



Myocardial hypothermia induced after reperfusion does not prevent adverse left ventricular remodeling nor improve cardiac function



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ABSTRACT

Aims: The purpose of the study was to determine whether late therapeutic hypothermia (LTH), administered after reperfusion, could prevent adverse left ventricular (LV) remodeling and improve cardiac function in the rat myocardial ischemia/reperfusion model.

Main methods: Rats were randomized to normothermia (n = 10) or LTH (initiated at 1 min after coronary artery reperfusion, n = 10) and subjected to 30 min of coronary occlusion followed by 6 weeks of reperfusion. Hypothermia was induced by pumping cold saline over the anterior surface of the LV until the temperature cooled to < 32 °C. In the normothermic group, the heart was bathed in saline at 38 °C.

Key findings: After 6 weeks of recovery, fractional shortening of the LV was comparable in the LTH (20.2 ± 0.6%) and normothermic group (20.0 ± 2.1%; p = 0.918). Postmortem LV volume (0.47 ± 0.04 ml in LTH and 0.44 ± 0.05 ml in normothermic group) and lung wet/dry weight ratio were similar in both groups. There were no significant differences in scar size, scar thickness, infarct expansion index, LV cavity or transmural (%) between groups. This data contrasts with our previous study showing that hypothermia administered during the ischemic phase significantly reduced the scar size; decreased LV cavity, infarct expansion index and transmural (%), and improved the scar thickness.

Significance: LTH did not prevent adverse LV remodeling nor improve cardiac function in the rat myocardial ischemia/reperfusion model. To have a long term benefit on remodeling, hypothermia must be administered during the ischemic phase and not just the reperfusion phase.

1. Introduction

A recent report [1] describes the facts that the annual incidence of a new myocardial infarction (MI) in the USA is 735,000; ~15% of those who experience a MI will die of it; in-hospital mortality rates for ST elevation myocardial infarction (STEMI) are still as high as 8%–10% in some series. Menees [2] has shown that further shortening door-to-balloon times to under 90 min has not further improved outcomes. Many adjunctive therapies to reduce MI size have been tested, but overall the results to date have been disappointing [3–5].

Previously our group showed that early therapeutic hypothermia, instituted during the ischemic phase of an experimental MI in rabbit or rat models limited myocardial infarct size as well as no-reflow (area of microvascular damage that does not reperfuse even after re-establishing patency of the large epicardial infarct related coronary artery) [6,7]. Therapeutic hypothermia administered during ischemia also reduced

long term adverse LV remodeling [8]. Recently we found that hypothermia initiated after reperfusion (late hypothermia) reduced the extent of early no-reflow during the first few hours of reperfusion without affecting infarct size [9,10]. These observations were made in both a rabbit model of 30 min of ischemia followed by 3 h of reperfusion [9] as well as a rat model of 60 min of ischemia and reperfusion [10]. This is important since both experimental and clinical studies show that the presence of early no-reflow after reperfusion results in worse left ventricular (LV) remodeling, a phenomenon that includes more infarct expansion with a thinner and stretched scar, more LV dilatation and worse LV function, and portends a poor prognosis [11,12] that is independent of the size of the infarction [13,14]. For example, Bolongnese et al. showed that early no reflow detected by echocardiographic contrast, just after percutaneous transluminal coronary angioplasty, predicted adverse left ventricular remodeling at 6 months [12]. Ndrepepa et al. diagnosed no reflow in the catheterization

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laboratory by angiographic criteria following percutaneous coronary intervention. This early no reflow phenomenon was an independent correlate of 5 year mortality [13].

One recent study showed that acute therapy for early no reflow that did not reduce infarct size was still capable of reducing long term adverse left ventricular remodeling [15]. Would the same phenomenon occur with late hypothermia which is known to reduce early no reflow but not infarct size? Because hypothermia started after reperfusion reduces early no reflow in 2 independent models, we hypothesized that late therapeutic hypothermia may reduce adverse left ventricular remodeling and preserve ventricular function, even though it does not reduce acute infarct size.

It is important to determine whether late hypothermia has long term benefits, since interventional cardiologists are often hesitant to delay primary percutaneous coronary intervention for STEMI for institution of adjunctive therapy while the coronary artery is still occluded. Instituting hypothermia after reperfusion would not delay door-to-balloon time; and by reducing no-reflow could improve adverse LV remodeling. The present study tested whether late therapeutic hypothermia, administered after reperfusion, prevents long-term adverse LV remodeling and preserves LV function. We chose the rat model of 30 min of ischemia and 6 weeks of reperfusion so that we could compare the results to a recent study that investigated the effects of early hypothermia in that same model [8] that assesses long term adverse ventricular remodeling. In addition, recent studies from our group showed that a 30 min occlusion plus reperfusion in the rat was associated with substantial early no reflow (33% of the risk zone in one study [6] and 36–40% of the risk zone in a very recent 2019 study (Dai W, Kloner RA, Hale S The effect of an experimental drug on infarct size and no reflow, unpublished results, 2019); which was comparable to the percentage of no reflow as a percent of the risk zone observed with a 60 min occlusion followed by reperfusion (39.5%) [10].

2. Methods

2.1. Surgical procedures

All procedures were approved by Huntington Medical Research Institute's Animal Care and Use Committee. Huntington Medical Research Institutes is an AAALAC approved facility. Female Sprague Dawley rats (~200 g) were anesthetized with an intraperitoneal injection of ketamine and xylazine and were mechanically ventilated. Under aseptic conditions, left thoracotomy was performed to expose the heart, and the pericardium was opened after the chest wall was infiltrated with 0.1 mg/kg bupivacaine for local analgesia. The left coronary artery was encircled with 4–0 silk suture that was threaded through a plastic tube. The artery was occluded by pulling the ends of the suture through the plastic tube and clamping. Successful coronary artery occlusion was confirmed by cyanosis and akinesis of the LV anterior wall. The artery was occluded for 30 min and then the clamp was released, allowing reperfusion of the epicardial artery. After treatment, the left thoracotomy was closed and the rat allowed to recover for 6 weeks. Analgesia (buprenorphine) was administered for a minimum of 2 days.

2.2. Experimental design

The rats were randomly assigned to normothermia or late therapeutic hypothermia (LTH) groups. The timing of onset of LTH was started at 1 min after coronary artery reperfusion, and continued for one hour. LTH was induced by continuously pumping saline (22 °C) directly over the anterior surface of the LV and removing an equivalent volume from the chest cavity using a suction catheter. A thermocouple was placed into the anterior wall of the LV (chest cavity temperature). The thermocouple measurement confirmed that the chest cavity temperature had been cooled to < 32 °C. Hearts in the normothermic group were bathed in saline at 38 °C. Rectal temperature was also monitored.

2.3. Cardiac function

At 6 weeks, rats were anesthetized and an echocardiogram was performed to measure LV end-diastolic diameter, LV end-systolic diameter, and LV fractional shortening. A jugular vein and carotid artery cut down were performed. A Millar catheter was placed into the carotid artery, advanced into the ascending aorta to record systemic arterial pressure and heart rate, and then advanced into the LV cavity to record end-diastolic pressure, end-systolic pressure, peak +dP/dt and peak -dP/dt.

2.4. Post-mortem LV volume and histological analysis

Under deep anesthesia, the rats were euthanized with intravenous KCl and the hearts excised. The hearts were pressure fixed in 10% formalin at 13 cm water for one hour and then immersed in formalin for 24 h. Postmortem LV volumes were measured by filling the dried cavity of the LV with water and weighing. The lungs were harvested and wet weight and then dry weight were measured in samples to determine potential fluid overload due to heart failure. A transverse cross sectional slice of the left ventricle from the center of scar was embedded in paraffin for histologic assessment. To delineate the scar size, histological slides (5 μ) were prepared and stained with hematoxylin and eosin and with picrosirius red stain, which stains collagen. Quantitative histologic analyses included: the infarcted endocardial circumference, scar size (% of scar endocardial plus epicardial circumference to total LV endocardial plus epicardial circumference), scar thickness (average of 5 equidistant measurements), septum thickness (average of 3 equidistant measurements), myocardial infarct expansion index (stretching and thinning of the infarcted wall, which is expressed as LV cavity area/total LV area x septum thickness/scar thickness) and transmural % (average of 5 equidistant measurements of LV scar thickness / LV wall thickness × 100). We also re-analyzed histological slides from rats from our previous study [8] that included early therapeutic hypothermia administered during the ischemic phase (hypothermia started at 2 min after coronary artery occlusion). This allowed us to compare the histological parameters from rats treated with early hypothermia [8] vs. LTH.

2.5. Statistical analyses

All results are expressed as means ± SEM and analysis using Student's *t*-test or 1 way ANOVA as appropriate. Statistically significant differences were established at $p < 0.05$.

3. Results

12 rats underwent surgical procedure in each group. There were a total of 10 successful studies in each group. 2 rats were excluded in each group due to failed occlusion.

3.1. Chest cavity temperature in hypothermic and normothermic groups

Chest cavity temperature was comparable in hypothermic (38.2 ± 0.3 °C) and normothermic group (38.3 ± 0.2 °C) at baseline. The change in chest cavity temperature in the LTH and normothermic groups are shown in Fig. 1. The chest cavity temperature remained ~38 °C during the surgical procedure in the normothermic group. In the LTH group, chest cavity temperature dropped to 26 °C and then gradually warmed up (Fig. 1A). The rectal temperature slowly dropped to 29 ° in the LTH group, while the rectal temperature maintained at 37 °C in the control group (Fig. 1B).

3.2. Cardiac function

After 6 weeks of recovery, echocardiographic data (Table 1)

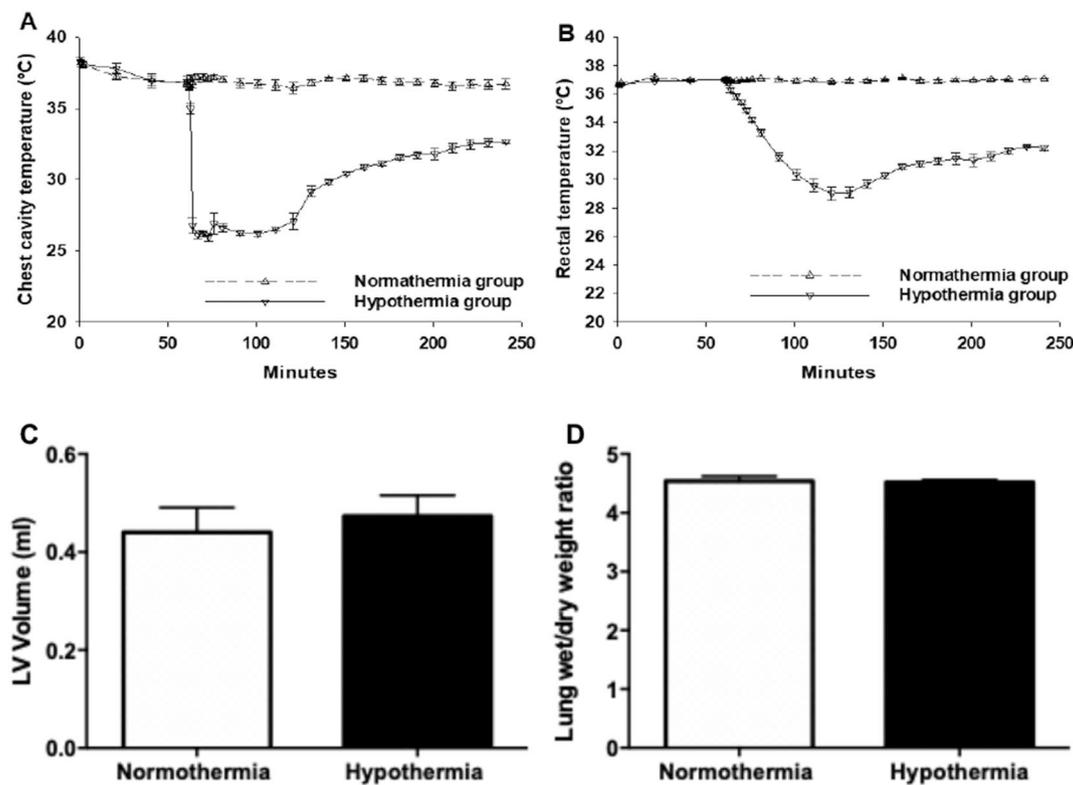


Fig. 1. A: The chest cavity temperature versus time. In the hypothermic (LTH) group, the saline (22 °C) was circulated to the rat chest cavity starting at 1 min after coronary artery reperfusion. The temperature in the chest cavity group dropped to 26 °C in 12 min of cooling and kept at 26 °C for 1 h. In the control group, chest cavity temperature maintained at 38 °C. B: The rectal temperature verse time. The rectal temperature slowly dropped to 29 °C within 60 min of cooling in the hypothermia group, while the rectal temperature maintained at 37 °C in the control group. C: Post-mortem left ventricular (LV) volume in the hypothermic and normothermic group. D: The lung wet/dry weight ratio in the hypothermic and normothermic group. Data are expressed as mean ± SE, N = 10 in each group.

Table 1
Cardiac function (echocardiography).

	Normothermia	Hypothermia	p value
LV diastolic ID (mm)	7.5 ± 0.2	8.2 ± 0.2	0.037
LV systolic ID (mm)	6.1 ± 0.3	6.6 ± 0.2	0.178
LVFS (%)	20.0 ± 2.1	20.2 ± 0.6	0.918

Data are expressed as mean ± SE. LV Diastolic ID: LV end-diastolic diameter; LV systolic ID (mm): LV end-systolic diameter; and LVFS: LV fractional shortening.

demonstrated that left ventricular fraction shorting (LVFS) was comparable in the LTH group (20.2 ± 0.6%) and normothermic group (20.0 ± 2.1%; p = 0.918). The diastolic internal dimension of the left ventricle was significantly larger in the LTH group (8.2 ± 0.2 mm compared to 7.5 ± 0.2 mm in normothermic group; p = 0.037). The systolic internal dimension of left ventricle (LVIDs mm) was similar in both groups.

3.3. Hemodynamics

There was no significant difference in heart rate between groups at 6 weeks (Table 2). The LV positive/negative dP/dt and Pes (end-systolic left ventricular pressure mm Hg) were comparable in the two groups. Diastolic systemic arterial blood pressure, systolic arterial blood pressure, mean arterial blood pressure and Ped (end diastolic left ventricular pressure mm Hg) were lower in the LTH vs. normothermic group. Tau, a measure of ventricular diastolic function, was significant lower in the LTH group (8.8 ± 0.5 ms) compared with normothermic group (11.7 ± 0.7 ms, p = 0.008). These data suggest that during diastole, the heart may have been capable of better relaxation in the

Table 2
Hemodynamics at 6 weeks.

	Normothermia	Hypothermia	p value
HR (bpm)	217 ± 6	209 ± 5	0.348
Systolic blood pressure (mm Hg)	94 ± 4	84 ± 2	0.064
Diastolic blood pressure (mm Hg)	66 ± 3	57 ± 2	0.018
Mean blood pressure (mm Hg)	79 ± 3	70 ± 2	0.033
Pes (mm Hg)	88 ± 3	84 ± 2	0.342
Ped (mm Hg)	4 ± 1	-4 ± 2	0.002
dP/dt max (mm Hg/s)	5243 ± 183	5449 ± 124	0.409
dP/dt min (mm Hg/s)	3767 ± 216	3826 ± 169	0.847
Tau (ms)	11.7 ± 0.7	8.8 ± 0.5	0.008

Data are expressed as mean ± SE. HR: heart rate (beats/min); Pes: end systolic left ventricular pressure; Ped: end diastolic left ventricular pressure; dP/dt max: LV positive change in pressure over time; dP/dt min: LV negative change in pressure over time; Tau: left ventricular diastolic time constant.

hypothermia group.

3.4. Post-mortem LV volumes and lung wet/dry weight ratio

There were no significant differences in post-mortem LV volumes between the LTH group (0.47 ± 0.04 ml) and normothermic group (0.44 ± 0.05 ml) (Fig. 1C). The lung wet/ dry weight ratio was comparable between the two groups (LTH, 4.5 ± 0.04; normothermia, 4.5 ± 0.1) (Fig. 1D).

3.5. Histological assessments

There were no significant differences between groups in infarcted endocardial circumference, scar size, scar thickness, myocardial infarct

Table 3
Histological assessment.

	Normothermia	Hypothermia	p value
Infarcted endocardial circumference (mm)	12.0 ± 0.7	12.0 ± 1.1	0.983
LV cavity area (mm ²)	31.8 ± 3.5	37.0 ± 2.9	0.267
Scar size (% of total LV circumference)	40.6 ± 4.2	44.9 ± 3.3	0.426
Scar thickness (mm)	1.3 ± 0.1	1.2 ± 0.1	0.877
Septum thickness (mm)	1.4 ± 0.1	1.4 ± 0.1	0.977
Myocardial infarct expansion index	0.57 ± 0.07	0.58 ± 0.05	0.914
Transmurality (%)	44.2 ± 6.6	54.2 ± 7.5	0.330

expansion index, LV cavity area or transmuralty (%) (Table 3). In contrast, our previous study [8] showed that initiating hypothermia during the ischemic phase (early hypothermia) significantly reduced the infarcted endocardial circumference (hypothermia, 5.1 ± 1.2 mm; normothermia, 11.9 ± 0.9 mm; $p = 0.0001$) and scar size (% of scar circumference to total LV circumference: LTH, 15.4 ± 3.3; normothermia, 39.0 ± 3.6; $p < 0.0001$); thickness of the anterior LV wall (hypothermia, 1.4 ± 0.1 mm, normothermia, 0.9 ± 0.1 mm; $p = 0.0003$); decreased LV cavity area (LTH, 30.2 ± 1.4 mm²; normothermia, 36.4 ± 1.4 mm²; $p = 0.0049$), myocardial infarct expansion index (LTH, 0.46 ± 0.1; normothermia, 0.85 ± 0.1; $p = 0.0013$) and transmuralty % (LTH, 27.8 ± 5.2; normothermia, 58.9 ± 3.0; $p < 0.0001$). Representative pictures of histological slides stained with picrosirius red are shown in Fig. 2.

3.6. Correlation between infarcted endocardial circumference and the other histological parameters

Infarcted endocardial circumference correlated well with the other parameters.

There was a positive correlation between infarcted endocardial circumference and scar size ($r = 0.7139$, $p = 0.0004$), myocardial expansion index ($r = 0.7093$, $p = 0.0005$), and transmuralty % ($r = 0.6235$, $p = 0.0033$). There was a negative correlation between infarcted endocardial circumference and scar thickness, $r = -0.673$, $p = 0.001$ (Fig. 3).

4. Discussion

It is well known that therapeutic hypothermia administered during ischemia limits infarct size, while therapeutic hypothermia administered only during the reperfusion phase does not limit infarct size. However LTH did decrease early no-reflow in our models. Early no-reflow is associated with increased adverse LV remodeling (increased scar circumference, increased infarct expansion and increased LV

dilation). Therefore, hypothermia initiated during reperfusion, although it will not decreased infarct size, has the potential to decrease remodeling; and that is what we sought to determine in this study.

However, our hypothesis that LTH would reduce adverse left ventricular remodeling and improve cardiac function, was disproved by our study. The major findings of the present study are that LTH, initiated after reperfusion, did not improve healing of myocardial infarction, did not prevent adverse LV remodeling and LV dilatation nor improve cardiac function in a chronic myocardial ischemia/reperfusion model in the rat. Our findings suggest that to have a long term benefit on remodeling hypothermia must be started during the ischemic phase and not just the reperfusion phase.

Our group has consistently shown that therapeutic hypothermia initiated during ischemia (early therapeutic hypothermia) limits myocardial infarct size. We observed that early hypothermia reduced infarct size and no-reflow in acute rabbit and rat MI models [6]. Our group also demonstrated that transient non-invasive surface cooling during the acute phase of MI improved cardiac function and resulted in long-term reduced remodeling in the chronic phase of experimental MI [8]. Other investigators testing different species and various techniques for inducing therapeutic hypothermia have also observed these benefits of hypothermia administered during ischemia [16–19]. As long as the hypothermia is present during the period of ischemia, myocardial infarct size and the zone of no-reflow (area of microvascular damage that does not reperfuse even after re-establishing patency of the large epicardial infarct related coronary artery) are both reduced.

Clinically, studies testing therapeutic hypothermia to reduce myocardial infarct size have had mixed results [20]. Some studies of large, anterior wall STEMIs have shown that if therapeutic hypothermia is successfully achieved prior to reperfusion, myocardial infarct size is reduced [21]. Instituting successful hypothermia prior to reperfusion in the clinical setting is not always possible and adds extra steps and time that can interfere with the interventional cardiologist's goal of opening up the infarct related coronary occlusion as soon as possible. On the other hand if there are new therapies that can postpone cardiac death while the occlusion is still in place (which may be the case for hypothermia as well as unloading of the left ventricle [22]); then while time to reperfusion is still important, these new therapies should be explored as they may reduce the “urgency” to immediately open the vessel and still salvage tissue.

Recently in a standardized rabbit model of experimental MI (30 min of coronary occlusion followed by 3 h of reperfusion), our research group made the observation that instituting LTH to cool the myocardium to 32 °C starting at 5 min before reperfusion, 5 min after reperfusion, or 30 min after reperfusion, failed to reduce myocardial infarct size but substantially reduced the size of the no-reflow zone, compared to animals randomized to normothermia [9]. We also showed

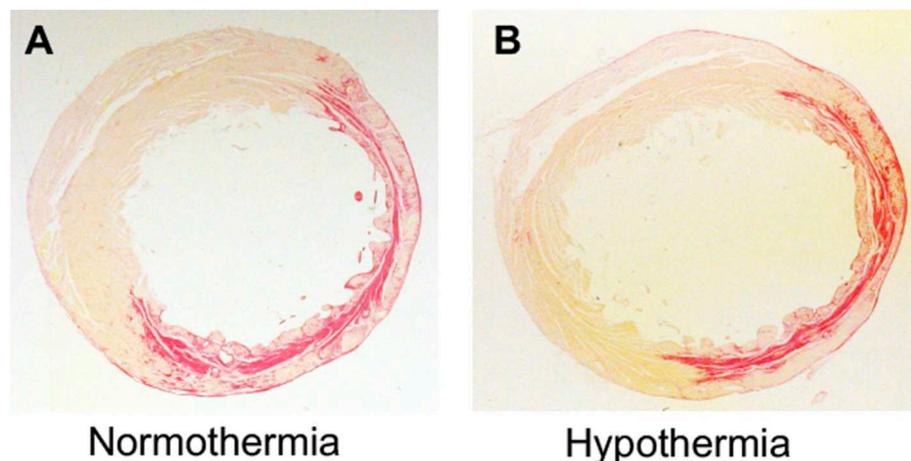


Fig. 2. Representative pictures stained with picrosirius red. The muscle stains yellow while scar stains red. A: Normothermia-treated heart from late hypothermia study; B: Hypothermia-treated heart from late hypothermia study. Scar is present in the sub-endocardium and midmyocardium. Portions of the subepicardial layer reveal viable yellow muscle.

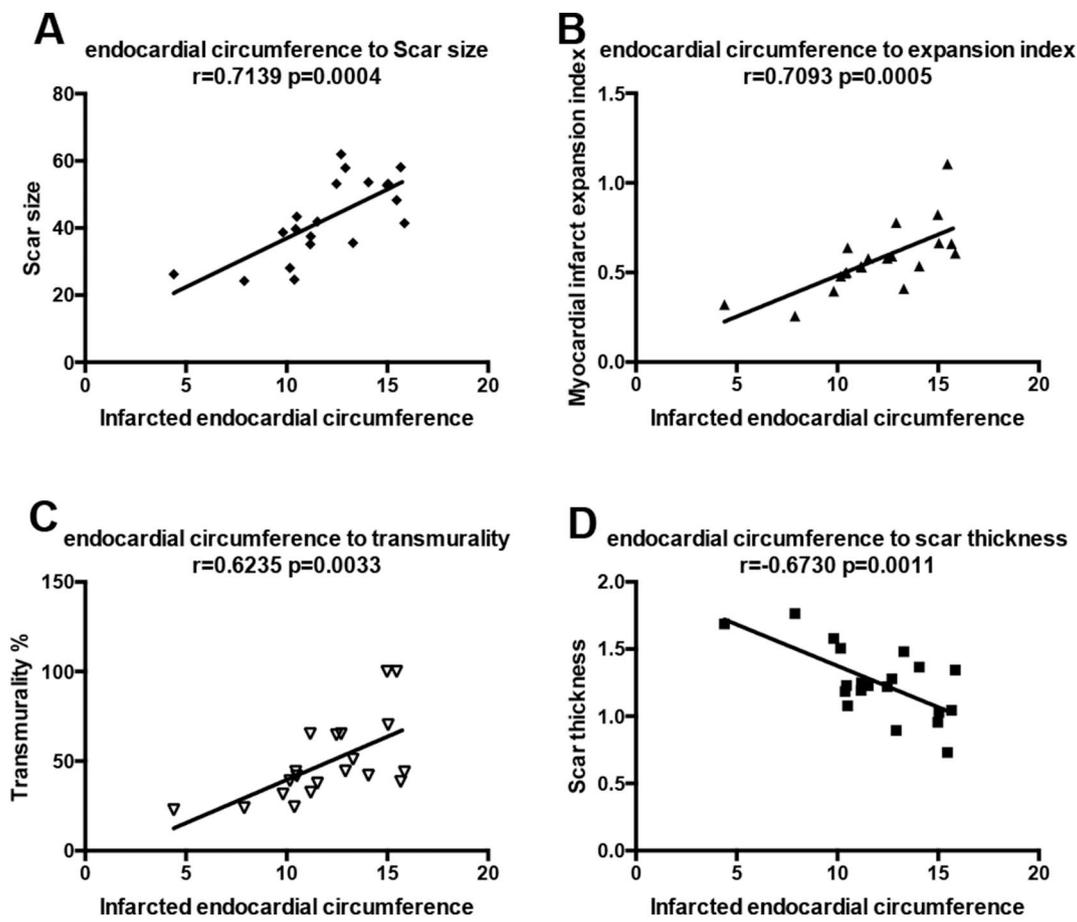


Fig. 3. Correlation between endocardial circumference and the other histological parameters. A: Correlation of infarcted endocardial circumference to scar size. B: Correlation of infarcted endocardial circumference to myocardial expansion index. C: Correlation of infarcted endocardial circumference to transmuralty %. D: Negative correlation of infarcted endocardial circumference to scar thickness.

this in the rat model [10]. However, until now, it was not known whether administering LTH was sufficient to reduce long term adverse remodeling. Our results suggest that even if LTH appears to reduce the early phase of no-reflow, it does not prevent adverse remodeling.

Areas of no-reflow most likely inhibit the ability of the infarct to heal by blocking removal of necrotic debris and by limiting access to the necrotic zone of blood-borne trophic factors and cells crucial to the healing phase of the infarct (neutrophils, macrophages, fibroblasts). In an experimental study reported in *Circulation* [11], we observed that rats subjected to proximal coronary artery occlusion (CAO) and reperfusion demonstrated persistent areas of no-reflow (late no-reflow) at one month. There was a marked reduction of microscopically perfused capillaries in the no-reflow zone. The fewer the number of perfused capillaries, the worse was myocardial infarct expansion and infarct thinning at one month. There are numerous, well-performed studies in the literature showing that no-reflow occurs in patients receiving reperfusion therapy for STEMI [23,24]. It occurs in > 30% of patients in some series, and has been documented by a number of imaging techniques [25]. Several carefully performed clinical studies have shown that early no-reflow occurring shortly after treatment of STEMI is associated with adverse LV dilatation and remodeling, which are known causes of worsened clinical outcome [12,26]. However, in the present study, LTH alone, a therapy that reduces no reflow acutely, was not sufficient to reduce adverse LV remodeling. It may be that the duration of hypothermia must be much longer and that once the hearts are rewarmed, no-reflow returns. There are no data available that show the optimal duration that hypothermia should be maintained following an acute myocardial infarction. In the present study though, hypothermia was maintained for at least 150 min. Keeping the heart hypothermic

much longer than this in the clinical situation would be difficult. However, we cannot rule out that a longer duration of hypothermia may have improved adverse LV remodeling. Or it is possible that to significantly reduce abnormal LV remodeling there must be an initial reduction in infarct size, which we know does not occur when hypothermia is started only after reperfusion. It is possible that other factors besides no-reflow may be important in causing adverse LV remodeling.

Myocardial infarct expansion is a complex phenomenon involving slippage of necrotic myocyte that results in a thinned and stretched scar, regional and global LV dilatation. The present histologic analysis suggests that endocardial scar circumference is a key measure that correlates closely with parameters of adverse LV remodeling including overall scar size (taking into account both endocardial and epicardial scar as defined in the [Methods](#) section), infarct expansion index, transmuralty of the infarct and inversely for scar thickness.

A limitation of this study was that it was not a head to head comparison of LTH versus early therapeutic hypothermia. Rather we compared LTH with an earlier study of early therapeutic hypothermia. In that early study we utilized a different technique to cool the heart (Thermosuit) [8] than in the current study. However, temperature monitoring revealed that both the Thermosuit technique and the continuous pumping of cooled saline on the surface of the heart were successful in inducing hypothermia in a rapid manner. Another limitation is that we did not specifically measure no reflow in the present study. However, 2 previous studies in both rabbit and rat showed that late hypothermia reduced acute no reflow. Whether late no reflow was affected was not studied in this model. However, based on scar size and morphology and the fact that most scar is relatively avascular, it is

unlikely that late no reflow was affected by late hypothermia in the present study.

5. Conclusion

In the present study, the diastolic arterial blood pressure, systolic arterial blood pressure, mean arterial blood pressure, Ped (end diastolic left ventricular pressure mmHg) and Tau were lower in the hypothermic group, which indicated that LTH might have some potential benefit on diastolic function and the ability of the heart to relax during diastole. However, myocardial hypothermia induced after reperfusion did not prevent adverse left ventricular remodeling and dilatation and did not improve cardiac systolic function. These findings, in contrast to our previous study, support the concept that hypothermia must be started during the ischemic phase and not just during reperfusion to demonstrate a long term benefit on remodeling.

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Conflict of interest statement

All authors report no conflicts of interest.

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