

Ischemic postconditioning decreases matrix metalloproteinase-2 expression during ischemia-reperfusion of myocardium in a rabbit model: A preliminary report

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OBJECTIVE: To investigate the effect of ischemic postconditioning on the expression of matrix metalloproteinase (MMP)-2 during ischemia-reperfusion of myocardium in a rabbit model.

METHODS: Thirty-six male New Zealand white rabbits were randomly divided into sham, ischemia-reperfusion and ischemic postconditioning groups. Myocardial ischemia-reperfusion was created by ligating the left anterior descending coronary artery for 30 min followed by 3 h of reperfusion. Myocardial infarction sizes were determined by dual staining with triphenyltetrazolium chloride and trypan blue. Plasma levels of MMP-2 were measured using ELISA. Myocardial MMP-2 messenger RNA was analyzed by reverse transcription polymerase chain reaction.

RESULTS: The mean (\pm SD) infarct size in the ischemic postconditioning group was significantly smaller compared with the ischemia-reperfusion group ($37.1 \pm 3.8\%$ versus $57.5 \pm 1.9\%$; $P=0.02$). The incidence of ventricular tachycardia in the ischemic postconditioning group was also lower than in the ischemia-reperfusion group (8.5% versus 75% ; $P=0.003$). MMP-2 messenger RNA expression in the ischemic postconditioning group was significantly lower compared with the ischemia-reperfusion group (0.4944 ± 0.0476 versus 0.6989 ± 0.0694 ; $P=0.02$).

CONCLUSION: Ischemic postconditioning reduces myocardial ischemia-reperfusion injury, possibly by inhibiting the expression of MMP-2.

Key Words: Acute myocardial infarction; Ischemia-reperfusion; Ischemic postconditioning; Matrix metalloproteinase-2; Rabbits

Acute myocardial infarction (AMI) is caused by coronary atherosclerosis and secondary thrombosis, which both interrupt coronary blood flow. Early opening of the occluded coronary artery and effective restoration of blood perfusion in ischemic myocardium is an important treatment in AMI (1). However, reperfusion injury can occur during ischemia-reperfusion, which may cause myocardial systolic and diastolic dysfunction, metabolic abnormalities and ultrastructural changes in the myocardium (2,3). Therefore, new modalities for preventing myocardial ischemia-reperfusion injury are required for the optimal treatment of AMI. Recent studies have shown that postconditioning promotes the recovery of cardiac function in ischemia-reperfusion injury in AMI (4,5) and also in cardiopulmonary bypass (6).

Matrix metalloproteinase (MMP)-2 plays an important role in acute mechanical dysfunction of the heart following ischemia and reperfusion (7,8). MMP-2 is activated intracellularly and cleaves troponin I during reperfusion (9). Inhibition of MMP-2 activity was shown to be correlated with decreased infarct size (10).

Ischemia postconditioning can reduce ischemia-reperfusion injury in myocardial infarction (11). Inhibition of the inflammatory response may be a mechanism after postconditioning in ischemia-reperfusion, in which ischemic postconditioning reduces tumour necrosis factor- α and interleukin-6 levels (12). A recent study showed that ischemic postconditioning decreases infarct size, possibly through the inhibition of MMP-2 activity induced by ischemia-reperfusion (13). However, the relationship between ischemic postconditioning and MMP-2 inhibition is currently unclear. In the present study, we explored MMP-2 expression during ischemic postconditioning in a rabbit model of AMI.

METHODS

The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the National Research Council (revised 1996) and was approved by the local Ethics Committee of Liaocheng People's Hospital, Shandong, China.

Preparation of the animal model

Male New Zealand white rabbits (2.0 kg to 2.5 kg, Animal Husbandry and Veterinary Institute, Academy of Agricultural of Shandong Province, China) were used for the experiments. After intravenous injection of 3% pentobarbital (30 mg/kg), the rabbits were fixed on an operating table. The trachea was cannulated for artificial ventilation with room air. The right carotid artery and jugular vein were also cannulated for measurement of arterial blood pressure and drug administration, respectively. Body temperature of the animals was maintained at a mean (\pm SD) temperature of $37 \pm 0.5^\circ\text{C}$ using an electrical heating pad. A multichannel physiological instrument was connected for electrocardiography recording. After skin preparation, the pericardium was cut along the left sternal incision, and the heart and blood vessels of the left ventricular surface were exposed. The left ventricular branch of the coronary artery was found between the left atrial appendage and the left ventricular apex. In the upper one-third of the plane connecting the left atrial appendage and the apex, a 5-0 needle was used to cross the left descending coronary artery (needle depth 0.2 cm to approximately 0.4 cm). Both ends of the line crossed through a small polyethylene tube (approximately 0.5 cm long, diameter 0.2 cm) to form a closed loop. The closed loop was tensed and fixed with a clamp that caused coronary artery occlusion and ischemia. AMI was defined as myocardial colour changes from red to dark gray in the region of the coronary occlusion, and ST segment elevation on electrocardiogram. Thirty minutes after ligation, the closed loop was released to induce reperfusion.

After the experiment, the animals were euthanized and the hearts were harvested. The ischemic myocardial tissues were stained with hematoxylin and eosin, and histopathological changes were observed under a light microscope under $\leq 400\times$ magnification.

Experimental protocol

Thirty-six male New Zealand white rabbits were randomly divided into three groups ($n=12$ per group). In the ischemia-reperfusion

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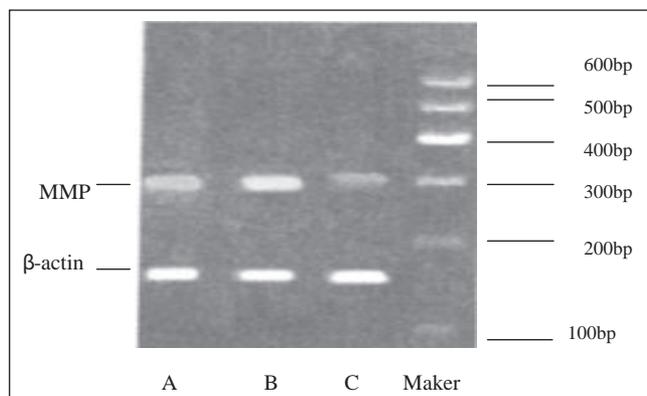


Figure 1 Effect of ischemia reperfusion and ischemia postconditioning on the matrix metalloproteinase (MMP)-2 messenger RNA expression levels. **A** Ischemic postconditioning group. **B** Ischemia-reperfusion group. **C** Sham group

group, hearts were subjected to 30 min of no-flow ischemia and 180 min of reperfusion. In the ischemia-conditioning group, hearts were subjected to 30 min of ischemia and, within the next 3 min, hearts were subjected to 30 s of ischemia and 30 s of reperfusion for three cycles. In the sham group, only coronary artery threading was performed and no ischemia or reperfusion was induced. In each group, six rabbits were used to determine myocardial infarct size after completion of perfusion, while the remaining six were used for myocardial tissue morphological analysis.

Determination of myocardial infarct size

Myocardial infarct size was determined using trypan blue staining. At the end of the experiment, the left anterior descending coronary artery was permanently ligated. The hearts were harvested and placed in saline to wash off residual intracardiac blood. One millilitre of 1% trypan blue was injected to the aorta retrogradely, and the hearts were refrigerated at -20°C for 2 h. The left ventricles were cut into eight to 10 pieces of equal thickness (approximately 2 mm) from the apex to the base parallel to the atrioventricular groove. The sections that were stained with trypan blue (ischemic) and not stained (nonischemic) were separated, and the area at risk (AAR = ischemic area/non-ischemic area) was calculated.

The slices were incubated for 15 min in a 1% triphenyltetrazolium chloride (triphenyltetrazolium chloride dissolved in pH 7.8 phosphate buffer) solution at 37°C . Dark red noninfarcted areas and gray infarcted areas were clearly apparent on the section. The blue nonischemic area, red ischemia without infarct area and gray ischemic infarct zone was separated using microscalpel, and was weighed using electronic scales after and dried with filter paper. The ratio of the myocardial infarct zone (AN) to ischemia zone was evaluated for infarct size.

MMP-2 messenger RNA expression in myocardial tissue

The target gene was searched for in the Genbank database. The MMP-2 upstream primer was 5'-AGC CTT CTC ACC CCC ACC TG-3', downstream 5'-GCC CTTATC CCA CTG CCC C-3', amplified length 313 bp; and β -actin upstream 5'-AGA AGC TGT GCT ACG TGG CG-3', downstream 5'-AGG AAG GAG GGC TGG AAC A-3', amplified length 160 bp, which was synthesized by Shanghai Public Health primers (China).

At the end of each experiment, the hearts were harvested and the infarct areas were measured. Thereafter, the fresh frozen myocardial tissues (100 mg) from the infarct areas were used to extract total tissue RNA using the TrizolRNA extraction kit (Invitrogen Ltd, China). The extracted RNA was assessed using a nucleic protein analyzer. The total RNA was added to $10\times$ reverse transcription buffer, 25 mM MgCl_2 , 10 mM dNTP, RNA inhibitor, AMV reverse transcriptase, Oligo (dT) 15 primer and double deionized water in a

TABLE 1
Serum levels of matrix metalloproteinase-2 ($\mu\text{mol/L}$) in the three groups of animals

	Group			P
	Sham (n=12)	IR (n=12)	IPC (n=12)	
Baseline	16.41 \pm 1.91	15.52 \pm 1.66	16.02 \pm 1.42	2.60
After MI	15.65 \pm 3.68	71.67 \pm 3.86	42.59 \pm 4.03	0.02

Data presented mean \pm SD unless otherwise indicated. MI Myocardial infarction; IR Ischemia-reperfusion; IPC Ischemic postconditioning

20 μL reaction volume. After incubation at 42°C for 15 min, 95°C for 5 min and 4°C 5 min, reverse transcription products were obtained. MMP-2 reaction conditions were: predenaturation at 95°C for 5 min, 95°C denaturation for 30 s, 64°C annealing for 30 s, 72°C extension for 1 min and 72°C extension for 10 min. β -actin response conditions were: 94°C predenaturation for 5 min, 94°C denaturation for 30 s, 60°C annealing for 30 s, 72°C extension for 1 min and 72°C extension for 10 min. Five microlitres of each polymerase chain reaction product were electrophoresed on a 1% agarose gel (Figure 1). After gel imaging was completed, the intensity of the target band was calculated and compared with β -actin as a standard.

Serum MMP-2 measurement

Blood samples were collected at the end of the experiment. All samples were taken from the ear vein and immediately stored at 4°C for 2 h, and then centrifuged after coagulation (2500 rpm \times 10 min). Serum was isolated and stored at -80°C for testing. Serum MMP-2 expression levels were determined using ELISA.

Statistical analysis

SPSS version 10.0 (IBM Corporation, USA) was used for statistical analysis. All data were expressed as mean \pm SD. Categorical data were analyzed using Fisher's exact test. Differences between the groups were analyzed using ANOVA, with statistical significance set at $P < 0.05$.

RESULTS

Comparison of myocardial infarction area and incidence of arrhythmias

Infarct size in the ischemic postconditioning group ($37.1 \pm 3.8\%$) was smaller than in the ischemia-reperfusion group ($57.5 \pm 1.9\%$; $P = 0.02$). No myocardial infarction was found in the sham group.

No ventricular arrhythmias were recorded in the sham group. Ventricular arrhythmias (nonsustained ventricular tachycardia) was recorded in nine (75%) hearts of the ischemia-reperfusion group and in one (8.3%) of the ischemic postconditioning group ($P = 0.003$).

Histological examination of myocardium

In the ischemic postconditioning group, scattered myocardial fibre swelling and mild cell interstitial edema were evident and the injury was less severe than in the ischemia-reperfusion group. In the ischemia-reperfusion group, myocardial fibres were disarranged, with breakage and dissolution. Interstitial edema and a large number of red blood cells were found within the tissue space, and foci of inflammatory cell infiltration were observed. In the sham group, myocardial cells were neatly arranged, with clear striations and no interstitial edema or hemorrhage.

Comparison of serum MMP-2 levels

As shown in Table 1, there was no statistically significant difference in the baseline serum MMP-2 levels among the three groups ($P > 0.05$). Following the ischemic event or sham operation, the serum levels of MMP-2 in the ischemia-reperfusion group ($71.67 \pm 3.86 \mu\text{g/L}$) were higher than in the sham group ($15.65 \pm 3.68 \mu\text{g/L}$; $P < 0.05$). However, serum levels of MMP-2 in the ischemic postconditioning ($42.59 \pm 4.03 \mu\text{g/L}$) group was lower than in the ischemia-reperfusion group ($P < 0.05$).

Myocardial MMP-2 messenger RNA expression

MMP-2 messenger RNA expression was higher in the ischemia-reperfusion group than in the sham group (0.6989 ± 0.0694 versus 0.2505 ± 0.0075 ; $P=0.02$). However, MMP-2 messenger RNA expression in the ischemic postconditioning group was lower than in the ischemia-reperfusion group (0.4944 ± 0.0476 versus 0.6989 ± 0.0694 ; $P=0.03$).

DISCUSSION

The present study showed that ischemia postconditioning attenuates the extent of myocardial infarction and reduces the incidence of ventricular arrhythmias induced by ischemia-reperfusion. In addition, ischemia postconditioning also inhibits the release of MMP-2 following ischemia-reperfusion.

Myocardial ischemia-reperfusion injury is mediated by several mechanisms including calcium overload, free radical damages and neutrophil infiltration (3). In recent years, a large number of experiments showed that MMPs may be involved in myocardial ischemia-reperfusion injury (9). Peroxynitrite anion generation not only activates MMPs but also inhibits tissue inhibitor of MMPs activity in isolated heart models of ischemia-reperfusion injury (14). Cheung et al (7) found that the production of MMP-2 increased significantly in the perfusion fluid after ischemia-reperfusion in the isolated heart. Duration of myocardial ischemia and increased MMP-2 levels after myocardial ischemia-reperfusion was correlated with reduced myocardial function (7). MMP inhibitors, such as doxycycline, phenanthroline or MMP-2 antibody, diminished or prevented myocardial stunning (7,9,14). In the isolated rabbit heart, 15 min of ischemia did not result in a significant increase of MMP-2 levels in the perfusion fluid. However, 60 min of ischemia and reperfusion led to a significant elevation in MMP-2 levels in the perfusion fluid (7). Wang et al (9)

found that MMP-2 and troponin I was present in the same region in the thin filaments. The intracellular troponin I degradation was significantly reduced after treatment with the MMP-2 inhibitors phenanthroline and doxycycline. This suggested that MMP-2 inhibition promotes cardiac function recovery after ischemia-reperfusion.

Ischemia postconditioning after ischemia-reperfusion has myocardial protection effects that may involve several mechanisms, such as stable endothelial cells, inhibition of the generation of reactive oxygen species and neutrophil activation, and aggregation in ischemic myocardium and reduction of microvascular injury (12,15). Ischemia postconditioning protection of myocardium has been studied via several pathways, such as the phosphatidylinositol 3-kinases-serine/threonine protein kinase (PI3K-PKB/Akt) PKB reperfusion injury salvage kinase pathway (16), reperfusion injury signalling kinase signal pathway (17) and the opioid receptor and Janus kinase-signal transducer and activator of transcription (JAK-STAT) signalling pathway (18). However, the intracellular mechanism of ischemic postconditioning is not completely understood. The present study suggested that ischemic postconditioning decreased infarct size through MMP-2 inhibition.

CONCLUSION

The present study demonstrated that ischemia postconditioning reduced MMP-2 expression in an ischemia reperfusion model. These results suggest that cardiac protection of postconditioning may be related to the reduction of MMP-2 expression in the myocardium.

DISCLOSURES: The authors have no financial disclosures or conflicts of interest to declare.

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