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Rapid cooling of the heart with total liquid ventilation prevents transmural myocardial infarction following prolonged ischemia in rabbits

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Abstract

Study aim: Total liquid ventilation (TLV) with cooled perfluorocarbons has been demonstrated to induce an ultrafast cardioprotective cooling in rabbits. However, it remains unknown whether this technically challenging strategy would be actually more potent than a conventional external cooling after a prolonged ischemia inducing transmural myocardial infarction. **Methods:** Anesthetized rabbits were randomly submitted to 60 min of coronary artery occlusion (CAO) under normothermic conditions (Control group, n=7) or with cooling started at the 5th min of CAO (target left atrial temperature: 32°C). Cooling procedures were either external cooling using cold blankets (EC group, n=7) or ultrafast cooling initiated by 20-min of TLV (TLV group, n=6). An additional group underwent a similar ultrafast cooling protocol started at the 20th min of CAO (TLV_{delayed} group, n=6). After reperfusion, all hypothermic animals were rewarmed and infarct size was assessed after 4 h. **Results:** In the EC group, the target temperature was reached only at 60-min of CAO whereas this time-interval was dramatically reduced to 15 and 25 min of CAO in TLV and TLV_{delayed}, respectively. Infarct sizes were significantly reduced in TLV and TLV_{delayed} but not in EC groups as compared to Control (45±18%, 58±5%, 78±10% and 82±7% of the risk zone, respectively). Similar significant differences were observed for the sizes of the no-reflow zones (15±9%, 23±8%, 49±11% and 58±13% of the risk zone, respectively). **Conclusion:** Cooling induced by TLV afforded a potent cardioprotection and prevented transmural infarction following prolonged and severe ischemia, even when started later than a surface cooling in rabbits.

Key Words :

Cardioprotection ; Cooling ; Hypothermia ; Liquid ventilation ; Myocardial infarction; Ischemia.

Introduction

Myocardial cooling is known to limit infarct size in animal models of coronary artery occlusion and reperfusion.^{1, 2} The maximal myocardial salvage is afforded for a cardiac temperature of 32°C without major hemodynamic adverse effect.² To exert an optimal protection, such a target temperature should however be reached very fast after the onset of ischemia to effectively shorten the normothermic ischemic time.¹⁻³ Many experimental studies investigating cooling for cardioprotection have been performed with topical cooling using, *e.g.*, bags of iced saline on the epicardium.¹ This allows a rapid and selective cooling of the heart but this would not be feasible in patients. Other cooling strategies have therefore been experimentally investigated such as closed circuit of pericardioperfusion,⁴ blood extracorporeal cooling² or endovascular thermodes.⁵ The latter strategy has been also tried in patients undergoing primary coronary intervention but no infarct size reduction was observed in the overall analyses.⁶⁻⁸ One might speculate that this negative result was related to an insufficient rate of cooling.

More recently, total liquid ventilation (TLV) with temperature controlled-perfluorocarbons has been proposed to induce ultra-fast cardioprotective cooling in the experimental setting.³ Our group demonstrated that this strategy decreases myocardial temperature within 5 to 10 min and potently protects the heart against infarction and subsequent dysfunction in rabbits.^{3, 9} These data were obtained using an experimental model in which a non transmural infarction (~50% of the risk zone in normothermic conditions) was obtained with 30-min of coronary artery occlusion followed by reperfusion. One could however argue that to further demonstrate the relevance of this technically challenging strategy, it would be critical to determine whether TLV is actually more protective than a simpler and conventional external cooling using cold blankets.⁷ To better predict the potential

clinical benefit, we also need to determine whether TLV is still protective with prolonged durations of ischemia which will induce transmural myocardial infarction in normothermic conditions (*e.g.*, 60-min in anesthetized rabbits). Another important aspect is to investigate whether TLV can also reduce the extent of the no-reflow size which predicts subsequent remodelling and outcome.¹⁰ Accordingly, the goal of the present study was to compare the effects of cooling induced by TLV *vs* external cooling on myocardial infarct and no-reflow sizes following a prolonged 60-min ischemia in rabbits.

Methods

The animal instrumentation and the ensuing experiments were conducted in accordance with French official regulations after approval by the local ethical committee.

Animal surgery

New Zealand rabbits (2.5-3.0 kg) were anesthetized using zolazepam, tiletamine and pentobarbital (all 20-30 mg/kg i.v.). The animals were intubated, mechanically ventilated and a left thoracotomy was performed. A thermal probe was implanted within the left atrium and a suture was passed beneath a major branch of the left coronary artery. The ends of the ligature were passed through a short propylene tubing to form a snare. A coronary artery occlusion (CAO) was induced during 60-min by pulling the snare through the tubing. Reperfusion was subsequently induced by releasing the snare. The chest was then closed in layers.

Experimental protocol

All rabbits were submitted to 60-min of CAO and 4-h of reperfusion. As shown in Figure 1 (upper panel), they randomly underwent either a normothermic CAO (Control group) or cooling from the 5th min to the end of CAO (target left atrial temperature: 32°C). Cooling procedures were either external cooling using cold blankets (EC group) or ultrafast cooling initiated by 20-min of total liquid ventilation (TLV group). An additional group underwent a similar ultrafast cooling started at the 20th min of CAO (TLV_{delayed} group). After the onset of reperfusion, all hypothermic animals were rewarmed using infra-red lights and thermal pads. Throughout the protocol, arterial blood pressure and heart rate were monitored from a catheter implanted in the ear artery and from the electrocardiogram, respectively. Left atrial temperature was also recorded.

In TLV and TLV_{delayed} groups, rabbits were switched to liquid ventilation by filling the lung with perfluorocarbon and then connecting the endotracheal tube to the liquid ventilator. The perfluorocarbon was a mixture of perfluorobutyltetrahydrofuran and perfluoropropyltetrahydropyrene (RM101, Miteni, Milano, Italy). The ventilator was set to a tidal volume of 10-15 ml/kg body weight with 6 breaths/min. The perfluorocarbon mixture was bubbled with 100% O₂. This protocol has previously been demonstrated to maintain normal blood gases.^{3, 9} The temperature of the heat exchanger was adjusted to maintain the left atrial temperature at ~32°C.

Measurement of risk area, infarct size and no-reflow zones

After completion of reperfusion, the chest was reopened and thioflavine S (4%; 1.5 ml/kg) was rapidly infused through the left atrium. Rabbits were then sacrificed using pentobarbital followed by potassium chloride. After excision, the hearts were perfused retrogradely with Alcian blue (0.5%) and cut into slices. The risk zone was identified as the non-blue region. Slices were photographed using UV light to identify the region of no-reflow as the dark zone. They were then incubated in 1% triphenyltetrazolium chloride, fixed in formaldehyde and rephotographed. Infarcted area was identified as the TTC-unstained tissue. The areas of no-reflow, infarct and risk zone were blindly determined from the pictures by a single observer using planimetry (Figure 2C).

Statistical analysis

Values are expressed as means±SD. Hemodynamic parameters were compared using a two-way ANOVA for repeated measures. This was performed at 6 time points at baseline, during CAO (at 15th, 30th and 60th min) and reperfusion (at 1st and 4th h), with pairwise comparisons to the Control group at each time-point using a Student *t*-test and Bonferroni

correction. Temperatures were also compared between hypothermic vs Control groups using a one-way ANOVA at baseline and a two-way ANOVA for repeated measures during CAO and reperfusion. As numerous time-points were used for this last analysis (*i.e.*, 8 during CAO and 7 during reperfusion), we did not perform pairwise comparison at each time-point in order to avoid multiple comparisons. Risk zone (% left ventricle) were compared between groups using a one-way ANOVA followed by Student *t*-tests with Bonferonni Correction. No reflow and infarct sizes (% left ventricle) were compared using an ANCOVA with risk zone size as covariate. Significant differences were determined as $p < 0.05$.

Results

Thirty rabbits were initially included in the present study (10, 8, 6 and 6 in the Control, EC, TLV and TLV_{delayed} groups, respectively). Three and one animals died from ventricular fibrillation in the Control and EC group, respectively. Finally, twenty-six rabbits successfully underwent the complete protocol, *i.e.*, 7, 7, 6 and 6 in Control, EC, TLV and TLV_{delayed} groups, respectively.

As illustrated in Figure 1 (lower panel), left atrial temperatures were not significantly different among groups at baseline. In the EC group, the left atrial target temperature of 32°C was reached only at 60-min of CAO whereas this time-interval was dramatically reduced to 15 and 25-min of CAO in TLV and TLV_{delayed}, respectively (Figure 1). After the onset of reperfusion and warm-up initiation, all hypothermic animals similarly recovered normothermic values within two hours.

As shown in Table I, heart rate and mean arterial pressure were not significantly different among groups at baseline. Mean arterial pressure was also not significantly different between groups throughout the experimental protocol. Conversely, as soon as hypothermia was induced in EC, TLV and TLV_{delayed} groups, heart rate significantly decreased by -20% to -34% as compared to the normothermic Control values.

Risk zones were not significantly different among Control, EC, TLV and TLV_{delayed} groups (37±5%, 39±7%, 38±8%, 39±9% of the left ventricle, respectively). As shown in Figure 2, infarct and no-reflow sizes were significantly decreased in TLV and TLV_{delayed} but not in EC as compared to Control.

Discussion

The present study demonstrates that rapid cooling of the ischemic heart with TLV reduced infarct and no-reflow sizes after a 60-min coronary artery occlusion in rabbits. Importantly, this duration of ischemia induced a sub-maximal and transmural myocardial infarction in normothermic rabbits, with virtually no myocardial salvage by reperfusion. This result demonstrates that the protective effect of ultrafast cooling of the heart is not limited by any ceiling of cardioprotection following prolonged ischemia, as opposed to almost all the other well known cardioprotective approaches which lose their effect with increasing durations of ischemia.¹¹⁻¹³ This was further confirmed by the powerful protection against no-reflow.¹⁰

Interestingly, external cooling started early during ischemia was able to reach hypothermic levels at the end of CAO but this was not able to afford any significant cardioprotection. This suggests that hypothermia limited to the beginning of the reperfusion time is not protective, as previously described.^{1-3,14} In contrast, cooling with TLV was so protective that it was still protective even started at the 20th of ischemia. This further emphasizes that a cooling strategy must be designed to decrease the cardiac temperature as fast as possible to effectively shorten the normothermic period of ischemia. One would argue however that a longer duration at target temperature in the EC group could have led to different results. Target temperature was indeed reached only at the end of the 60-min CAO and rewarming was immediately started, concomitantly with reperfusion. We speculate however that this did not impact our results as hypothermia during only reperfusion failed to reduce infarct size in most experimental reports.^{1-3,14} We should also emphasize that we induced a fast rewarming in comparison to the clinical recommendations. This fast rewarming

can be considered as a limitation but this was used in order to recover normothermic values at the end of the protocol.

The present study further supports the clinical relevance of rapid cooling by liquid ventilation, as previously suggested.^{9,15,16} This would be of importance as several prototypes of total liquid ventilators have been developed and may be used in adults after technical improvements.^{17,18} By the present time, no clinical reports have properly investigated cooling using TLV in humans but several studies have tested other cooling strategies such as external or endovascular cooling. The first large scale clinical trial implementing cooling in the treatment of myocardial infarction was done using endovascular cooling devices in the COOL-MI and ICE-IT-1 studies.⁶⁻⁸ In both studies, there was no overall benefit regarding infarct size with cooling, probably according to insufficient cooling rate. Ultra-fast cooling with TLV would therefore afford a greater protection even if technically challenging. It would be even more relevant to protect the heart against myocardial infarction in patients resuscitated from cardiac arrest with underlying coronary disease.⁷ In this setting, one might predict that TLV would protect both the heart and the brain and would be less challenging than for the treatment of myocardial infarction. Recently, it was also importantly demonstrated that intra-arrest hypothermia induced by liquid ventilation facilitates resumption of spontaneous circulation in a swine cardiac arrest model.^{19,20}

In conclusion, the present study demonstrates that rapid cooling induced by TLV affords cardioprotection against prolonged and severe ischemia in rabbits, even when started later than a low rate surface cooling. This result also supports the need for further investigations of TLV in the resuscitation setting.

Conflict of Interest

No disclosure.

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Legends of figures

Figure 1

Experimental protocol (upper panel) and left atrial temperature recording throughout the protocol (lower panel)

*CAO, coronary artery occlusion; CAR, coronary artery reperfusion; EC, external cooling using ice-filled cold blankets from the 5th min of CAO to the end of CAO; TLV, cooling instituted by a 20 min episode of total liquid ventilation initiated at the 5th min of CAO; TLV_{delayed}, cooling instituted by a 20 min episode of total liquid ventilation initiated at the 20th min of CAO. * $p < 0.05$ vs Control throughout the protocol using a two-way ANOVA for repeated-measures with 8 time points during CAO and 6 during reperfusion, respectively. Pairwise comparisons at each time-point were not performed in order to avoid multiple comparisons.*

Figure 2

Panel A and B: Infarct and no-reflow sizes expressed as percentage of the risk zone in the different groups of rabbits. Open circles represent individual infarct sizes and closed circles represent the mean \pm SD of the corresponding group.

Panel C: Photographs of representative left ventricle slices of a rabbit in each group. Under halogen light (left column), areas lacking blue dye show the risk zone. Within this zone, the territories that were stained in red by triphenyltetrazolium chloride are salvaged. In contrast the gray territories are infarcted. Under UV light (right column), the myocardium that recovered perfusion after the ischemic episode is fluorescent and the areas of no-reflow are dark.

*See legend of Figure 1; * $p < 0.05$ vs Control and EC.*

Table I

Heart rate and mean arterial pressure during the experimental protocol

Groups	Baseline	Coronary artery occlusion			Reperfusion	
		15 min	30 min	60 min	1 h	4 h
<i>Heart rate (beats/min)</i>						
Control	240±23	236±17	236±21	216±16	235±18	230±33
EC	251±35	233±30	214±34	180±23*	223±37	252±28
TLV	244±23	159±19*	161±18*	173±20*	225±26	239±17
TLV _{delayed}	248±26	242±21	156±15*	160±18*	206±40	229±29
<i>Mean arterial pressure (mmHg)</i>						
Control	63±8	65±10	68±11	57±14	62±14	58±6
EC	66±8	64±6	61±6	63±9	59±6	60±8
TLV	67±9	60±2	58±8	58±10	59±16	57±9
TLV _{delayed}	68±10	71±4	61±9	64±12	66±6	58±4

CAO, coronary artery occlusion; EC, external cooling using ice-filled cold blankets from the 5th min of CAO to the end of CAO; TLV, cooling instituted by a 20 min episode of total liquid ventilation initiated at the 5th min of CAO; TLV_{delayed}, cooling instituted by a 20 min episode of total liquid ventilation initiated at the 20th min of CAO. * $p < 0.05$ vs corresponding Control value.

FIGURE 1

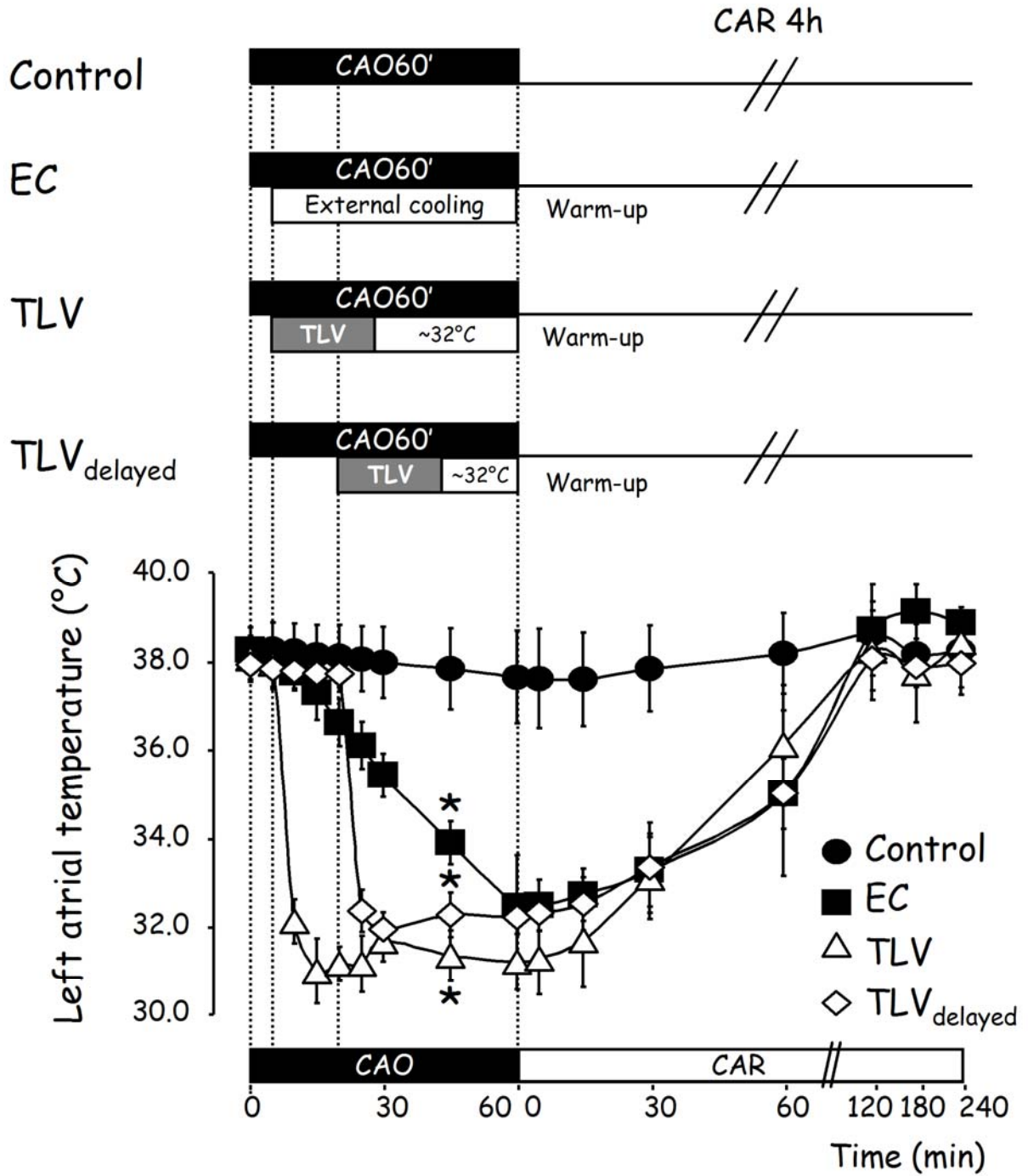


FIGURE 2

