의학석사 학위논문

Effect of ischemic preconditioning on myocardial protection in rat heart; Comparison of direct versus remote ischemic preconditioning

허혈 전처치에 따른 쥐 심근 보호 효과; 직접 및 원격 허혈 전처치 방법의 비교

2013년 2월

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오세진 의 의학석사 학위논문을 인준함
2013년 2월

위 원 장 _____ (인)
부위원장 _____ (인)
위 원 _____ (인)

ABSTRACT

Background: Ischemic preconditioning, direct or remote, has been known to protect the myocardium against lethal ischemic injury. Expression of neural cell adhesion molecule (NCAM) is also known to have protective and regulating effects on myocardial infarction. The aims of the present study are; (1) to compare the effect of direct versus remote type of ischemic preconditioning on myocardial protection, (2) to examine the expression level of neural cell adhesion molecule (NCAM) in acute stage of myocardial infarction, and (3) to demonstrate if the expression of NCAM is involved in myocardial ischemic preconditioning models in rat.

Methods: Ischemic preconditioning was induced in Sprague-Dawley (SD) rat by three cycles of 3 minutes occlusion of the left anterior descending coronary artery (Direct group, n=7) or left femoral artery (Remote group, n=7), followed by 5 minutes of reperfusion. Myocardial ischemia was produced by ligation of the left anterior descending coronary artery for 50 minutes, followed by reperfusion for 50 minutes (Control group, n=7). Infarct and at risk area was determined by 2,3,5-triphenyltetrazolium chloride staining. Immunohistochemistry was performed with anti-NCAM rabbit polyclonal antibody H-300. Expression level of NCAM of infarct area was presented as the ratio of intensity of infarct area to intensity of the reference area.

Results: The direct ischemic preconditioning significantly decreased the infarct size (p = 0.003) when compared with that of the control group. The infarct size was also decreased in the remote group, however, there was no significant difference when compared with that of the control group (p = 0.269). The infarct size in the direct group was smaller than that in the remote group without significant difference between two groups (p = 0.097). In the immunohistochemistry, the expression of NCAM in the direct group was lower than the other 2 groups; however, there was no significant difference in the NCAM expression level among the three groups.

Conclusions: Ischemic preconditioning to the left anterior descending coronary artery territory provided a myocardial protection for ischemia-reperfusion injury to the same coronary artery territory. However, the protective effect of ischemic preconditioning to the femoral artery was not significant in the rat myocardium infarction model. The expression of NCAM seemed to be down-regulated in the acute stage of myocardial infarction and showed no differences in the groups of ischemic preconditioning in rat.

Keywords: heart, ischemic preconditioning, neural cell adhesion molecules

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INTRODUCTION

Ischemic preconditioning (IPC) is defined as some kind of myocardial protection by rendering the ischemic territory resistant to a subsequent longer period of ischemia after the transient episodes of myocardial ischemia and reperfusion. Since the concept of ischemic preconditioning was firstly described by Murry and colleagues [1], numerous animal and clinical studies has been performed to identify the basic mechanism of IPC and to evaluate the cardioprotective effects on myocardial ischemic injury. In the experimental studies, almost all previous reports concluded that the IPC improved the myocardial preservation while the value of IPC remained controversial in the clinical investigations [2].

On the other hand, there are two types of IPC, which are direct and remote IPC. Despite a variety of results favoring the cardioprotective effect of the direct type of IPC, the clinical application is restricted, due to the direct invasiveness to the myocardium. Therefore, the notion of remote ischemic preconditioning (RIPC) applied in other coronary arteries, including the circumflex artery (intra-cardiac IPC), mesenteric or renal artery (inter-organ IPC), and femoral or brachial artery (distant IPC) has been well established, and several underlying mechanisms have also been investigated [3-5]. In RIPC to the far distant organs, including limbs, however, some arguments regarding the true protective effect on ischemic myocardium still remain.

Moreover, there were lacked studies that compared direct IPC with remote IPC.

Expression of neural cell adhesion molecule (NCAM, CD 56) is also known to have protective and regulating effects on myocardial infarction and congestive heart failure [6]. However, there was no study with regard to the role of NCAM in acute stage of myocardial infarction and ischemic preconditioned heart.

The aims of the present study are; (1) to compare the effect of direct versus remote type of ischemic preconditioning on myocardial protection, (2) to examine the expression level of neural cell adhesion molecule (NCAM) in acute stage of myocardial infarction, and (3) to demonstrate if the expression of NCAM is involved in myocardial ischemic preconditioning model in rat.

METHODS

All animals were treated in compliance with the Guide for the Care and Use of Laboratory Animals (National Academy of Science, Washington, D.C.). Animal use protocols were approved by the Institutional Animal Care and Use Committee (IACUC) in Seoul National University Hospital Biomedical Research Institute (Approval Number: 04-2010-095-0).

1. Surgical procedures

Female Sprague-Dawley (SD) rats weighing 250g to 300g were used in the present study. Anesthesia of rats was performed with inhalation of isoflurane for induction, followed by intraperitoneal administration with combination of Zoletil 50 and Rompun for maintenance. A tracheostomy was performed and the rat was intubated with a cannula connected to a ventilator. To prevent the atelectasis, positive pressure ventilation was maintained during the operation. A left thoracotomy was performed and approached via the fifth intercostals space. After the pericardium was opened, the left atrial appendage was moved to expose the left coronary artery, close to the place of origin. Myocardial infarction was induced by ligating the left anterior descending coronary artery (LAD), approximately 2mm from its origin with 6-0 nylon suture.

2. Experimental protocols

In the ‘Control group’ (n=7), rats underwent 50 minutes of ischemia, followed by 50 minutes of reperfusion. After reperfusion, the heart was quickly excised and heparinized via coronary ostium, while clamping the ascending aorta. The coronary artery, which had been ligated for infarction, was again occluded by 6-0 nylon suture, and then 1% Evans blue was perfused to stain the myocardium. The heart was cut into 2mm slices and the slices were then incubated in 2,3,5,-triphenyltetrazolium chloride (TTC, Sigma Chemical, St. Louis, MO), which was dissolved in a 100 mmol/L phosphate buffer, for 15 minutes. In the ‘Direct group’ (n=7), the LAD close to its origin was snared with 6-0 nylon double suture. After a snare occluder was placed into the ring formed by double suture, the suture ends were crossed and tugged from both sides for 3 minutes of LAD occlusion, and then were released for 5 minutes of reperfusion. Ischemic preconditioning was induced by three cycles of LAD occlusion, followed by reperfusion. In the ‘Remote group’ (n=7), the femoral artery of the left hind limb was dissected and snared with a 6-0 nylon suture for occlusion by crossing the suture. RIPC was elicited by three cycles of 3 minutes of the left femoral artery occlusion interspersed with 5 minutes of reperfusion before 50 minutes of regional ischemia in the heart. The experimental protocols and time-lines are shown in Fig. 1.

3. Determination of Infarct size and area at risk

We always used the mid portion of left ventricle among all sliced sections of myocardium for measuring the infarct size, and comparing it between three groups on the same cross section. Infarct (white zone) and at risk (red zone) areas were measured by plainmetry, using Image J v1.44 software (NIH, Bethesda, MD) [7]. Infarct size was expressed as the ratio of infarct area (white zone) to whole the ischemia area, which consisted of infarct and at risk areas (white plus red zone) (Fig. 2).

4. Immunohistochemistry

The tissue was fixed by immersion in 10% neutral buffered formalin for 24~36 hours at room temperature. Fixed tissue specimens were dehydrated and embedded in paraffin, according to the standard procedures. Paraffin sections of 4 μm thickness were cut and placed on silane-coated glass slides. After deparaffinization, using serial xylene and alcohol, antigen was retrieved by heating for 10 minutes. Endogeneous peroxidase activity was blocked, using 0.3% H_2O_2 in distilled water for 5 min. The glass slides were washed in phosphate- buffered saline (PBS, 3 times, 5 min each) and mounted with 1% normal serum in PBS for 30 min. Anti-NCAM rabbit polyclonal antibody H-300 (Santa Cruz, CA, USA) diluted to 1:50 in PBS was applied for two hours at room temperature. The slides were incubated

with biotinylated anti-rabbit serum (second antibody) diluted to 1:100 in PBS for 40 min, followed by washes in PBS (3 times, 5 min each). Avidin-biotin-peroxidase complex (ABC) (ABC-Elite, Vector Laboratories, CA, USA) at a dilution of 1:100 in PBS was applied for 20 min. After washing in PBS (3 times, 5 min each), the detection reaction was carried out with 3,3-diaminobenzidine (DAB), and nuclei were counterstained with Meyer's hematoxylin.

5. Image analysis of immunohistochemistry

Images were captured with Olympus light microscope BX43 (Olympus, Japan) and a digital camera DP26 (Olympus, Japan) with CellSens software (Olympus, Japan) with pixel size 1280x960. Three images at the lateral wall and anterior wall of the left ventricle with mid portion in between were obtained in each case. After the same image adjustment parameter was applied to all images with Image J v1.44 (NIH, Bethesda, MD) [7], pixels over the threshold were counted. To adjust inter-individual variation in immunostaining pattern of NCAM, lateral wall of left ventricle with non-ischemic change confirmed in chemical staining was used as a reference area. Pixel count of lateral wall was used as a reference value in each case for the correction of the level of NCAM expression in the tissue.

Expression level of NCAM of infarct area was presented as ratio of intensity of anterior wall (infarct area) to intensity of reference point (non-ischemia

area) (Fig. 2).

6. Statistical analysis

Statistical analysis was performed using the SPSS software package (Version 12.0, SPSS Inc, Chicago, IL). Continuous variables were expressed as the mean ± standard deviation, median and ranges, or proportions. Comparison between the 3 groups was performed with the one-way ANOVA. All statistical tests were two-tailed, and all P values of less than 0.05 were considered statistically significant.

RESULTS

1. Myocardial infarct size

Mean myocardial infarct size in control, direct, and remote group were 0.68±0.13, 0.35±0.19, and 0.54±0.17, respectively. The infarct size of the direct group, which underwent the LAD IPC, was significantly decreased compared with those of no IPC group (Control group) ($p = 0.003$). However, RIPC to the femoral artery (Remote group) before regional heart ischemia slightly decreased the infarct size. Therefore, there was no significant difference between the remote and direct groups ($p =0.097$), and the infarct size of the remote group was similar with those of the control group ($p =0.269$). Infarct size of all groups and comparison analysis between the groups were presented in Table 1.

2. Expression level of CD 56 (NCAM) in infarct area

Ventricular myocardial tissues of three rats included in each group were stained with anti-NCAM rabbit polyclonal antibody H-300 for immunohistochemistry. Level of expression in intercalated discs of myocardial cell showed the decreasing patterns in infarct area compared with non-ischemia area in all myocardium (Fig. 3). While the NCAM expression level between the control and remote groups was similar

(Control vs. Remote group; 0.74 ± 0.23 vs. 0.87 ± 0.35, p=0.589), the level of NCAM expression in direct group was decreased compared with those in other two groups. However, the statistical significance in each comparison analysis was not existed (Direct vs. Control group; 0.50 ± 0.13 vs. 0.74 ± 0.23, p=0.233, Direct vs. Remote group; 0.50 ± 0.13 vs. 0.87 ± 0.35, p=0.063). Expression level of NCAM in infarct and non-ischemia area, and group comparison were shown in Table 2.

DISCUSSION

The current study revealed three main findings. First, the direct IPC significantly decreased the myocardial infarct size, while the RIPC to the femoral artery did not show the significant reduction of infarct size in rat heart. Second, the expression level of NCAM in infarct area showed the decreasing pattern compared with that of the non-ischemia area in the acute stage of myocardial infarction. Third, the NCAM expression was more down-regulated in infarct area of direct group than in infarct area of other two groups without the statistical significance.

A brief period of repeated ischemia and reperfusion has been shown to protect the myocardium against lethal injury, following subsequent sustained ischemia, and the phenomenon is termed as ischemic preconditioning (IPC) [1]. According to the results of a variety of previous experimental studies related with IPC, it has been known that myocardial infarct size is significantly decreased after IPC. Moreover, the concept of IPC at a remote site from the target organ was first described by Przyklenk and colleagues, and termed remote ischemic preconditioning (RIPC) [8]. RIPC has been applied in non-targeted coronary arteries (intra-cardiac IPC), mesenteric or renal artery (inter-organ IPC), and femoral or brachial artery (distant IPC). Since the clinical application of direct IPC induced by transient ischemia and

reperfusion to the target coronary territory may be limited due to the practical difficulties and the possibilities of myocardial dysfunction resulted from the procedure itself, currently, RIPC appears to be more attractive and popular in the diverse clinical situation, including myocardial infarction, coronary artery bypass surgery [9], abdominal aortic aneurysm repair [10], and even in orthotopic heart transplantation [11]. However, there were some arguments regarding the true protective effect of RIPC to the limbs because the transient limb ischemia usually showed to be a potent preconditioning stimulus in humans and larger animals [12]. In addition, there were lacked studies that compared direct IPC with remote IPC.

In the present study, the three cycles of direct IPC to the LAD, which was a coronary territory subjected to the sustained ischemia, significantly decreased the myocardial infarct size while the RIPC to the femoral artery showed the slightly decreased or similar patterns of infarct size compared with no IPC group. Rather, there was much difference in the infarct size between direct and remote IPC group despite no statistical difference. Therefore, the cardioprotective effect of IPC may be reduced in RIPC to the far distant organs, such as limbs, especially in rats that have smaller proportion of blood flow in limbs than other major organs.

Although it is well established by numerous experimental studies that ischemic preconditioning provides the enhanced tolerance of the myocardium to the prolonged ischemic insult, however, the precise

mechanisms are still unknown. Basically, ischemic preconditioning is considered to cause an increase in adenosine monophosphate (AMP) and cyclic AMP, thereby attenuating the degradation of adenosine triphosphate (ATP) and the accumulation of glycolytic intermediates and lactate production during sustained ischemia [13] in the view of metabolic theory. Furthermore, there have been presented with several possible and multifactorial mechanisms related with the ischemic preconditioning, such as stimulation of beta adrenergic receptors [13], activation of signaling pathways, including mitochondrial K_{ATP} channels and protein kinase C (PKC) [14,15], generation of reactive oxygen species (ROS) and endogenous nitric oxide (NO) [16], innate immunity pathways activated by tumor necrosis factor alpha (TNF-α) in leukocyte [17], modification of gene expression in myocardium [18], and Akt pathway and glycogen synthase kinase-3β (GSK-3β) activation [19]. Therefore, various types of endo- or exogenous substances, including κ- or δ- opioid receptor agonists [14,20,21], adenosine [15], bradykinin agonist [16,22-25], and adenylate cyclase agonist [13] have been investigated, which are able to mimic the protective effect of ischemic preconditioning. However, the complete signaling pathway of protective effect remains unclear, and the exact relationship between these mechanisms is not clearly defined. In addition, the function and importance of mechanisms and various endo- or exogenous triggers are controversial. These uncertainties may reflect multiple signaling paths in preconditioning. It

is also possible different paths may mediate the differing protective effects [15].

In this regard, we hypothesized that neural cell adhesion molecule (NCAM, CD56), which was known for a regulating factor of myocardial protection, would be involved in the mechanisms related to the myocardial response against acute myocardial infarction and IPC.

Although the basic role of NCAM was presented as a major regulator of development, cell survival, migration, and neurite outgrowth in the nervous system [6], and mediates the intercellular adhesion in the nervous system and skeletal muscle [26], several previous studies demonstrated that the expression of NCAM was enhanced and played a protective role against the metabolic stress, such as congestive heart failure (CHF) and myocardial infarction (MI) in rat and mouse cardiomyocytes. There was significant up-regulation of NCAM in infarct area at subacute (7 days after surgery) and chronic phase (12 months after surgery) of MI, which was induced by ligating the left anterior descending coronary artery [6]. Moreover, the overexpression of NCAM was identified as a local response of cardiomyocyte to scar formation in ischemic cardiomyopathy of human and rat models [26]. However, there was no previous report with regard to the role of NCAM in ischemic preconditioned MI heart. It has been wondered whether the similar patterns of NCAM expression level would be observed in acute MI model. In our experiment, the NCAM staining of infarct area in the

direct group showed much weaker than those in the control and femoral group, although there was no statistical significant difference due to a very small number of cases. Interestingly, the NCAM expression of infarct area was decreased than those of non-ischemic area in all the groups. From these results, therefore, we deduced that the NCAM, which was a kind of cell surface protein up-regulated under subacute and chronic stress conditions, was down-regulated in cardiac myocytes under acute infarction phase because of the active degradation of the myocardial cell. Moreover, NCAM might not be relative to the mechanism of ischemic preconditioning on myocardial protection because NCAM expression of infarct area was down-regulated in the direct group, while the infarct size of the same group was significantly decreased. It is possible that the up-regulation of NCAM may be impeded by the enhancement of ischemic preconditioning, which has the other cardioprotective mechanism on myocardial protection.

In conclusion, the direct ischemic preconditioning provides the myocardial protection for sustained ischemia while the remote ischemic preconditioning to the femoral artery shows no significant cardioprotective effect in rats. NCAM may be down-regulated in acutely stressed cardiomyocytes, and the protective role may not be associated with ischemic preconditioning.

Limitation

There are limitations to the present study that must be recognized. First, we

failed to demonstrate the protective effect of remote preconditioning although that of direct ischemic preconditioning was significant. This might result from small sample size in the rat study. Second, immunohistochemistry is qualitative or quasi-quantitative rather than quantitative, thus it is appropriate for localization of macromolecules in tissue or subcellular organization. For the precise quantitation of molecules, western blotting for protein, and northern blotting or quantitative RT-PCR for mRNA should be used together with tissue IHC. Third, the present animal model is an acute infarction model demonstrating biochemical alterations instead of identifiable morphological changes. In this regard, the current model and results might be somewhat different from those of previous models.

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Table 1. Infarct size data and group comparison

	Control group		Direct group		Remote group	
	Weight(g)	Infarct size	Weight(g)	Infarct size	Weight(g)	Infarct size
1	257	0.702	251	0.609	253	0.688
2	254	0.630	233	0.593	263	0.549
3	258	0.426	248	0.330	254	0.799
4	263	0.750	265	0.228	258	0.469
5	253	0.683	260	0.109	248	0.594
6	260	0.779	253	0.276	249	0.392
7	250	0.801	262	0.301	259	0.302
Mean		0.68±0.13*		0.35±0.19		0.54±0.17

Comparison of mean infarct size of myocardiums without ischemic preconditioning (IP) (control group), those with ischemic preconditining of LAD (direct group), ischemic preconditioned myocardiums of femoral artery (remote group).

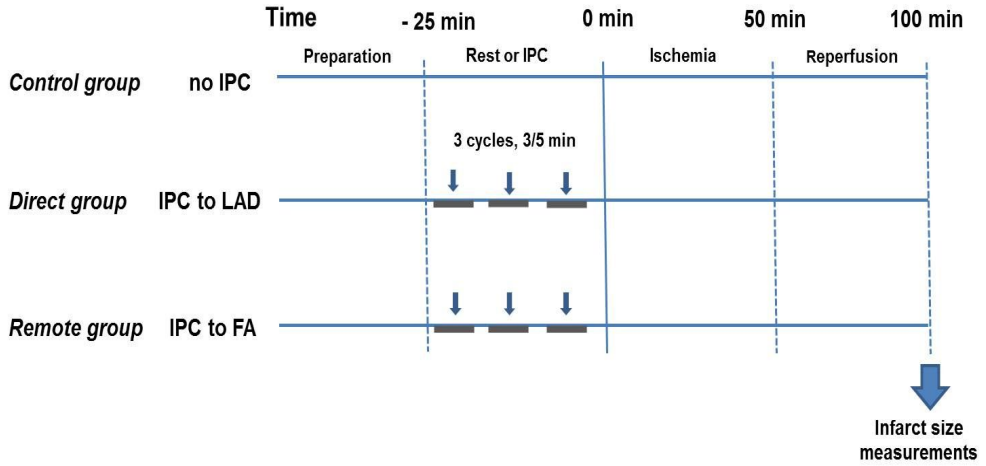
*Significant difference compared with the direct group (p =0.003).

Table 2. Expression level of CD 56 (NCAM) in infarct area and group comparison

	Control group	Direct group	Remote group
1	0.95	0.65	0.84
2	0.50	0.42	0.84
3	0.78	0.44	0.78
Mean	0.74 ± 0.23	0.50 ± 0.13	0.87 ± 0.35

Comparison of NCAM expression level in infarct area (Intensity of infarct area / intensity of reference area, measured by Image J) between three groups.

Figure 1. Diagram of experimental protocols and time-lines

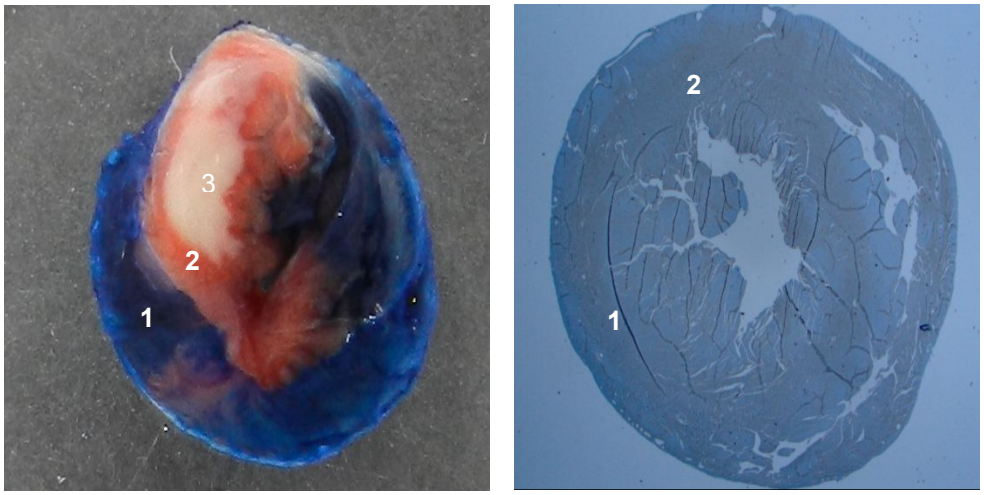


IPC = ischemic preconditioning, LAD = left anterior descending, FA = femoral artery

In all experiments, rats underwent 50 minutes of ischemia followed by 50 minutes of reperfusion after the rest (Control group) or ischemic preconditioning (IPC, Direct and Remote group) period. IPC was induced by three cycles of 3 minutes of occlusion and 5 minutes of reperfusion to left anterior descending coronary artery (LAD) or left femoral artery (FA). After then, heart was quickly excised and stained with 1% Evans blue and 2,3,5,-triphenyltetrazolium chloride (TTC) for infarct size measurement.

Figure 2. Measurement of infarct area and NCAM expression in myocardium using chemical and immunohistochemical staining

(A) (B)



1: Non-ischemia area

2: at risk area (red zone)

3: infarct area (white zone)

* Infarct size: white zone / (red + white zone)

1. Reference point (non-ischemia area)

2. Anterior wall (infarct area)

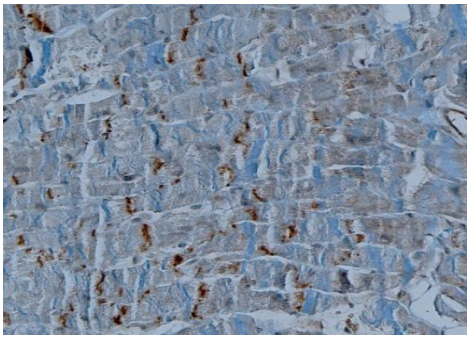
(A) Photograph obtained after staining with Evans blue and TTC of the infarcted heart subjected to ischemic preconditioning. Hearts were cut into 2mm of transverse slices and stained with TTC to differentiate infarct area (white zone) from at risk area (red zone). Infarct size was expressed as ratio of infarct area (white zone) to whole ischemic area (white plus red zone).

(B) Slice of tissue was sectioned in 4μm thickness and stained with anti-

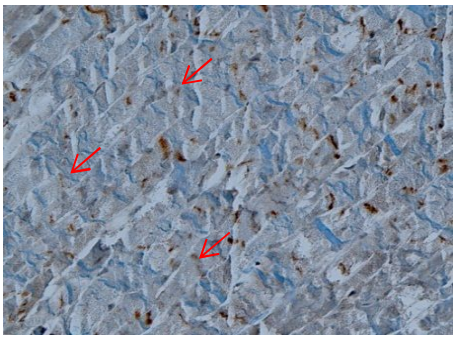
NCAM rabbit polyclonal antibody diluted to 1:50 in PBS. Level of NCAM protein expression was evaluated by comparing pixel counts using image analysis at corresponding areas marked in (B).

Figure 3. Immunohistochemical study of myocardium from different experimental groups using rabbit anti-rat NCAM antibody

1. Control group

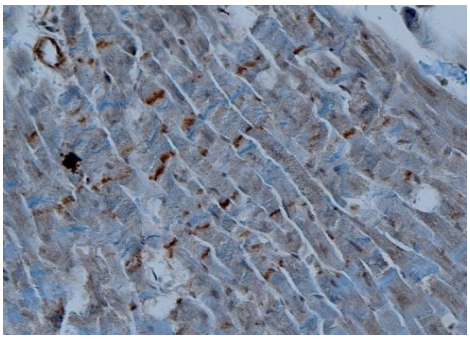


(A) Non-ischemia area (reference point)

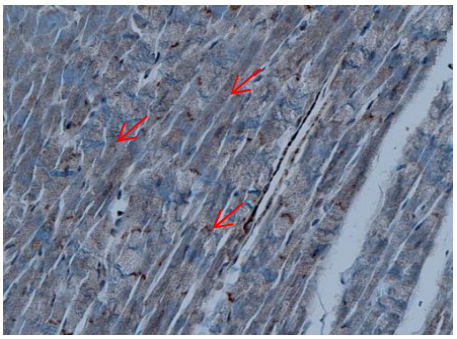


(B) Infarct area (anterior wall)

2. Direct group

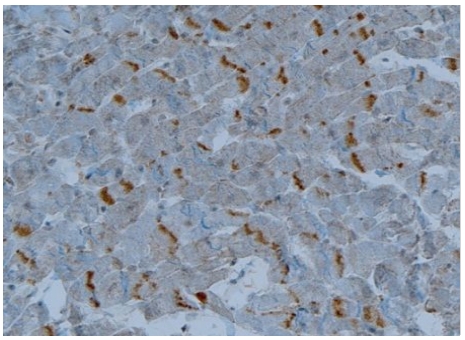


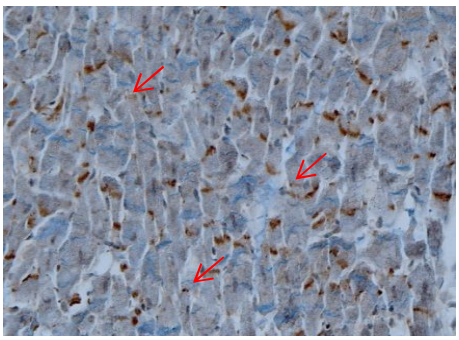
(A) Non-ischemia area (reference point)



(B) Infarct area (anterior wall)

3. Remote group





(A) Non-ischemia area (reference point) (B) Infarct area (anterior wall)

Intense expression of NCAM is noted in intercalated discs of ventricular myocardial cells. Staining intensity at the intercalated discs is mildly decreased and patch or incomplete staining pattern (arrow) is more frequent in anterior wall (infarct area, (A)) compared to reference point (non-ischemia area, (B)) in all groups. Representative figures are arranged in order of control, direct and remote groups from top to bottom row.

국문 초록

목적: 쥐 심장의 좌전하행지 관상동맥에 직접 허혈 전처치를 한 경우와 대퇴동맥에 원격 허혈 전처치를 한 경우에 좌전하행지 관상동맥 심근영역의 허혈에 대해 미치는 심근 보호 효과를 비교하고자 하였다. 그리고 심근 보호의 조절 인자로서 알려진 neural cell adhesion molecule (NCAM) 의 발현 정도를 확인하여, 급성 심근경색 하에서의 NCAM 의 심근 보호 효과 및 허혈 전처치와의 관련성, 그리고 허혈 전처치 방법에 따른 차이를 규명해 보고자 하였다.

방법: Sprague-Dawley (SD) 랫드를 이용하여, 대조군은 허혈 전처치 없이 좌전하행지 관상동맥을 50분간 결찰하여 심근 허혈을 유발하였고 (Control group, N=7), 실험군은 두 군으로 나누어 각 군의 해당 동맥에 3분의 허혈과 5분의 재관류를 3번씩 반복하여 허혈 전처치를 시행한 후 대조군과 마찬가지로 좌전하행지 관상동맥의 허혈을 유발하였다. 실험군은 좌전하행지 관상동맥에 직접 허혈 전처치를 시행하거나 (Direct group, N=7), 대퇴동맥에 원격 허혈 전처치를 시행하였다 (Remote group, N=7). 심장을 적출한 후 1% Evans blue 및 2,3,5-triphenyltetrazolium chloride (TTC) 염색을 시행하여 경색이 일어난 부위 (Infarct area) 와 경색 주변부위 (at risk area) 를 측정하여 그 비

율로 허혈 전처치의 효과를 평가하였다. 각 군당 3마리에서는 Neural cell adhesion molecule (NCAM; CD 56) 에 대한 항체를 이용하여 면역조직화학염색을 시행한 후 그 발현 정도를 비교하였다.

결과: 1% Evans blue 및 2,3,5-triphenyltetrazolium chloride (TTC) 염색에서 Direct group 의 경색부위 비율은 Control group 에 비해 유의하게 감소하였다 (P=0.003). Remote group 에서 경색 부위는 Control group 보다 감소하였으나 통계적으로 유의한 차이를 보이지 않았다 (P=0.269). 한편, Direct group 의 경색부위는 Remote group 에 비해 감소한 양상을 보였으나 두 군간 통계적으로는 유의한 차이는 관찰되지 않았다. 한편 CD 56 항체를 이용한 면역조직화학염색 결과, 경색부위의 NCAM 발현은 비경색 부위에 비해 감소하였고, 세 군간 비교에 있어 Direct group은 다른 두 군에 비해 NCAM 발현이 감소하였으나 유의한 차이를 보이지 않았다.

결론: 쥐에서 좌전하행지 관상동맥에의 직접 허혈 전처치는 허혈 전처치를 시행하지 않은 군에 비해 심근보호 효과가 있었으나, 대퇴동맥에의 원격 허혈 전처치는 쥐 심근경색 모델에서 유의한 보호 효과를 보이지 않았다. NCAM 발현은 급성 심근경색 상태에서는 감소하는 양상을 보였고 허혈전처치를 시행한 군들에서 유의한 차이를 보이지 않았다. 따라서 허혈 전처치 기전과는 다른 기전을 통해 작동할 것으로 추정된다.

주요어 : 심장, 허혈 전처치, 신경세포 유착분자

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