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Original Article

Preconditioning threshold of brief pressure overload of the left ventricle

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Abstract

Background: We previously reported that pressure overload of the left ventricle reduced myocardial infarct (MI) size in rabbits. The threshold of pressure overload was investigated in this study.

Methods: Pressure overload of the left ventricle was induced by partial snare of the ascending aorta in anesthetized, open-chest rabbits. Systolic left ventricular pressure (SLVP) was elevated 50% or 30% above baseline value by varying the degree of partial snaring. Different duration of pressure overload, including 10 minutes, 5 minutes, 3 minutes, or 2 minutes, was applied to determine the threshold of protective effects. Ischemic preconditioning was elicited by two 10-minute coronary artery occlusions and reperfusions. Ten minutes after different pretreatment, 1 hour occlusion of the left anterior descending coronary artery followed by 3 hours reperfusion was done to induce MI. The size of area at risk and MI were determined by blue dye injection and triphenyl tetrazolium chloride staining after experiments.

Results: Pressure overload increase of SLVP 50% above baseline value for 10 minutes, 5 minutes, and 3 minutes significantly reduced MI size ($18.5 \pm 3.6\%$, $21.4 \pm 1.9\%$ and $21.6 \pm 1.7\%$, respectively, vs. $26.6 \pm 1.0\%$ in the control group, mean $\pm$ standard deviation, $p < 0.01$). A 30% increase of SLVP by pressure overload for 10 minutes, 5 minutes and 3 minutes also significantly decreased MI size ($20.5 \pm 2.5\%$, $21.6 \pm 2.3\%$, and $21.5 \pm 2.3\%$, $p < 0.01$). Ischemic preconditioning significantly decreased MI size ($19.9 \pm 2.8\%$, $p < 0.001$). Pressure overload to elevate SLVP 50% or 30% above baseline value for 2 minutes did not significantly alter MI size ($25.0 \pm 2.3\%$ and $26.0 \pm 1.7\%$, $p = 0.122$ and $p = 0.457$). Two episodes of 2 minutes pressure overload did not significantly decrease MI size ($25.0 \pm 2.2\%$ and $25.5 \pm 2.2\%$, $p = 0.118$ and $p = 0.281$). The hemodynamics, area at risk, and mortality were not significantly different among all groups of animals.

Conclusion: Pressure overload to raise SLVP either 50% or 30% above baseline value reduced MI size. A minimum duration of 3 minutes was necessary to induce the protective effects.

Keywords: ischemic preconditioning; left ventricular pressure overload; myocardial infarction; rabbits

1. Introduction

Brief reversible ischemia by short durations of coronary artery occlusion and reperfusion render myocardium resistant to subsequent prolonged ischemic insult and decreased myocardial infarct (MI) size.[1] Although the phenomenon of ischemic preconditioning was first found in dogs,[1] it was also found in other animals, including rabbits,[2,3] rats,[4] sheep,[5] and pigs.[6–8] This experimental finding was also reported in clinical care of patients.[9] In addition to limiting MI size, ischemic preconditioning attenuates arrhythmia[10] and improves recovery from postischemic myocardial stunning.[8] To avoid the ischemic insult, nonischemic stimuli, including rapid cardiac

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pacing,[11] heat stress,[12] pharmacological administration,[6] and myocardial stretch,[2,3,13] were designed to mimic preconditioning effects and limit MI size.

The minimum duration and number of cycles of coronary artery occlusion required to induce ischemic preconditioning is not certain. Schulz et al reported that ischemic preconditioning by 3 minutes low-flow ischemia followed by 15 minutes reperfusion significantly reduced MI size in swine.[8] Yao and Gross showed that 3 minutes ischemia did not limit MI size in dogs.[14] Li et al demonstrated that preconditioning with one episode of 5 minutes ischemia followed by 10 minutes reperfusion was as effective as preconditioning with 6 and 12 episodes in dogs.[15] However, more effective protection against MI by three cycles of 5 minutes ischemia and 10 minutes reperfusion than a single cycle was reported in rabbits by Sandhu et al.[16]

We previously demonstrated that brief pressure overload of the left ventricle by two episodes of 10 minutes partial snaring of the ascending aorta reduced MI size in anesthetized, open-chest rabbits.[2,3] In our previous studies, we induced pressure overload of the left ventricle by partial snaring of the ascending aorta to raise the systolic left ventricular pressure (SLVP) 50% above baseline. It is not certain if milder pressure overload by less elevation of SLVP reduces MI size or not. The minimum duration of pressure overload is not known.

Therefore, the purpose of this study is to determine the preconditioning threshold of pressure overload of the left ventricle. Different degrees of pressure overload were induced by raising SLVP either 50% or 30% above baseline. Different duration of pressure overload, including 10 minutes, 5 minutes, 3 minutes, or 2 minutes, was applied to determine the threshold of protective effects.

2. Methods

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

2.1. Animal preparation

The methods for animal preparation have been reported previously.[17,18] New Zealand White rabbits (body weight 2.3 ± 0.4 kg) were anesthetized by pentobarbiturate (30 mg/kg, intravenous) and maintained by 5 mg/kg/h infusion. Body temperature was maintained at 37°C with a heating pad. After tracheotomy, animals were intubated and ventilated with a ventilator (Harvard Apparatus, Natick, MA, USA). Femoral artery was cannulated for arterial pressure monitoring. 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 limbs. A median sternotomy was performed. The heart was exposed. A 2-0 silk suture was passed around the proximal left anterior descending coronary artery. The ends of the silk suture were threaded through a small vinyl tube to form a snare. Meticulous dissection of the ascending aorta was performed. A ribbon tape was passed around the ascending aorta distal to the orifices of the coronary arteries. A microtip-manometer (Millar, Houston, TX, USA) was inserted into the left ventricle to measure pressure. The first derivative of left ventricular pressure (dP/dt) was obtained by electronic differentiation using a differentiator (Gould Instruments). Arterial pressure, heart rate, electrocardiography, left ventricular pressure, and dP/dt were recorded on a pressurized ink-chart recorder (Gould Instruments) and on a personal computer with waveform data analysis software (AcqKnowledge; Biopac System, Goleta, CA, USA).

2.2. Experimental protocol

After hemodynamics were stable for 30 minutes, rabbits were randomly allocated to 12 groups. Fig. 1 showed the experimental protocol. Group 1 received no intervention (control group) and Group 2 received ischemic preconditioning by two episodes of 10 minutes coronary artery occlusion (ischemic preconditioning group). Group 3 received brief pressure overload of the left ventricle by partial snaring of the ribbon tape around the ascending aorta to increase SLVP 50% above the baseline value for 10 minutes (50% LVPO, 10-min group). Group 4 received pressure overload to increase SLVP 50% above the baseline value for 5 minutes (50% LVPO, 5-min group). Group 5 received pressure overload to increase SLVP 50% above the baseline value for 3 minutes (50% LVPO, 3-min group). Group 6 received pressure overload to increase SLVP 50% above the baseline value for 2 minutes (50% LVPO, 2-min group). Group 7 received pressure overload to increase SLVP 50% above the baseline value for 2 minutes. Two episodes of pressure overload were done (50% LVPO, 2-min*2 group). Group 8 received pressure overload to increase SLVP 30% above the baseline value for 10 minutes (30% LVPO, 10-min group). Group 9 received pressure overload to increase SLVP 30% above the baseline value for 5 minutes (30% LVPO, 5-min group). Group 10 received pressure overload to increase SLVP 30% above the baseline value for 3 minutes (30% LVPO, 3-min group). Group 11 received pressure overload to increase SLVP 30% above the baseline value for 2 minutes (30% LVPO, 2-min group). Group 12 received pressure overload to increase SLVP 30% above the baseline value for 2 minutes. Two episodes of pressure overload were done (30% LVPO, 2-min*2 group). Ten minutes after above treatments, 1 hour coronary artery occlusion was applied 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 development of ST-segment elevation and changes in the QRS complex on electrocardiography, and cyanotic change in the myocardium in the occluded area. After 1 hour coronary artery occlusion, the snare was released for reperfusion for 3 hours. No antiarrhythmic agents were given at any time. Arterial pressure, heart rate, electrocardiography, LVP, and dP/dt were recorded simultaneously and continuously throughout the experiments,

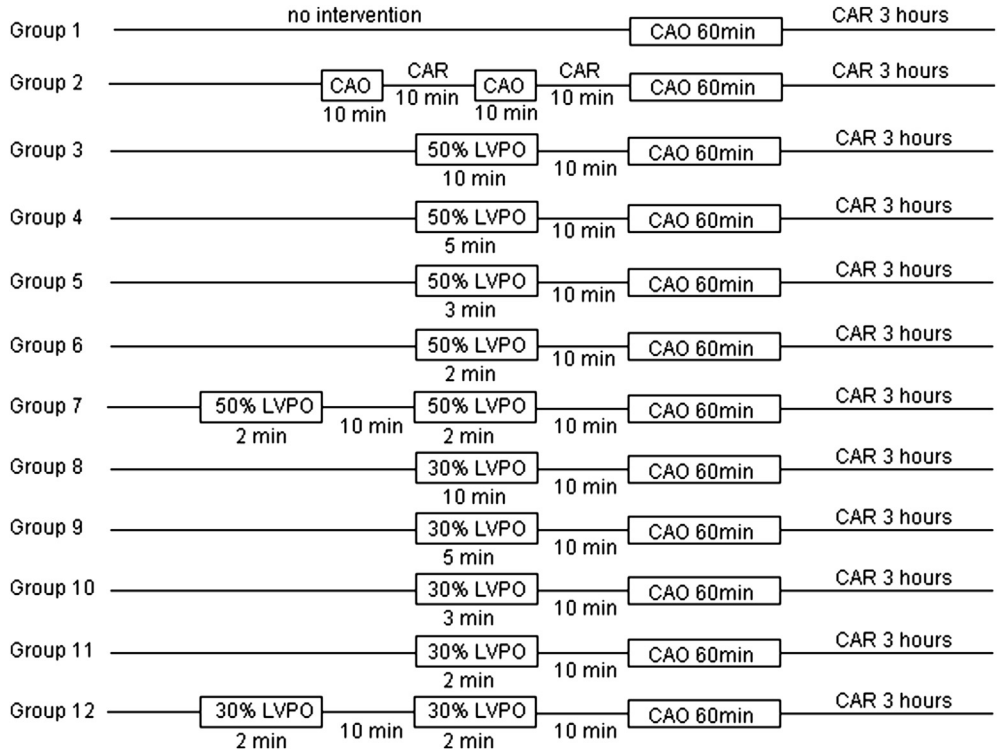

Fig. 1. Experimental protocol. All rabbits underwent 1 hour coronary artery occlusion (CAO) and 3 hours coronary artery reperfusion (CAR) after different treatments. In the treatment period, Group 1 received no intervention, whereas Group 2 received ischemic preconditioning by two 10-minute CAOs. Group 3 received pressure overload of left ventricle to increase systolic left ventricular pressure (SLVP) 50 % above baseline for 10 minutes. Group 4 received pressure overload to increase SLVP 50% above baseline for 5 minutes. Group 5 received pressure overload to increase SLVP 50% above baseline for 3 minutes. Group 6 received pressure overload to increase SLVP 50% above baseline for 2 minutes. Group 7 received pressure overload to increase SLVP 50% above baseline for 2 minutes. Two episodes of pressure overload were applied. Group 8 received pressure overload to increase SLVP 30% above baseline for 10 minutes. Group 9 received pressure overload to increase SLVP 30% above baseline for 5 minutes. Group 10 received pressure overload to increase SLVP 30% above baseline for 3 minutes. Group 11 received pressure overload to increase SLVP 30% above baseline for 2 minutes. Group 12 received pressure overload to increase SLVP 30% above baseline for 2 minutes. Two episodes of pressure overload were applied.

including baseline (prior to treatment), treatment period, 1 hour coronary artery occlusion, and 3 hours reperfusion.

2.3. Determination of area at risk and MI size

At the end of the experiments, 2000 U heparin were administered. The heart was rapidly excised and placed on a perfusion apparatus. The methods for Evans blue dye perfusion have been reported previously.[2,3,6] 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. After perfusion was completed, the atria and right ventricular free wall were removed. The left ventricle plus septum was then cut into 6−7 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 by triphenyl tetrazolium chloride). The slices were weighed and fixed in 10% formalin solution. The basal surfaces were photographed. Images were traced with a digitizer to calculate both the area at risk (AAR), identified by the Evans blue dye exclusion, and the MI area. The AAR and MI areas of each slice were calculated from planimetry and the values for each slice summed for the entire left ventricle plus septum. AAR was reported as percentage of left ventricle plus septum, with MI size as percentage of AAR.

2.4. Exclusion criteria

Rabbits with AAR $<$ 10% of left ventricular weight and rabbits that exhibited ST segment shift on electrocardiography during pressure overload period were excluded.

2.5. Statistics and data analysis

All values were expressed as mean $\pm$ standard deviation. Hemodynamic variables were analyzed by a two-way analysis of variance (ANOVA) with repeated measures. The multiple comparisons of repeated measures were tested by within-subjects contrasts. The difference of AAR and MI size among groups was analyzed by one-way ANOVA. If the ANOVA results were significant, a multiple comparison using a *post hoc* Bonferroni test was made to assess which group was significantly different. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Mortality and exclusions

There were 96 rabbits that entered this study (Table 1). Four rabbits exhibited ventricular fibrillation and died. Another six rabbits died of heart failure, which was defined as a progressive decrease of arterial pressure to a systolic value < 50 mmHg with global left ventricular dilatation and poor contraction. One rabbit had electrocardiography changes during the pressure overload period. One rabbit in Group 2 suffered from bleeding of the sternum and hypotension. They were excluded. The mortality was not significantly different among all groups ($p = 0.914$).

3.2. Characteristics of brief pressure overload

During partial snaring of the ascending aorta, SLVP increased significantly from 92 ± 12 mmHg to 135 ± 16 mmHg ($p < 0.001$), and systolic arterial pressure decreased significantly from 92 ± 12 mmHg to 78 ± 11 mmHg ($p < 0.001$) in Groups 3–7 (Table 2). The SLVP was increased 50% above baseline. In Groups 8–12, SLVP increased significantly from 89 ± 14 mmHg to 116 ± 17 mmHg ($p < 0.001$), whereas systolic arterial pressure decreased significantly from 89 ± 14 mmHg to 80 ± 12 mmHg ($p < 0.001$) during partial snaring of the ascending aorta. SLVP was increased 30% above baseline. Both 50% and 30% elevation of SLVP caused obvious pressure overload of the left ventricle. There was no significant change in heart rate during the pressure overload period. After brief pressure overload, LVP, arterial pressure, and heart rate returned to baseline levels.

3.3. Hemodynamic changes during experiments

The baseline arterial pressure and heart rate of the 12 groups were not significantly different (Table 3). The treatments prior to 1 hour coronary artery occlusion did not cause any significant hemodynamic changes. Except during pressure overload of the left ventricle, there was no significant change in arterial pressure and heart rate throughout the experiment. The hemodynamic variables among the 12 groups were not significantly different during the experiments.

Table 1
Mortality and exclusions.

Group	Treatment	Total no.	Mortality[a]			ECG changes in LVPO	No. included
			VF	HF	Other		
1	Control	8	0	1	0	0	7
2	IPC	7	0	0	0	0	7
3	50% LVPO, 10-min	9	1	1	0	0	7
4	50% LVPO, 5-min	8	0	1	0	0	7
5	50% LVPO, 3-min	9	1	1	0	0	7
6	50% LVPO, 2-min	7	0	0	0	0	7
7	50% LVPO, 2-min*2	7	0	0	0	0	7
8	30% LVPO, 10-min	9	0	1	1	0	7
9	30% LVPO, 5-min	8	0	0	0	1	7
10	30% LVPO, 3-min	8	0	1	0	0	7
11	30% LVPO, 2-min	8	1	0	0	0	7
12	30% LVPO, 2-min*2	8	1	0	0	0	7

ECG = electrocardiography; HF = heart failure; IPC = ischemic preconditioning; LVPO = left ventricle pressure overload; VF = ventricular fibrillation.

[a] There was no significant difference in mortality; Pearson χ^2 $p = 0.914$.

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

Hemodynamics	Baseline 1	LVPO	Baseline 2
50% LVPO Groups (Groups 3–7)			
SLVP	92 ± 12	135 ± 16[a]	95 ± 13
SAP	92 ± 12	78 ± 11[a]	95 ± 13
MAP	74 ± 9	66 ± 8[b]	73 ± 9
HR	243 ± 25	241 ± 27	243 ± 29
30% LVPO Groups (Groups 8–12)			
SLVP	89 ± 14	116 ± 17[a]	89 ± 13
SAP	89 ± 14	80 ± 12[b]	89 ± 13
MAP	73 ± 9	67 ± 9[c]	72 ± 10
HR	230 ± 25	229 ± 24	232 ± 23

Data are presented as mean ± standard deviation. There were significant changes in SLVP, SAP, and MAP during left ventricular pressure overload. Baseline 1 = baseline prior to left ventricular pressure overload; Baseline 2 = baseline after left ventricular pressure overload; HR = heart rate; LVPO = left ventricular pressure overload; MAP = mean arterial pressure; SAP = systolic arterial pressure; SLVP = systolic left ventricular pressure.

[a] Multiple comparison $p < 0.001$ versus baseline 1.

[b] Multiple comparison $p = 0.001$ versus baseline 1.

[c] Multiple comparison $p = 0.25$ versus baseline 1. There was no significant change in HR ($p = 0.966$ in 50% LVPO groups, $p = 0.872$ in 30% LVPO groups, respectively).

3.4. AAR and MI size analysis

The AAR (expressed as percentage of left ventricular weight) of the 12 groups averaged 47.8 ± 3.6% (control group), 48.0 ± 5.8% (ischemic preconditioning group), 45.6 ± 3.6% (50% LVPO, 10-min group), 47.8 ± 3.2% (50% LVPO, 5-min group), 49.2 ± 5.1% (50% LVPO, 3-min group), 45.0 ± 2.9% (50% LVPO, 2-min group), 45.1 ± 3.3% (50% LVPO, 2-min*2 group), 48.2 ± 1.4% (30% LVPO, 10-min group), 46.7 ± 3.9% (30% LVPO, 5-min group), 50.3 ± 5.7% (30% LVPO, 3-min group), 49.1 ± 2.5% (30% LVPO, 2-min group), and 47.4 ± 2.7% (30% LVPO, 2-min*2 group) (Table 4). The AAR was not significantly different among the groups ($p = 0.235$). The infarct area was located in subepicardial, midmyocardial, and subendocardial areas in both groups of animals. The infarct area pattern did not differ between both groups. There was also no significant difference in reduction of MI size. MI size, expressed as percentage of AAR, was 26.6 ± 1.0% in the control group (Table 4). Ischemic preconditioning significantly decreased MI size (19.9 ± 2.8%, $p < 0.001$). Pressure overload of the left ventricle to raise SLVP 50% above baseline value for 10 minutes, 5 minutes, and 3 minutes significantly reduced MI size (18.5 ± 3.6%, 21.4 ± 1.9%, and 21.6 ± 1.7%, respectively, $p < 0.01$). A 30% increase in SLVP by pressure overload for 10 minutes, 5 minutes, and 3 minutes also significantly decreased MI size (20.5 ± 2.5%, 21.6 ± 2.3%, and

Table 3
Hemodynamic changes during the experiment.

Group	Treatment protocol	No.	Baseline 1	Baseline 2	CAO		CAR		
					30 min	60 min	1 h	2 h	3 h
MAP (mmHg)									
1	Control	7	70 ± 12	71 ± 11	69 ± 14	68 ± 14	65 ± 14	67 ± 18	68 ± 17
2	IPC	7	70 ± 8	70 ± 8	67 ± 8	68 ± 9	71 ± 7	71 ± 7	70 ± 9
3	50% LVPO, 10-min	7	74 ± 7	75 ± 9	71 ± 7	71 ± 8	69 ± 11	69 ± 10	68 ± 14
4	50% LVPO, 5-min	7	73 ± 10	71 ± 9	67 ± 11	71 ± 5	70 ± 7	69 ± 11	69 ± 10
5	50% LVPO, 3-min	7	72 ± 9	73 ± 10	65 ± 9	69 ± 11	71 ± 9	67 ± 12	67 ± 17
6	50% LVPO, 2-min	7	79 ± 11	71 ± 10	67 ± 10	70 ± 11	71 ± 8	71 ± 9	68 ± 9
7	50% LVPO, 2-min*2	7	70 ± 6	71 ± 7	65 ± 9	66 ± 10	67 ± 8	68 ± 13	70 ± 15
8	30% LVPO, 10-min	7	75 ± 9	77 ± 13	71 ± 13	74 ± 10	70 ± 9	66 ± 13	69 ± 19
9	30% LVPO, 5-min	7	71 ± 8	71 ± 7	68 ± 8	67 ± 11	71 ± 9	71 ± 11	68 ± 13
10	30% LVPO, 3-min	7	70 ± 10	68 ± 9	69 ± 9	71 ± 7	66 ± 12	65 ± 12	67 ± 16
11	30% LVPO, 2-min	7	76 ± 8	74 ± 7	71 ± 8	71 ± 11	70 ± 10	69 ± 10	71 ± 8
12	30% LVPO, 2-min*2	7	77 ± 6	76 ± 3	74 ± 6	71 ± 9	72 ± 9	69 ± 11	70 ± 14
HR (beats/min)									
1	Control	7	259 ± 19	260 ± 19	263 ± 19	251 ± 25	243 ± 21	233 ± 26	236 ± 28
2	IPC	7	243 ± 35	239 ± 34	226 ± 33	225 ± 36	223 ± 41	223 ± 44	223 ± 44
3	50% LVPO, 10-min	7	246 ± 32	247 ± 38	234 ± 42	239 ± 38	230 ± 16	237 ± 21	234 ± 23
4	50% LVPO, 5-min	7	241 ± 20	250 ± 30	246 ± 28	244 ± 27	234 ± 27	234 ± 25	230 ± 28
5	50% LVPO, 3-min	7	247 ± 30	237 ± 29	236 ± 20	236 ± 26	223 ± 31	226 ± 22	222 ± 27
6	50% LVPO, 2-min	7	237 ± 18	236 ± 20	237 ± 14	240 ± 16	233 ± 10	233 ± 13	236 ± 16
7	50% LVPO, 2-min*2	7	237 ± 24	240 ± 28	237 ± 31	233 ± 24	230 ± 26	231 ± 16	224 ± 21
8	30% LVPO, 10-min	7	229 ± 16	233 ± 15	227 ± 17	226 ± 18	219 ± 23	210 ± 18	216 ± 10
9	30% LVPO, 5-min	7	237 ± 29	243 ± 31	240 ± 29	229 ± 25	216 ± 30	226 ± 28	229 ± 25
10	30% LVPO, 3-min	7	219 ± 29	222 ± 25	221 ± 25	218 ± 24	222 ± 18	215 ± 21	223 ± 15
11	30% LVPO, 2-min	7	243 ± 14	237 ± 14	236 ± 16	230 ± 17	227 ± 17	224 ± 11	226 ± 15
12	30% LVPO, 2-min*2	7	233 ± 13	227 ± 13	229 ± 15	220 ± 17	221 ± 23	219 ± 20	227 ± 21

Data are presented as mean ± standard deviation. There was no significant change in MAP and HR throughout the experiment. There was no significant difference in hemodynamic variables among the three groups of animals during the experiment. Baseline 1 = baseline prior to treatment; Baseline 2 = baseline after treatment; CAO = coronary artery occlusion; CAR = coronary artery reperfusion; HR = heart rate; IPC = ischemic preconditioning; LVPO = left ventricular pressure overload; MAP = mean arterial pressure.

21.5 ± 2.3%, $p < 0.01$). Pressure overload to elevate SLVP 50% or 30% above baseline value for 2 minutes did not significantly change MI size (25.0 ± 2.3% and 26.0 ± 1.7%, $p = 0.122$ and $p = 0.457$). Two episodes of 2 minutes pressure overload did not significantly decrease the MI size (25.0 ± 2.2% and 25.5 ± 2.2%, $p = 0.118$ and $p = 0.281$).

Table 4
AAR size, expressed as percentage of LV, and MI size, expressed as percentage of AAR.

Group	Treatment protocol	No.	AAR/LV (%)	MI/AAR (%)
1	Control	7	47.8 ± 3.6	26.6 ± 1.0
2	IPC	7	48.0 ± 5.8	19.9 ± 2.8[a]
3	50% LVPO, 10-min	7	45.6 ± 3.6	18.5 ± 3.6[a]
4	50% LVPO, 5-min	7	47.8 ± 3.2	21.4 ± 1.9[b]
5	50% LVPO, 3-min	7	49.2 ± 5.1	21.6 ± 1.7[b]
6	50% LVPO, 2-min	7	45.0 ± 2.9	25.0 ± 2.3
7	50% LVPO, 2-min*2	7	45.1 ± 3.3	25.0 ± 2.2
8	30% LVPO, 10-min	7	48.2 ± 1.4	20.5 ± 2.5[a]
9	30% LVPO, 5-min	7	46.7 ± 3.9	21.6 ± 2.3[b]
10	30% LVPO, 3-min	7	50.3 ± 5.7	21.5 ± 2.3[b]
11	30% LVPO, 2-min	7	49.1 ± 2.5	26.0 ± 1.7
12	30% LVPO, 2-min*2	7	47.4 ± 2.7	25.5 ± 2.2

Data are presented as mean ± standard deviation. There was no significant difference in AAR size. The one-way analysis of variance p value of AAR was 0.235. There was a significant difference in MI size. The one-way analysis of variance p value of myocardial infarct size was < 0.001.
AAR = area at risk; IPC = ischemic preconditioning; LV = left ventricle; LVPO = left ventricular pressure overload; MI = myocardial infarct.
[a] Multiple comparison $p < 0.001$ versus control group.
[b] Multiple comparison $p < 0.01$ versus control group.

4. Discussion

In the current study, reduction of MI size was not observed in 2-minute pressure overloads (Table 4). One more episode of 2 minutes did not significantly decrease MI size either (Groups 7 and 12). If the duration of pressure overload was ≥ 3 minutes, either 50% or 30% elevation of SLVP above baseline by pressure overload significantly decreased MI size. Reduction of MI size was comparable to that induced by ischemic preconditioning. Only one animal exhibited ST segment shift on electrocardiography during pressure overload (Table 1). It was excluded. The protection of pressure overload was not induced by transient ischemia. Therefore, pressure overload to raise SLVP either 50% or 30% above baseline reduced MI size. A minimum duration of 3 minutes was necessary to induce the protective effects.

The minimum duration of ischemic preconditioning varied in different studies. Schulz et al reported that 2 minutes of low-flow ischemia followed by 15 minutes reperfusion did not reduce MI size in swine. MI size was significantly limited by 3 minutes ischemic preconditioning and reduced further by 10 minutes ischemia. They concluded that MI size reduction by ischemic preconditioning was a graded phenomenon.[8]

Tsuchida et al also showed that 2 minutes ischemia preconditioning was not long enough to decrease MI size significantly in rabbits.[19] In a report by Mizumura et al, they demonstrated that MI size was not significantly affected by 2.5 minutes ischemic preconditioning in dogs. MI size was significantly reduced by 5 minutes preconditioning.[20] Yao and Gross reported that 3 minutes coronary occlusion did not significantly reduce MI size in dogs.[14] Van Winkle et al suggested that there was a threshold of preconditioning between 2 minutes and 5 minutes ischemia.[21] Our study showed that MI size was not significantly reduced by 2 minutes pressure overload. A minimum duration of 3 minutes was required.

The effects of single or multiple episodes of ischemic preconditioning were not consistent in various reports. Li et al reported that preconditioning with one brief ischemic interval was as effective as preconditioning with multiple ischemic periods.[15] Bell et al showed that reducing the number of preconditioning cycles from four to three to two continued to result in attenuation of MI size reduction in mouse hearts.[22] Miura et al demonstrated that preconditioning with a single and two cycles of 5 minutes ischemia followed by 5 minutes reperfusion limited MI size to the same extent in rabbits. Administration of inhibitors of protein kinase C significantly decreased MI size reduction by a single cycle of preconditioning. However, MI size reduction by repetitive preconditioning was not abolished. They concluded that the effect of protein kinase C inhibitors on cardioprotection of ischemic preconditioning depended on the number of preconditioning cycles.[23] Sandhu et al reported that one or three cycles of ischemic preconditioning significantly reduced MI size in rabbits. However, the extent of MI size reduction by three cycles of preconditioning was significantly greater than that by one cycle. They also showed that three cycles of preconditioning were less susceptible to blockade by protein kinase C inhibitors.[16] Interestingly, Cohen et al reported that conscious rabbits became tolerant to multiple episodes of ischemic preconditioning. [24] Although we did not perform repetitive cycles of pressure overload in this study, the extent of MI size reduction by two cycles of pressure overload in our previous studies was not significantly different from the results for one cycle of pressure overload in this study.[2,3]

Adenosine and ATP-sensitive potassium channels are important mediators of ischemic preconditioning.[7,25−27] Administration of activators of adenosine receptors or ATP-sensitive potassium channels has been shown to lower the threshold of ischemic preconditioning. Mizumura et al reported that preconditioning with 2.5 minutes ischemia alone did not significantly reduce MI size. Intracoronary infusion of an A1-adenosine activator during 2.5 minutes ischemia significantly limited MI size.[20] Yao and Gross reported that preconditioning with 3 minutes ischemia alone did not significantly decrease MI size. Intracoronary infusion of an opener of ATP-sensitive potassium channels during 3 minutes ischemia significantly limited MI size.[14] Our previous study showed that protection of pressure overload was independent of adenosine receptor activation.[2] We did not perform administration of activators of ischemia preconditioning in this study.

Determination of threshold is of importance in both animal research and clinical application of myocardial preconditioning. The preconditioning stimuli must be stronger or more powerful than the preconditioning threshold to induce protection in animal experiments. However, clinicians try to induce protection in human patients by the minimal stimuli or insult.[9,28] In this study, we found that pressure overload by raising SLVP either 50% or 30% above baseline significantly reduced MI size. Pressure overload for < 2 minutes failed to decrease MI size significantly. The findings may be of significance for clinical application of pressure overload.

There were several limitations to this study. The effects of pressure overload on myocardial blood flow were not investigated. We verified the performance of successful coronary artery occlusion by observing the development of ST-segment elevation and changes in the QRS complex on electrocardiography and the cyanotic changes in the myocardium in the occluded area. No myocardial blood flow data were provided. However, rabbits have a minimal collateral coronary circulation.[29] Besides, the rabbit model was widely used in experiments on myocardial ischemia and preconditioning.[30−33] The current study was conducted on anesthetized, open-chest rabbits. The acute effects of anesthesia and surgery should be taken into account when interpreting the results.[33,34] Although the triphenyl tetrazolium chloride technique is commonly used in MI experiments, the infarct size might have been underestimated.[35]

In conclusion, we demonstrated pressure overload to raise SLVP either 50% or 30% above baseline value reduced MI size. However, reduction of MI size was not observed after 2 minutes pressure overload. Repetitive episode of 2 minutes also did not significantly decrease MI size. A minimum duration of 3 minutes is necessary for pressure overload to induce protective effects.

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