



Original Article

Brief pressure overload of the left ventricle reduces myocardial infarct size via activation of protein kinase C

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Abstract

Background: We have previously reported that brief pressure overload of the left ventricle reduced myocardial infarct (MI) size. However, the role of protein kinase C (PKC) remains uncertain. In this study, we investigated whether pressure overload reduces MI size by activating PKC.

Methods: MI was induced by a 40-minute occlusion of the left anterior descending coronary artery and a 3-hour reperfusion in anesthetized Sprague-Dawley rats. MI size was determined using triphenyl tetrazolium chloride staining. Brief pressure overload was achieved by two 10-minute partial snarings of the ascending aorta, raising the systolic left ventricular pressure 50% above the baseline value. Ischemic preconditioning was elicited by two 10-minute coronary artery occlusions and 10-minute reperfusion. Dimethyl sulfoxide (vehicle) or calphostin C (0.1 mg/kg, a specific inhibitor of PKC) was administered intravenously as pretreatment.

Results: The MI size, expressed as the percentage of the area at risk, was significantly reduced in the pressure overload group and the ischemic preconditioning group ($19.0 \pm 2.9\%$ and $18.7 \pm 3.0\%$ vs. $26.1 \pm 2.6\%$ in the control group, where $p < 0.001$). Pretreatment with calphostin C significantly limited the protection by pressure overload and ischemic preconditioning ($25.2 \pm 2.4\%$ and $25.0 \pm 2.3\%$, where $p < 0.001$). Calphostin C itself did not significantly affect MI size ($25.5 \pm 2.4\%$). Additionally, the hemodynamics, area at risk, and mortality were not significantly different.

Conclusion: Brief pressure overload of the left ventricle reduced MI size. Since calphostin C significantly limited the decrease of MI size, our results suggested that brief pressure overload reduces MI size via activation of PKC.

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Keywords: ischemic preconditioning; myocardial infarction; pressure overload; protein kinase C

1. Introduction

Transient preceding ischemia protects the myocardium against subsequent sustained ischemic insult and reduces

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myocardial infarct (MI) size.¹ The phenomenon of ischemic preconditioning also suppresses postischemic arrhythmia and improves myocardial recovery from stunning.^{2,3} These laboratory findings have been used to inform and enhance strategies to protect human myocardium in cardiac surgery.^{4–7} To avoid ischemic insult, several nonischemic maneuvers, including rapid cardiac pacing,⁸ heat stress,⁹ pharmacological administration,¹⁰ and volume overload,^{11,12} have been developed to mimic ischemic preconditioning and to protect myocardium.

The mechanisms of ischemic preconditioning have been extensively investigated. Activation of protein kinase C (PKC) has been reported to play an important role in ischemic preconditioning protection.^{12–15} Administration of PKC activators mimics ischemic preconditioning and decreases MI size.^{13,15} Some studies have demonstrated that pretreatment with PKC inhibitors significantly limits the MI size reduction caused by ischemic preconditioning.^{12–15} However, other studies have reported that administration of PKC inhibitors did not limit the MI size reduction, and did not support a role of PKC in ischemic preconditioning protection.^{16,17}

We previously reported that brief pressure overload of the left ventricle by two episodes of 10-minute partial snaring of the ascending aorta preconditioned myocardium and significantly reduced MI size.^{18–20} In our previous reports, we demonstrated that the protective effects of brief pressure overload is dependent on activation of stress-activated ion channels, and is independent on activation of adenosine receptors.^{18–20} We recently reported that inhibition of apoptosis plays an important role in the myocardial protection of left ventricular pressure overload.²¹ The role of PKC in the myocardial protection of brief pressure overload is not certain. Therefore, we conducted this study to investigate whether brief pressure overload reduces MI size via activation of PKC.

2. Methods

This study was approved by the Animal Experiment Committee of Taipei Veterans General Hospital, Taipei, Taiwan. Animals used in this study were cared for humanely in accordance with the “Guide for the Care and Use of Laboratory Animals” (National Academic Press, 1996).

2.1. Animal preparation

The techniques of animal preparation have been previously reported.^{22,23} Short male Sprague-Dawley rats (250–300 g) were anesthetized by intraperitoneal pentobarbiturate (30 mg/kg). After undergoing tracheotomy, each rat was intubated and ventilated with a ventilator (Harvard Apparatus, South Natick, MA, USA) with a tidal volume of 0.5–1.5 mL and a respiratory rate of 95–105 breaths per minute. The descending abdominal aorta was cannulated for arterial pressure monitoring. The arterial pressure was measured using a Statham P23 pressure transducer coupled to a pressure processor amplifier (Gould Instruments, Cleveland, OH, USA). Electrocardiography leads were placed on the limbs. After median sternotomy, the heart was exposed. A 4-0 silk suture was passed around the proximal part of the left anterior descending coronary artery. The ends of the silk suture were threaded through a small vinyl tube to form a snare. A ribbon tape was passed around the ascending aorta. A microtip-manometer (Millar Inc., Houston, TX, USA) was inserted into the left ventricle to measure the left ventricular pressure. The arterial pressure, heart rate, electrocardiography, and left ventricular pressure were recorded on a pressurized ink-chart recorder (Gould Instruments, Cleveland, OH, USA) and on a personal

computer with a waveform data analysis software program (AcqKnowledge; Biopac System, Goleta, CA, USA). The body temperature was monitored by a rectal thermometer and maintained at 37°C by heating pads throughout the experiment.

2.2. Experimental protocol

After hemodynamics were stable for 30 minutes, the rats were randomly allocated into six groups (Fig. 1). Group 1 received no intervention except a 10-minute intravenous infusion of 10% dimethyl sulfoxide (DMSO, 0.1 mL/kg, vehicle; control group). Group 2 received a 10-minute intravenous infusion of DMSO followed by brief left ventricular pressure overload via partial snaring of the ribbon tape around the ascending aorta to raise the systolic left ventricular pressure 50% above the baseline value for 10 minutes. Two episodes of this pressure overload were conducted (LVPO group). Group 3 received a 10-minute intravenous infusion of DMSO followed by ischemic preconditioning via two episodes of 10-minute coronary artery occlusion and 10-minute reperfusion (IPC group). Group 4 received a 10-minute intravenous infusion of calphostin C (0.1 mg/kg, Kyowa Medex, Tokyo, Japan), a specific inhibitor of PKC,²⁴ 10 minutes before left ventricular pressure overload (CPS-LVPO group). Group 5 received a 10-minute intravenous infusion of calphostin C 10 minutes before ischemic preconditioning (CPS-IPC group). Group 6 received an intravenous infusion of calphostin C for 10 minutes (CPS-control group). Ten minutes after the above treatments, an episode of 40-minute coronary artery occlusion was conducted by pulling the snare around the left anterior descending coronary artery, which was then fixed by clamping the vinyl tube with a mosquito clamp. The performance of successful occlusion was verified by observing the development of ST-segment elevation and changes in the QRS complex on the electrocardiography, and cyanotic change of

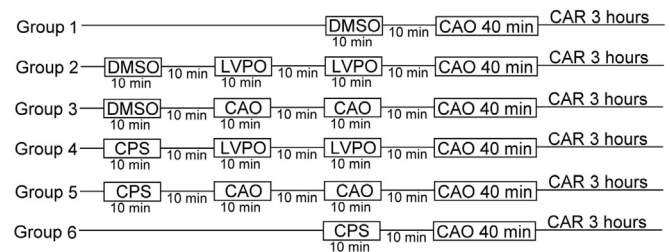


Fig. 1. The experimental protocol. All rats underwent a 40-minute coronary artery occlusion (CAO) and 3-hour reperfusion (CAR) after different treatments. In the treatment period, Group 1 received no pretreatment except a 10-minute intravenous infusion of 10% DMSO (dimethyl sulfoxide, vehicle). Group 2 received two episodes of 10-minute left ventricular pressure overload (LVPO) by partial snaring of the ascending aorta after infusion of DMSO. Group 3 received infusion of DMSO followed by ischemic preconditioning by two episodes of 10-minute coronary artery occlusion and 10-minute reperfusion. Calphostin C (CPS), a specific inhibitor of protein kinase C, was intravenously administered in the remaining three groups. Then, Group 4 received left ventricular pressure overload, Group 5 received ischemic preconditioning, and Group 6 received no other pretreatment.

myocardium in the occluded area. After the 40-minute coronary artery occlusion, the snare was released for reperfusion for 3 hours. Reperfusion was confirmed by refilling the coronary artery and visualizing a reactive hyperemic response. No anti-arrhythmic agents were given at any time. The arterial pressure, heart rate, electrocardiography, and the left ventricular pressure were recorded simultaneously and continuously throughout the experiments, including baseline (before treatment), treatment period, 40-minute coronary artery occlusion, and 3-hour reperfusion.

2.3. Determination of area at risk and MI size

The methods for determination of area at risk (AAR) and MI size have been previously reported.^{25,26} At the end of the experiment, 1000 units of heparin were administered. The heart was rapidly excised and placed on a perfusion apparatus. The left anterior descending coronary artery was ligated at the site of previous occlusion. The ascending aorta was cannulated (distal to the sinus of Valsalva) and perfused with 1% Evans blue dye (Sigma-Aldrich, St. Louis, MO, USA). After perfusion was completed, the atria and right ventricular free wall were removed. The left ventricle plus septum was then cut into six to seven transverse slices, which were incubated at 37°C in 1% triphenyl tetrazolium chloride solution in phosphate buffer (pH = 7.4) for 20 minutes to visualize MI (area unstained using triphenyl tetrazolium chloride). The slices were weighed and fixed in a 10% formalin solution for 24 hours. The basal surfaces were photographed. Images were traced with a digitizer to calculate both the AAR, identified by Evans blue dye exclusion, and the MI area. The AAR and MI area of each heart slice were calculated from planimetry. The percentage of area of AAR and MI in each heart slice was then multiplied by the weight of each slice to determine the weights of AAR and MI in that heart slice. Total weights of AAR and MI were then calculated and summed for the entire left ventricle plus septum. AAR was reported as the percentage of left ventricle plus septum, with MI size as the percentage of AAR.

2.4. Chemicals

Calphostin C (Kyowa Medex, Tokyo, Japan) is a specific inhibitor of PKC.²⁴ It was dissolved in DMSO at a concentration of 2 mg/mL, and the solution was diluted with sterilized water to the concentration of 0.1 mg/mL before use.

2.5. Exclusion criteria

Rats with AAR <10% of the left ventricular weight and rats exhibiting S-T segment shift on the electrocardiography during pressure overload period were excluded.

2.6. Statistical analysis

All values were expressed as mean value \pm standard deviation. Hemodynamic variables were analyzed using a one-way analysis of variance with repeated measures. The differences

in AAR and MI size among groups were analyzed using a one-way analysis of variance. If the analysis of variance results were significant, a multiple comparison using a post-hoc Bonferroni test was made to assess which group was significantly different. The analysis of covariance was performed to evaluate the group difference of MI (expressed as the percentage of the left ventricular weight) with AAR (expressed as the percentage of the left ventricular weight) as covariates. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Mortality and exclusions

Fifty-five rats were used in this study (Table 1). One rat in Group 1 suffered from ventricular fibrillation and died. One rat in Group 5 suffered from bleeding from the sternum and hypotension during sternotomy. One rat in Group 6 died of heart failure, which was defined as a progressive decrease of the systolic arterial pressure <50 mmHg with global left ventricular dilatation and poor contraction. The data for these three rats were excluded. The mortality rate among the six groups was not significantly different ($p = 0.688$).

3.2. Characteristics of brief pressure overload

During pressure overload, the systolic left ventricular pressure increased significantly from 107 ± 9 mmHg to 157 ± 13 mmHg ($p < 0.001$), and the systolic arterial pressure decreased significantly from 107 ± 9 mmHg to 75 ± 5 mmHg ($p < 0.001$) in Group 2 (Table 2). In Group 4, the systolic left ventricular pressure increased significantly from 108 ± 9 mmHg to 160 ± 15 mmHg ($p < 0.001$), whereas the systolic arterial pressure decreased significantly from 108 ± 9 mmHg to 78 ± 10 mmHg ($p < 0.001$) during pressure overload. Thus, the systolic left ventricular pressure was raised 50% above the baseline value during the pressure overload period, causing obvious pressure overload of the left ventricle in Group 2 and Group 4. There was no significant change of heart rate during the pressure overload period. After brief pressure overload, the left ventricular pressure, the arterial pressure and the heart rate returned to baseline levels.

Table 1
The mortality and exclusion data.

| Group | Treatment protocol | No. | Mortality* | | | No. included |
|-------|--------------------|-----|------------|----|-------|--------------|
| | | | VF | HF | Other | |
| 1 | Control | 9 | 1 | 0 | 0 | 8 |
| 2 | LVPO | 9 | 0 | 0 | 0 | 9 |
| 3 | IPC | 9 | 0 | 0 | 0 | 9 |
| 4 | CPS-LVPO | 9 | 0 | 0 | 0 | 9 |
| 5 | CPS-IPC | 10 | 0 | 0 | 1 | 9 |
| 6 | CPS-Control | 9 | 0 | 1 | 0 | 8 |

* There was no significant difference in the mortality rate among the six groups. Pearson Chi-square $p = 0.688$.

CPS = calphostin C; HF = heart failure; IPC = ischemic preconditioning; LVPO = left ventricular pressure overload; VF = ventricular fibrillation.

Table 2
Hemodynamic changes during pressure overload of the left ventricle.

| Hemodynamics | Baseline 1 | LVPO | Baseline 2 | <i>p</i> |
|----------------------------------|------------|------------|------------|----------|
| Group 2 (LVPO), <i>n</i> = 9 | | | | |
| MAP (mmHg) | 76 ± 7 | 64 ± 6* | 77 ± 5 | <0.001 |
| SAP (mmHg) | 107 ± 9 | 75 ± 5** | 109 ± 9 | <0.001 |
| SLVP (mmHg) | 107 ± 9 | 157 ± 13** | 109 ± 9 | <0.001 |
| HR (beats/min) | 429 ± 73 | 424 ± 55 | 447 ± 56 | 0.705 |
| Group 4 (CPS-LVPO), <i>n</i> = 9 | | | | |
| MAP (mmHg) | 79 ± 6 | 65 ± 6*** | 80 ± 6 | <0.01 |
| SAP (mmHg) | 108 ± 9 | 78 ± 10** | 107 ± 11 | <0.001 |
| SLVP (mmHg) | 108 ± 9 | 160 ± 15** | 107 ± 11 | <0.001 |
| HR (beats/min) | 412 ± 72 | 427 ± 66 | 438 ± 66 | 0.726 |

Data are presented as mean ± standard deviation. There were significant changes in MAP, SAP, and SLVP during pressure overload.

* Multiple comparison *p* < 0.01, as compared with baseline 1.

** Multiple comparison *p* < 0.001, as compared with baseline 1.

*** Multiple comparison *p* < 0.05, as compared with baseline 1. There was no significant change in HR.

Baseline 1 = baseline before left ventricular pressure overload; Baseline 2 = baseline after left ventricular pressure overload; CPS = calphostin C; HR = heart rate; LVPO = left ventricular pressure overload; MAP = mean arterial pressure; SAP = systolic arterial pressure; SLVP = systolic left ventricular pressure.

3.3. Hemodynamic changes during the experiments

The baseline arterial pressures, heart rates, and mean arterial pressure-heart rate products of the six groups were not significantly different (Table 3). Treatments before 40-minute coronary artery occlusion did not elicit any significant

hemodynamic changes. There was no significant change in the arterial pressure and heart rate throughout the experiments, except during pressure overload of the left ventricle. The hemodynamic variables among the six groups were not significantly different during the experiments.

3.4. AAR and MI size analysis

The AAR values, expressed as the percentage of left ventricular weight, of the six groups averaged 50.2 ± 2.6% (control group), 52.5 ± 3.0% (LVPO group), 51.5 ± 3.4% (IPC group), 52.8 ± 3.1% (CPS-LVPO group), 51.0 ± 3.3% (CPS-IPC), and 52.8 ± 2.9% (CPS-control group) (Fig. 2). These AAR values were not significantly different among the six groups (*p* = 0.416). MI size, expressed as the percentage of AAR, was 26.1 ± 2.6% in Group 1 (Fig. 2). Pressure overload significantly reduced MI size in Group 2 (19.0 ± 2.9%, *p* < 0.001). Ischemic preconditioning also significantly decreased MI size in Group 3 (18.7 ± 3.0%, *p* < 0.001). Administration of calphostin C significantly limited the reduction of MI size induced by pressure overload in Group 4 (25.2 ± 2.4%, *p* < 0.001 vs. Group 2). The MI size reduction by ischemic preconditioning was also significantly decreased by pretreatment with calphostin C in Group 5 (25.0 ± 2.3%, *p* < 0.001 vs. group 3). Administration of calphostin C itself did not significantly affect MI size in Group 6 (25.5 ± 2.4%, *p* = 0.792 vs. Group 1).

Since AAR is the main determinant of MI size,²⁷ we performed analysis of covariance with MI size (expressed as

Table 3
Hemodynamic changes during the experiments.

| Group | Treatment protocol | No. | Baseline 1 | Baseline 2 | CAO | | | CAR | |
|---------------------------|--------------------|-----|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| | | | | | 20 min | 40 min | 1 h | 2 h | 3 h |
| MAP (mmHg) | | | | | | | | | |
| 1 | Control | 8 | 73 ± 6 | 74 ± 7 | 68 ± 5 | 73 ± 5 | 73 ± 7 | 74 ± 7 | 74 ± 7 |
| 2 | LVPO | 9 | 76 ± 7 | 77 ± 5 | 73 ± 6 | 73 ± 7 | 76 ± 7 | 75 ± 6 | 73 ± 7 |
| 3 | IPC | 9 | 76 ± 10 | 75 ± 8 | 68 ± 7 | 77 ± 11 | 77 ± 10 | 76 ± 8 | 73 ± 10 |
| 4 | CPS-LVPO | 9 | 76 ± 9 | 79 ± 7 | 73 ± 4 | 77 ± 6 | 75 ± 3 | 78 ± 4 | 79 ± 11 |
| 5 | CPS-IPC | 9 | 78 ± 6 | 76 ± 3 | 73 ± 4 | 77 ± 7 | 79 ± 7 | 79 ± 9 | 75 ± 4 |
| 6 | CPS-Control | 8 | 75 ± 7 | 77 ± 7 | 69 ± 5 | 78 ± 7 | 76 ± 5 | 77 ± 3 | 73 ± 3 |
| HR (beats/min) | | | | | | | | | |
| 1 | Control | 8 | 429 ± 42 | 439 ± 47 | 424 ± 55 | 431 ± 43 | 449 ± 37 | 452 ± 61 | 449 ± 39 |
| 2 | LVPO | 9 | 430 ± 73 | 447 ± 56 | 448 ± 65 | 438 ± 54 | 436 ± 58 | 428 ± 68 | 435 ± 70 |
| 3 | IPC | 9 | 423 ± 43 | 444 ± 46 | 435 ± 42 | 442 ± 58 | 457 ± 39 | 451 ± 52 | 439 ± 38 |
| 4 | CPS-LVPO | 9 | 419 ± 63 | 443 ± 63 | 436 ± 72 | 444 ± 64 | 428 ± 42 | 445 ± 63 | 430 ± 66 |
| 5 | CPS-IPC | 9 | 454 ± 46 | 455 ± 42 | 446 ± 44 | 449 ± 44 | 460 ± 31 | 461 ± 41 | 454 ± 42 |
| 6 | CPS-Control | 8 | 425 ± 45 | 433 ± 58 | 436 ± 68 | 455 ± 61 | 453 ± 58 | 432 ± 69 | 433 ± 67 |
| PRP (mmHg beats/min/1000) | | | | | | | | | |
| 1 | Control | 8 | 31.12 ± 4.36 | 32.72 ± 5.42 | 28.95 ± 4.90 | 31.51 ± 3.79 | 32.52 ± 4.13 | 33.52 ± 6.38 | 33.42 ± 4.44 |
| 2 | LVPO | 9 | 32.30 ± 5.44 | 34.45 ± 4.28 | 32.72 ± 4.77 | 31.85 ± 4.72 | 33.00 ± 3.73 | 32.04 ± 5.05 | 31.67 ± 5.96 |
| 3 | IPC | 9 | 31.94 ± 5.66 | 33.50 ± 5.95 | 29.77 ± 4.47 | 34.07 ± 7.84 | 35.06 ± 5.38 | 33.99 ± 4.45 | 31.93 ± 4.64 |
| 4 | CPS-LVPO | 9 | 32.14 ± 7.19 | 35.34 ± 6.72 | 31.73 ± 5.34 | 34.02 ± 5.19 | 32.05 ± 2.42 | 34.92 ± 5.79 | 34.28 ± 7.47 |
| 5 | CPS-IPC | 9 | 35.29 ± 4.14 | 34.55 ± 2.86 | 32.41 ± 2.96 | 34.44 ± 4.88 | 36.18 ± 2.12 | 36.28 ± 4.72 | 34.00 ± 3.08 |
| 6 | CPS-Control | 8 | 31.87 ± 4.82 | 33.46 ± 6.68 | 30.49 ± 6.42 | 35.39 ± 6.84 | 34.39 ± 4.07 | 33.13 ± 5.12 | 31.29 ± 4.52 |

Data are presented as mean ± standard deviation. There were no significant changes in MAP, HR, and PRP throughout the experiment. There were no significant differences in hemodynamic variables among the six groups of animals during experiments.

Baseline 1 = baseline before treatment; Baseline 2 = baseline after treatment; CAO = coronary artery occlusion; CAR = coronary artery reperfusion; CPS = calphostin C; HR = heart rate; IPC = ischemic preconditioning; LVPO = left ventricular pressure overload; MAP = mean arterial pressure; PRP = mean arterial pressure-heart rate product.

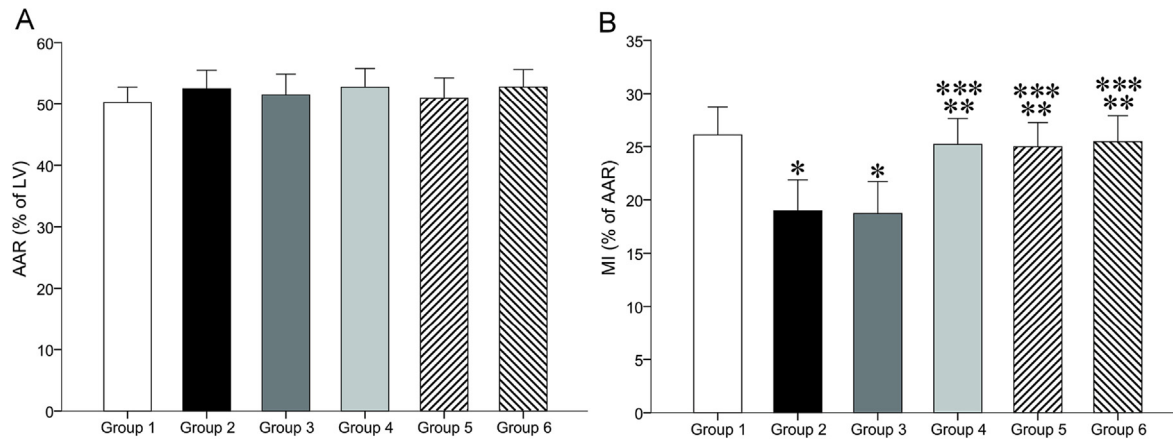


Fig. 2. (A) Size of area at risk (AAR), expressed as a percentage of the left ventricle (LV); (B) size of myocardial infarct (MI), expressed as a percentage of AAR. * $p < 0.001$ versus Group 1. ** $p < 0.001$ versus Group 2. *** $p < 0.001$ versus Group 3.

the percentage of left ventricular weight) as the dependent variable and AAR (expressed as the percentage of left ventricular weight) as a covariate to determine the effect of AAR on MI size. The data points and the regression line of each group are shown in Fig. 3. After adjusting MI for AAR, analysis of covariance showed that the regression lines for MI/LV for the control group, CPS-LVPO group, CPS-IPC group, and CPS-control group were not significantly different. However, the regression lines for MI/LV for the LVPO group and the IPC group were significantly different from those of the control group, CPS-LVPO group, CPS-IPC group, and CPS-control group ($p < 0.001$). The results demonstrated that pressure overload and ischemic preconditioning reduced MI size and that the protective effects of pressure overload and ischemic preconditioning were dependent upon activation of PKC.

4. Discussion

In the current study, we demonstrated that brief pressure overload of the left ventricle by two episodes of partial snaring of the ascending aorta, raising the systolic left ventricular pressure 50% above the baseline value, significantly reduced MI size in anesthetized rats. The myocardial protective effects were comparable to those of ischemic preconditioning (Fig. 2). There were no significant hemodynamic changes during the experiments (Table 3), so the reduction of MI size could not be ascribed to hemodynamic changes. Although the underlying mechanisms are not certain, administration of calphostin C, a specific inhibitor of PKC,²⁴ significantly limited the MI size reduction. Our results suggested that brief left ventricular pressure overload might reduce MI size via activation of PKC.

PKC has been reported to play an important role in myocardial protection.^{12–15} It has been shown that ischemic preconditioning activates PKC by translocation of inactive cytosolic PKC to particulate fractions, where the kinase activity of PKC occurs.^{28–30} Administration of PKC activators mimics ischemic preconditioning and reduces MI size in rabbits and rats.^{13,15} Pretreatment with PKC inhibitors

prevents the protection induced by ischemic preconditioning.^{12–15} However, Przyklenk et al¹⁶ reported that PKC inhibitors did not prevent the MI size reduction by ischemic preconditioning, and that ischemic preconditioning did not to induce translocation of PKC in dogs. Vahlhaus et al¹⁷ demonstrated that PKC inhibitors did not prevent ischemic preconditioning protection in swine. Although there are discrepancies among the effects of PKC inhibitors on MI size reduction by ischemic preconditioning, our results

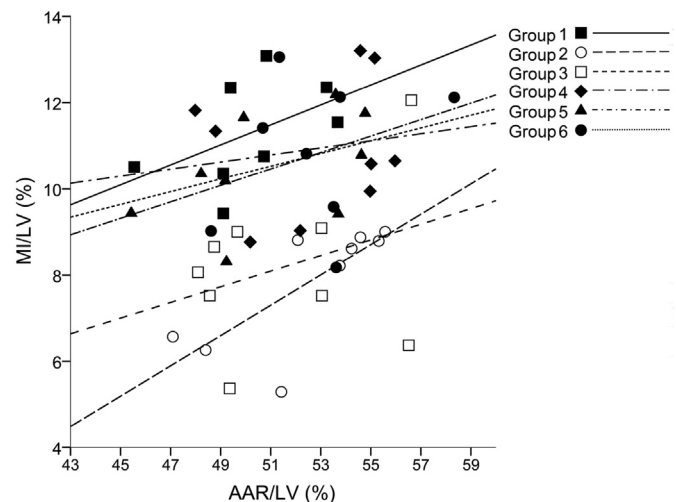


Fig. 3. Myocardial infarct (MI) size was plotted as a function of area at risk (AAR). Both MI and AAR were expressed as the percentage of left ventricular (LV) weight. “—”, regression line of Group 1 (control group, “■”, “MI/LV = $-0.33+0.23 \cdot \text{AAR/LV}$ ”, $p = 0.226$, $R^2 = 0.23$). “- - -”, regression line of Group 2 (LVPO group, “○”, “MI/LV = $-10.64+0.35 \cdot \text{AAR/LV}$ ”, $p = 0.017$, $R^2 = 0.58$). “· · · · ·”, regression line of Group 3 (IPC group, “□”, “MI/LV = $-1.16+0.18 \cdot \text{AAR/LV}$ ”, $p = 0.395$, $R^2 = 0.10$). “- · - · -”, regression line of Group 4 (CPS-LVPO group, “◆”, “MI/LV = $6.60+0.08 \cdot \text{AAR/LV}$ ”, $p = 0.684$, $R^2 = 0.03$). “- - - - -”, regression line of Group 5 (CPS-IPC group, “▲”, “MI/LV = $0.74+0.19 \cdot \text{AAR/LV}$ ”, $p = 0.175$, $R^2 = 0.25$). “- - - - -”, regression line of Group 6 (CPS-control group, “●”, “MI/LV = $2.99+0.15 \cdot \text{AAR/LV}$ ”, $p = 0.557$, $R^2 = 0.06$). The slopes of the regression equations of the six groups were not significantly different.

supported a significant role of PKC activation in the myocardial protection by brief pressure overload.

We previously reported that administration of gadolinium, a blocker of stretch-activated ion channels, significantly limits the MI size reduction by brief pressure overload and ischemic preconditioning. Both brief pressure overload and ischemic preconditioning may share activation of stretch-activated ion channels as common mechanisms.¹⁹ It has been shown that ischemic preconditioning protects the myocardium via activation of adenosine receptors.^{10,12,18,20} We demonstrated that brief pressure overload reduces MI size independent of activation of adenosine receptors in rabbits and rats.^{18,20} Brief pressure overload might protect the myocardium through some pathways different from ischemic preconditioning. Although PKC plays an important role in ischemic preconditioning,^{12–15} the role of PKC in brief pressure overload protection is not fully understood. In this study, we demonstrated that brief pressure overload reduces MI size dependent on activation of PKC.

In contrast to our finding that activation of PKC mediates myocardial protection by brief left ventricular pressure overload, activation of PKC has been reported to contribute to hypertrophy and heart failure after chronic pressure overload.³¹ Bayer et al³² reported that supra-renal abdominal aorta constriction in rats induced hypertension, left ventricular hypertension, and heart failure over time. Increased expression and phosphorylation of various PKC isoenzymes were found. Liu et al³³ reported that transgenic PKC α -deletion mice were less susceptible to heart failure following chronic pressure overload induced by transverse aortic arch constriction. They also demonstrated that administration of a PKC inhibitor, ruboxistaurin, prevented death in wild-type mice throughout 10 weeks of chronic pressure overload. Reduced ventricular dilation, enhanced ventricular performance, reduced fibrosis, and decreased pulmonary edema were also found. Accordingly, they suggested that PKC inhibitors might represent a novel therapeutic approach in treating heart failure.

Brief myocardial stretch has been shown to induce myocardial protection. We stretched myocardium by pressure overload of the left ventricle in this study. Ovize et al¹¹ reported that rapid infusion of normal saline, which they described as “volume overload,” significantly reduced MI size. Gysembergh et al¹² demonstrated that infusion of a PKC inhibitor significantly decreased the MI size reduction by volume overload. In the current study, we demonstrated that calphostin C, a specific inhibitor of PKC, significantly limited the reduction of MI size induced by pressure overload. Activation of PKC may mediate the myocardial protective effects of both pressure overload and volume overload.

There were limitations in this study. Firstly, there were several PKC isoforms,^{34,35} and the effects of specific PKC isoforms were not examined in our study. Calphostin C, a specific inhibitor of PKC,²⁴ was used in this study. However, the expression of PKC itself was not examined in this study. The effects of pressure overload and calphostin C on myocardial blood flow were not determined. The performance of successful coronary artery occlusions was verified by

observing the development of ST-segment elevation and changes in the QRS complex on electrocardiography, and cyanotic change of myocardium in the occluded area. No definite myocardial blood flow data were collected. However, rats have a limited collateral coronary circulation,³⁶ and our model is comparable to that widely used in experiments on myocardial ischemia and preconditioning.^{2,9,14,15,20,21,25–27,29,30,32} The current study was conducted on anesthetized, open-chest rats. The acute effects of anesthesia and surgery should be taken into account when interpreting the results.³⁷ Although triphenyl tetrazolium chloride staining technique is commonly used to determine MI size, the infarct size might have been underestimated.³⁸

In conclusion, brief pressure overload of the left ventricle significantly reduced MI size. The MI size reduction was significantly limited by administration of calphostin C, a specific PKC inhibitor. Our results suggested that brief pressure overload might reduce MI size via activation of PKC. Further studies are required to investigate the underlying mechanisms and signal transduction pathways involved.

Acknowledgments

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