  $\mu\text{mol/L}$ ) for 10 or 20 min increased the NO content in the hearts [(2.63 $\pm$ 0.40) and (2.89 $\pm$ 0.30) nmol/g protein] compared with those in  $\text{H}_2\text{O}_2$  group [(1.36 $\pm$ 0.23) nmol/g protein,  $P<0.05$ ], and this increase was abolished by *L*-NAME (Fig.6).

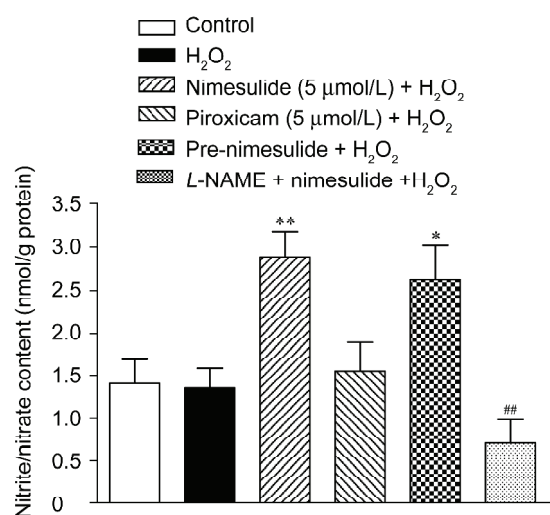


Fig. 6. Concentration of nitrite/nitrate in the isolated rat hearts. means $\pm$ SEM,  $n=6-8$ . \* $P<0.05$ , \*\* $P<0.01$  vs  $\text{H}_2\text{O}_2$ ; ## $P<0.01$  vs pre-nimesulide +  $\text{H}_2\text{O}_2$ .

### 3 DISCUSSION

The present study found that pretreatment with a low concentration of nimesulide (5  $\mu\text{mol/L}$ ) for only 10 min improved both systolic and diastolic function in rat hearts exposed to  $\text{H}_2\text{O}_2$ . However, perfused with nimesulide (25  $\mu\text{mol/L}$ ) for 20 min had a deleterious effect on diastolic function. The same phenomena occurred in piroxicam-pretreated rat hearts. The difference in the effect of exposure to nimesulide for 10 min at low dose and for 20 min at high dose may have a clinical relevance when the drug is administered for chronic use.

Studies on the relationship between NSAIDs and cardiovascular diseases are not consistent. Although non-aspirin NSAIDs have anti-inflammatory and anti-platelet effects similar to those of aspirin, many case-control studies reported that non-aspirin NSAIDs do not confer clinical cardiovascular protection comparable to that achieved by low dose of aspirin<sup>[11-13]</sup>. But others found that some NSAIDs such as naproxen, are associated with a reduced rate of acute major cardiovascular events (myocardial infarction, sudden death, and stroke)<sup>[14,15]</sup>. Some animal studies also

found that selective inhibition of COX-2 constitutes an important therapeutic target for the treatment of myocardial infarction and congestive heart failure in rabbits and rats<sup>[4,16,17]</sup>. But Ray *et al.* reported that users of NSAIDs at high dose are more likely to have coronary heart disease than non-users, although there was no evidence of raised risk of coronary heart disease among users of NSAIDs at low dose<sup>[18]</sup>. Our results suggested that COX inhibitors can act as a protective or a toxic molecule depending on the concentration or duration of perfusion, and on the isoform of COX inhibitor involved. Although many clinical studies have shown increased cardiovascular risk with higher dose of NSAIDs, this study deals with acute effects of nimesulide and the effects may be different in a chronic model.

It had been reported that coronary flow is initially increased during infusion of H<sub>2</sub>O<sub>2</sub>, and thereafter decreased<sup>[19]</sup>. The decrease in coronary flow further aggravates the cardiac dysfunction induced by H<sub>2</sub>O<sub>2</sub>. The decrease in coronary flow may be due to: (1) coronary arteries are squeezed due to edema of cells and/or intercellular space; (2) the smooth muscle of coronary arteries is damaged, which enhances the tension of vessels and vasorelaxation function declines; (3) the endothelium of coronary arteries is injured. Our study demonstrated that the coronary flow decline was not due to edema because myocardial water content did not differ among groups. 5-HT and SNP are coronary vasodilators in isolated vessel preparations and in the isolated perfused heart model<sup>[20,21]</sup>. In this study, the vasodilation to 5-HT was used as an index of endothelial function. This response is an indicator of the ability of endothelial cells to generate and release NO. This study showed that endothelium-dependent vasodilatation decreased after H<sub>2</sub>O<sub>2</sub> exposure, which suggested that the coronary flow decline was due to the dysfunction of coronary artery endothelium. COX inhibitors prevent the reduction in vasodilatation to 5-HT after ischaemia-reperfusion, suggesting that COX inhibitors can protect endothelial function in coronary arteries<sup>[22]</sup>. Such protection was observed in rat heart in the present study. A protective effect of COX inhibitors on endothelium was also found in isolated porcine<sup>[23]</sup> and bovine coronary arteries<sup>[24]</sup>. The vasodilatation to SNP was used as an index of smooth muscle function. This study suggested that the decline of coronary flow was also due to the dysfunction of smooth muscle in coronary arteries. But nimesulide and piroxicam could not protect smooth muscle function in coronary arteries against the deleterious effects of H<sub>2</sub>O<sub>2</sub>. Therefore,

this study suggested that nimesulide may ameliorate the injury of oxidative stress by improving endothelium-dependent relaxation.

NO is an important regulator in myocardial function and vascular tone under physiological conditions. However, its role in pathological situations, such as myocardial ischemia, is equivocal. Both positive and negative effects have been demonstrated in different experimental settings, including human pathology. Some studies found that overexpression of myocyte-specific endothelial NOS provides protection against ischemia-induced left ventricular dysfunction in non-diabetic or diabetic hearts<sup>[6]</sup>. Receiving the NO donor nitroglycerin for 24 h had reduced left ventricular infarct size in isolated perfused hearts subjected to global ischemia/reperfusion<sup>[25]</sup>. But some studies indicate that administration of *L*-NAME may be cardioprotective in normal hearts exposed to ischemia/reperfusion, while blockade of the cardioprotective effect of ischemic preconditioning by *L*-NAME points to a dual role of NO in the heart<sup>[26]</sup>. In this study, an increase of NO was observed in rat hearts pretreated with nimesulide, and the NOS inhibitor *L*-NAME partially abolished the protection induced by low dose of nimesulide. This suggested that low dose of nimesulide may provide cardioprotection against oxidative stress through the NO pathway. A study by Taubert *et al.* also showed that low dose of aspirin may improve vascular endothelial function by inducing an immediate concentration-dependent NO release from porcine coronary arteries<sup>[27]</sup>. So, we speculate that nimesulide-induced protection against oxidative stress may contribute to the increase in NOS/NO in cardiomyocytes and/or endothelium. And the mechanisms are needed to investigate further. Now a new class of agents named COX-inhibiting nitric oxide donors (CINODs) are being designed for the treatment of pain and inflammation. CINODs have a multi-pathway mechanism of action that involves COX inhibition and NO donation. CINODs have reduced gastrointestinal toxicity and cardiorenal protection has been established in animal models; early clinical results suggest a favorable gastrointestinal safety profile in humans. The potential for CINODs to provide cardiorenal protection in humans is currently being investigated<sup>[28]</sup>.

In conclusion, these data suggest that the COX-2 inhibitor nimesulide can improve myocardial function in isolated rat hearts suffering from oxidative stress. The mechanisms may be through an improvement in endothelium-dependent arterial relaxation and an increase of NO content in the heart.

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