Original Article
Intracoronary hypothermia for acute myocardial infarction in the isolated beating pig heart

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Abstract: Hypothermia may attenuate reperfusion injury and thereby improve acute myocardial infarction therapy. Systemic cooling trials failed to reduce infarct size, perhaps because the target temperature was not reached fast enough. The use of selective intracoronary hypothermia combined with intracoronary temperature monitoring allows for titrating to target temperature and optimizing the cooling rate. We aimed to the test the feasibility of intracoronary cooling for controlled, selective myocardial hypothermia in an isolated beating pig heart. In five porcine hearts the left anterior descending artery (LAD) was occluded by an over-the-wire balloon (OTWB). After occlusion, saline at 22°C was infused through the OTWB lumen for 5 minutes into the infarct area at a rate of 30 ml/min. Thereafter the balloon was deflated but infusion continued with saline at 4°C for 5 minutes. Distal coronary temperature was continuously monitored by a pressure/temperature guidewire. Myocardial temperature at several locations in the infarct and control areas was recorded using needle thermistors. In the occlusion phase, coronary temperature decreased by 11.4°C (range 9.4-12.5°C). Myocardial temperature throughout the infarct area decreased by 5.1°C (range 1.8-8.1°C) within three minutes. During the reperfusion phase, coronary temperature decreased by 6.2°C (range 4.1-10.3°C) and myocardial temperature decreased by 4.5°C (range 1.5-7.4°C). Myocardial temperature outside the infarct area was not affected. In the isolated beating pig heart with acute occlusion of the LAD, we were able to rapidly "induce, maintain, and control" a stable intracoronary and myocardial target temperature of at least 4°C below body temperature without side effects and using standard PCI equipment, justifying further studies of this technique in humans.

Keywords: Intracoronary hypothermia, acute myocardial infarction, reperfusion injury

Introduction

Early reperfusion by primary percutaneous coronary intervention (PCI) remains the cornerstone of treatment for acute myocardial infarction (AMI) [1-3]. Nevertheless, despite timely reperfusion 7-10% of AMI patients die during the index hospitalization and 23% of survivors develop heart failure within one year [4, 5].

Besides ischemia-induced myocardial necrosis, considerable further damage to the myocardium may occur due to the reperfusion process itself [6]. This reperfusion injury contributes substantially to the final infarct size [7]. Despite many advances made in revascularization therapies, the prevention and treatment of reperfusion injury in AMI has not proven successful in clinical practice.

Mild hypothermia, i.e. cooling of endangered myocardium to 33°C (91.4°F), may attenuate reperfusion injury [8]. Animal studies in a variety of species have shown that induction of hypothermia prior to reperfusion reduces infarct size [9-23]. However, if hypothermia is initiated after the onset of reperfusion, this benefit is not observed [12, 24-26]. Human studies in patients with AMI have not demonstrated a reduction in infarct size using different methods of systemic hypothermia [27-36]. This is likely related to the fact that, in the majority of patients, sufficient hypothermia was not reached before the onset of reperfusion [27-31]. In most of these studies, whole body hypothermia was induced by an endovascular cooling system in combination with administration of cold saline or noninvasive surface cooling [29]. This mode of delivery hampered rapid

and sufficient cooling of the endangered myocardium and led to adverse systemic reactions such as shivering, enhanced adrenergic state, or volume overload [31, 37].

Selective intracoronary induction of hypothermia preceding primary PCI may greatly reduce the time needed to achieve therapeutic hypothermia while simultaneously preventing adverse systemic effects. The intracoronary application of hypothermia has already been investigated in two animal studies demonstrating safety, feasibility, and a significant reduction in myocardial necrosis [9, 38].

In those studies, however, achievement of sufficient hypothermia required up to 60 minutes-too much delay for clinical application in humans with AMI. Furthermore, only myocardial temperature was monitored by external thermistors implanted in the myocardium, which is fundamentally not possible during primary PCI in humans.

In the present study we aimed to: (1) evaluate the feasibility and reproducibility of selective intracoronary hypothermia in isolated beating pig hearts during occlusion and subsequent reperfusion of the left anterior descending coronary artery using regular PCI equipment; and (2) study the relationship between intracoronary temperature distal to the occlusion, measured by a pressure/temperature guidewire (PTW), and distribution of hypothermia across the endangered myocardium, measured by thermistors. Knowledge of such distribution is mandatory to apply hypothermia safely in humans, where only intracoronary temperature can be measured.

This study was performed to test only the methodology of rapid selective intracoronary cooling using regular PCI equipment but did not aim to say anything about outcome. That should be the goal of further studies.

Methods

In humans a target temperature of 33°C (4°C below normal blood temperature) has been suggested to protect against reperfusion injury and has proven to be safe [39-41]. By analogy our present study sought to lower the myocardial temperature throughout the infarct area by 4°C. Such a decrease of temperature should be

obtained quickly during occlusion and maintained after reperfusion. Moreover, the temperature decrease should be limited to the infarct area.

Therefore, prior to the present study, we performed 2 dose-finding experiments to search for the most appropriate saline temperature and infusion rate. Our goals included both induction and maintenance: to reach the target temperature rapidly but without the risk of decreasing temperature too much; and to keep this temperature stable in the infarct-related artery and its dependent myocardium throughout the occlusion and reperfusion phases. The infusion rate and temperature of saline in this study are based upon those dose-finding experiments.

Isolated beating pig heart model

In this study hearts were obtained from Dutch landrace hybrid pigs slaughtered for human consumption. Following regular slaughterhouse protocols, the chest was opened to extract the heart. The isolated heart was immediately cooled and cannulated to administer 1 L of cold cardioplegic solution (4°C modified St. Thomas 2 added with 5000 IU of heparin) via the coronary arteries, such that warm ischemic time never exceeded 5 min. Further preparation was carried out according to the standards of our isolated beating heart pig model as previously described [42, 43].

After preparation the isolated pig heart was fixed in a supine position and mounted on an external circulation platform [42]. The circulation loop consisted of a centrifugal pump (Sarns 9000 Perfusion System, 3 M, St. Paul, MN, USA) that drew blood from a venous reservoir through an arterial filter (AFFINITY Arterial 38 mm blood filter, Medtronic, Minneapolis, MN, USA) and a combined oxygenator-heat exchanger (AFFINITY NT Oxygenator, Medtronic) retrogradely into the aortic cannula. Blood was kept at 38°C and oxygenated with a 20% O_2, 5% CO_2, 75% N_2 carbogen gas mixture. The heart started to have spontaneous contractions and gradually regained sinus rhythm after coronary perfusion was reinstated. In some cases, defibrillation was required with 10-30 J to restore sinus rhythm. An external pacemaker (Medtronic Model 5388) was connected to the left atrium to maintain a stable heart rate as necessary.
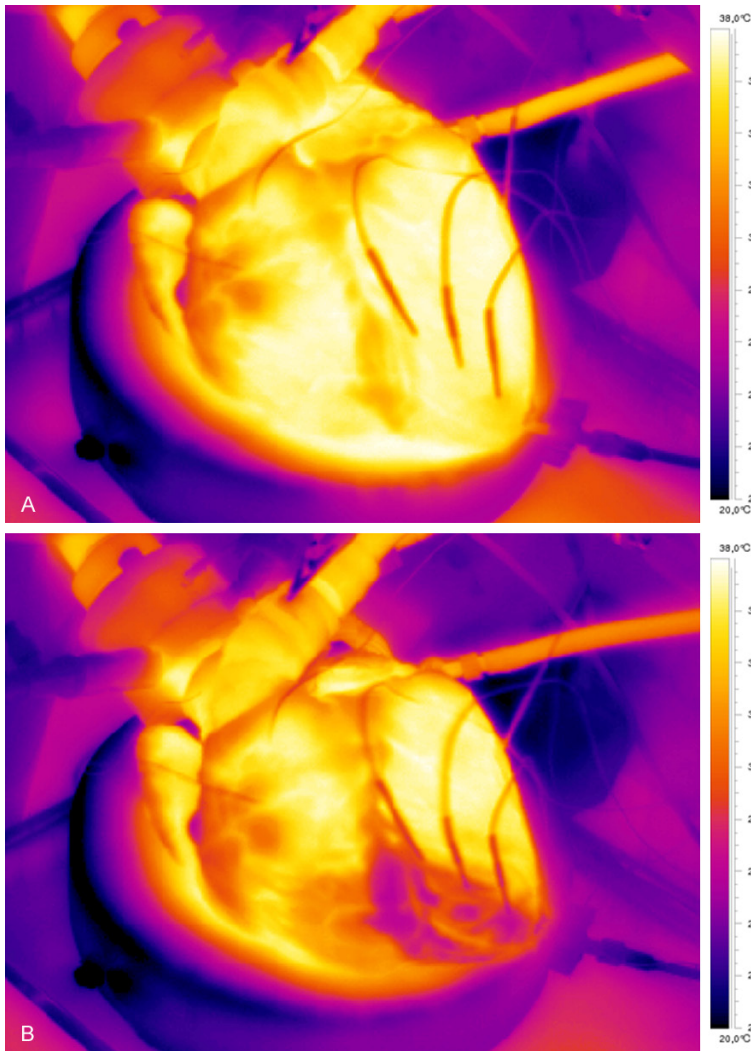
Figure 1. Visualization of the infarct area by infrared camera before (A) and during (B) selective infusion of cold saline through the balloon catheter in the occluded coronary artery. Moving images of the isolated beating heart and recordings of the cooled infarct area by the infrared camera are presented in the supplemental appendix online.

St Jude Medical, Minneapolis, MN) was advanced through this guiding catheter and positioned in the distal LAD. Next, a regular guide wire was also advanced into the distal LAD. Next an over the wire balloon (OTWB, Apex, Boston Scientific, 12×2.5-3.5 mm) was advanced over this second wire and positioned in the mid LAD, whereafter that second wire was removed. In this way the OTWB allowed us both to create a mid-LAD occlusion, and to infuse saline through its lumen selectively into the infarct area. The pressure/temperature guidewire was kept in place in the distal coronary artery for continuous temperature recording. Saline was infused using an infusion pump (Medrad® Mark V ProVis® Angiographic system) with an adjustable infusion rate.

A thermal imaging camera (FLIR ThermaCam Researcher Professional S65HS) was used to delineate the cooled infarct area after a short infusion of cold saline (**Figure 1**). Based upon those images, three intramyocardial thermistors (HYP-1 connected to TC-08 thermocouple data logger, OMEGA Engineering INC, Connecticut, USA) were placed within the infarct area: proximal (close to the occlusion), mid (center of infarct area) and distal (close to the apex), respectively (**Figure 2**). A fourth thermistor was placed in the non-infarcted myocardium as a control and a fifth in the proximal great cardiac vein to determine coronary venous blood temperature (**Figure 2**).

After having completed these preparations, the beating heart was completely submerged in a 38°C (100°F) water bath to simulate the surrounding body temperature. Finally, 100 mg of amiodarone was added to the circulating blood to prevent ischemia-related rhythm disturbanc-

Blood gas values and electrolytes were monitored using a VetScan i-STAT 1 (Abaxis, Union City, CA, USA). Coronary blood flow was measured by an ultrasonic flow probe (em-tec Sonno TT 1/2"×3/32", em-tec GmbH, Finning, Germany) with a flow amplifier (HFM-10-1, Life-Tec Group, Eindhoven, The Netherlands).

Catheterization and thermistors

The following specific instrumentation was applied in this study: A 6 F guiding catheter was advanced to the ostium of the left coronary artery and affixed to the aortic wall. The pressure/temperature wire (Pressurewire Certus®,
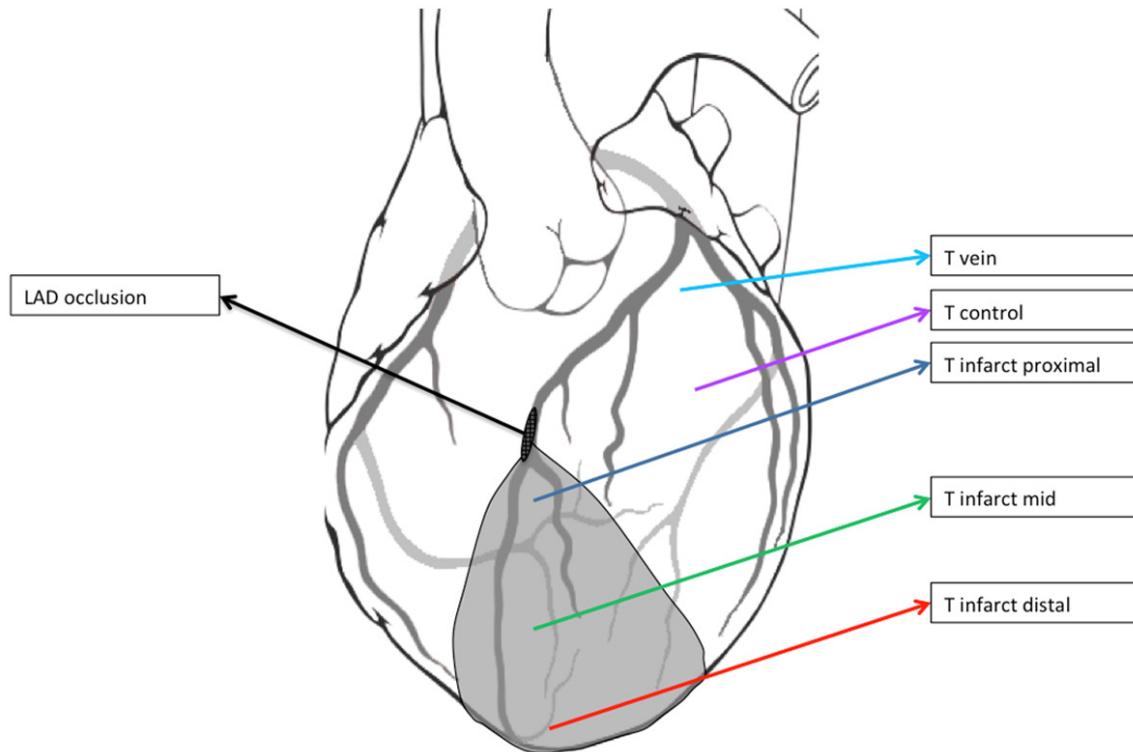
Figure 2. Overview of the thermistor position. The grey zone indicates the infarct area. The black oval indicates the site of occlusion in the Left Anterior Descending Artery (LAD). *T infarct proximal*: thermistor close to the site of the LAD occlusion; *T infarct mid*: thermistor at the mid infarct area; *T infarct distal*: thermistor at the distal infarct area; *T control*: thermistor in the non-infarcted myocardium; *T vein*: thermistor in the proximal great cardiac vein. In addition, the guidewire with temperature sensor is present within the distal lumen of the coronary artery (Not shown).
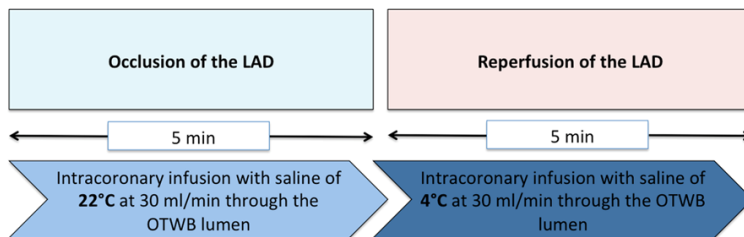

Figure 3. Intracoronary hypothermia protocol.

es as these frequently occur in pigs during occlusion of a coronary artery.

Using this setup, temperature was continuously monitored at 6 locations: distal coronary artery, 3 myocardial positions throughout the infarct area, reference myocardium, and the great cardiac vein.

Experimental protocol

At first the balloon in the LAD was inflated to 4 atmospheres to create coronary occlusion. Immediately thereafter, infusion of 22°C saline

(room temperature) at a rate of 30 ml/min was started and maintained during the next 5 minutes of balloon occlusion. Next, the balloon was deflated, and anterograde blood flow was restored while the infusion of saline was continued at a temperature of 4°C at a rate of 30 ml/min for another 5 minutes (**Figure 3**). After an interval of 20 minutes, the whole sequence was repeated.

At the end of the experiment after the heart had died, methylene blue staining was administered through the inflated balloon to delineate the infarct area post-mortem and to determine the mass of the infarct related territory.

Statistics

Data are expressed as mean with range. Repeatability within an experiment was assessed

Table 1. Baseline characteristics of the five isolated pig heart models

Heart	1	2	3	4	5
Heart rate (bpm.)	126	91	77	102	105
Blood temp (°C)	38.3	38.5	37.7	37.2	38
Area at risk (g, %)	42 (17%)	38 (15%)	55 (22%)	60 (24%)	45 (18%)

The area at risk was determined at the end of the experiments by methylene blue staining of the infarct area and is expressed as a percentage of the mean left ventricular mass of a domestic pig. Bpm = beats per minute.

Table 2. Top, time to induce the target temperature in the distal coronary artery and at 3 sites of the infarct area

	Occlusion mean [range]		Reperfusion mean [range]	
Time to achieve 34°C (s)				
Coronary artery	4.6	[3.6-5.1]	-	-
Infarct area				
Proximal	56	[37.5-89.5]	-	-
Mid	77.1	[42.3-130.6]	-	-
Distal	110.1	[62.9-176.4]	-	-
Temperature in °C				
Coronary artery	26.5	[25.4-28.5]	31.7	[27.6-33.8]
Infarct area				
Proximal	32.6	[29.9-36.4]	33.8	[30.0-37.7]
Mid	33.0	[29.3-35.6]	33.7	[30.5-37.7]
Distal	32.7	[30.3-36.4]	32.8	[30.9-33.7]
Great cardiac vein	34.4	[33.5-36.0]	34.7	[33.6-35.5]

Bottom, blood and myocardial temperatures during the occlusion and reperfusion phases. Temperature in the reference myocardium remains unchanged and normal during all experiments.

as the relative difference between two consecutive measurements. In this study, reproducibility was defined as the capability to replicate the same temperature courses among the five experiments using the predefined protocol.

Results

Baseline characteristics and procedural results

Baseline characteristics of the five hearts are presented in **Table 1**. The isolation, instrumentation, resuscitation, and catheterization was uncomplicated and the experimental protocol could be performed in all 5 hearts.

In all hearts, infrared camera recordings allowed excellent delineation of the infarct area (**Figure 1**, Supplementary Video).

Coronary and myocardial temperature during saline infusion

The target myocardial temperature of 4°C below blood temperature was obtained in all hearts with the exception of the first one, in which the mean temperature decrease was 3.5°C. The mean time intervals to target temperature in the coronary artery and the different parts of the infarct area are listed in **Table 2**.

An example of the temperature recordings at all relevant sites during occlusion and reperfusion is illustrated in **Figure 4** and a mean graph of all hearts is shown in **Figure 5**.

During the occlusion phase, temperature in the distal coronary artery decreased by 11.4°C (range 9.4-12.5°C) and myocardial temperature by 5.3°C (range 1.5-8.0°C), 4.9°C (range 2.3-8.6°C) and 5.2°C (range 1.5-7.6°C) in the proximal, mid, and distal infarct areas, respectively. Temperature in the non-infarct reference area was not affected and remained unchanged during occlusion. Temperature in the great cardiac vein decreased by 2.9°C (range 1.0-4.2°C).

During the reperfusion phase, temperature in the distal coronary artery decreased by 6.2°C (range 4.1-10.3°C) and myocardial temperature decreased by 4.1°C (range 0.2-7.9°C), 4.2°C (range 0.2-7.4°C) and 5.1°C (range 4.2-7.0°C) in the proximal, mid, and distal infarct areas, respectively. Temperature in the non-infarct reference area was not affected and remained unchanged during reperfusion. Temperature in the great cardiac vein decreased by 2.6°C (range 1.5-3.4°C) (**Table 2**).

During the very short time needed to change the infusion of saline from room temperature to 4°C, only coronary temperature increased, but myocardial temperature remained close to target temperature (**Figures 4**, **5**).
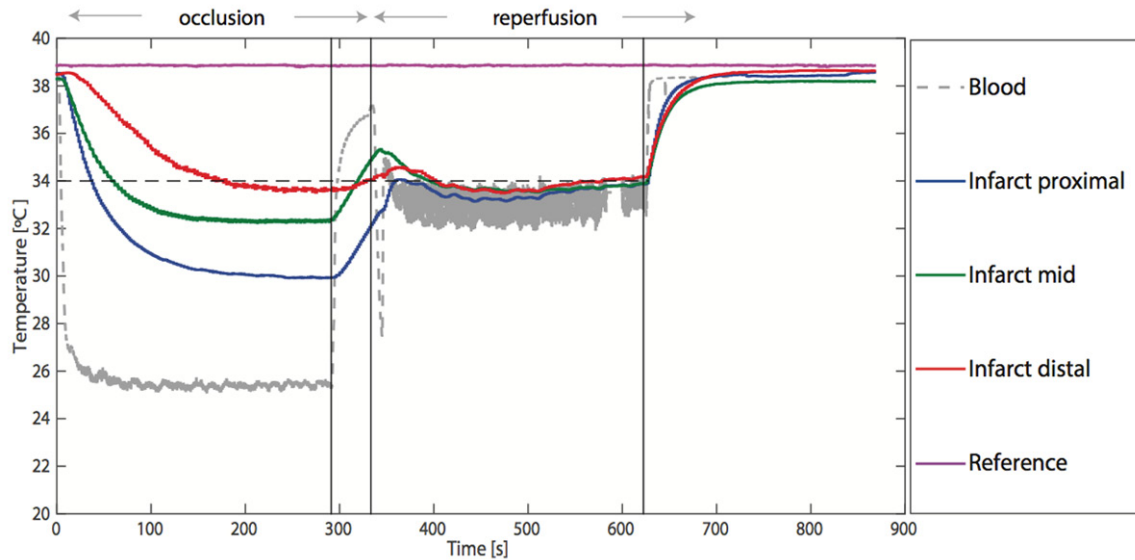
Figure 4. Continuous registration of the temperatures in the coronary arterial blood, the proximal infarct area, the mid infarct area, the distal infarct area, and the reference myocardium during one experiment. After the coronary artery is occluded, there is a rapid induction of the target temperature in the infarcted myocardium during an infusion of 30 ml/min saline at 22°C. The temperature remains stable during the occlusion phase. In the reperfusion phase (middle vertical line), the infusion rate is maintained at 30 ml/min, but the temperature is lowered to 4°C. This results in a stable myocardial temperature in the infarct area. In the reperfusion phase, the temperatures at the different sites within the infarct area are closer to the temperature in the coronary artery. This is explained in the discussion. During the very short time needed to change the temperature of the infused saline (between the left and middle vertical line), only coronary temperature increases rapidly, but myocardial temperature remains at the target temperature. Temperatures normalize rapidly after stopping saline infusion during the reperfusion phase (right vertical line).

After stopping the infusion of saline, all temperatures returned to normal within 60 seconds.

The relation between distal coronary temperature and myocardial temperature-important for safe application of this technique in humans with AMI-was reproducible in all hearts, but differed between the occlusion and reperfusion phase (**Figure 4**), which will further be explained in the discussion.

Repeatability and reproducibility

With the exception of the first heart, the cooling protocol was performed twice in each heart with an interval of 20 minutes as described in the methods. Relative differences between the two measurements of myocardial temperatures were small and are shown in Supplementary Table 1. The temperature courses of the 5 experiments were similar and reproducible.

Discussion

In this beating pig heart model we demonstrated the feasibility of selective intracoronary infusion of cold saline as a method to achieve myocardial hypothermia for potentially limiting reperfusion injury. Using routine equipment of an over-the-wire balloon infusion catheter and an intracoronary pressure/temperature wire, we were able to cool the endangered myocardium selectively, rapidly, and stability without side effects. At an infusion rate of 30 ml/min saline at a temperature of 22°C in the occlusion phase and 4°C in the reperfusion phase, the target temperature of 34°C was rapidly achieved and maintained. Repeatability was excellent.

In addition, and in contrast to earlier studies in this field, the saline infusion rate was primarily determined and safe-guarded by intracoronary temperature measurement using routine PCI equipment. Our experimental setup allowed the relationship between coronary temperature distal to the occlusion and myocardial temperature to be carefully assessed.

Currently there is no effective therapy for preventing reperfusion injury in patients with acute myocardial infarction. Factors responsible for
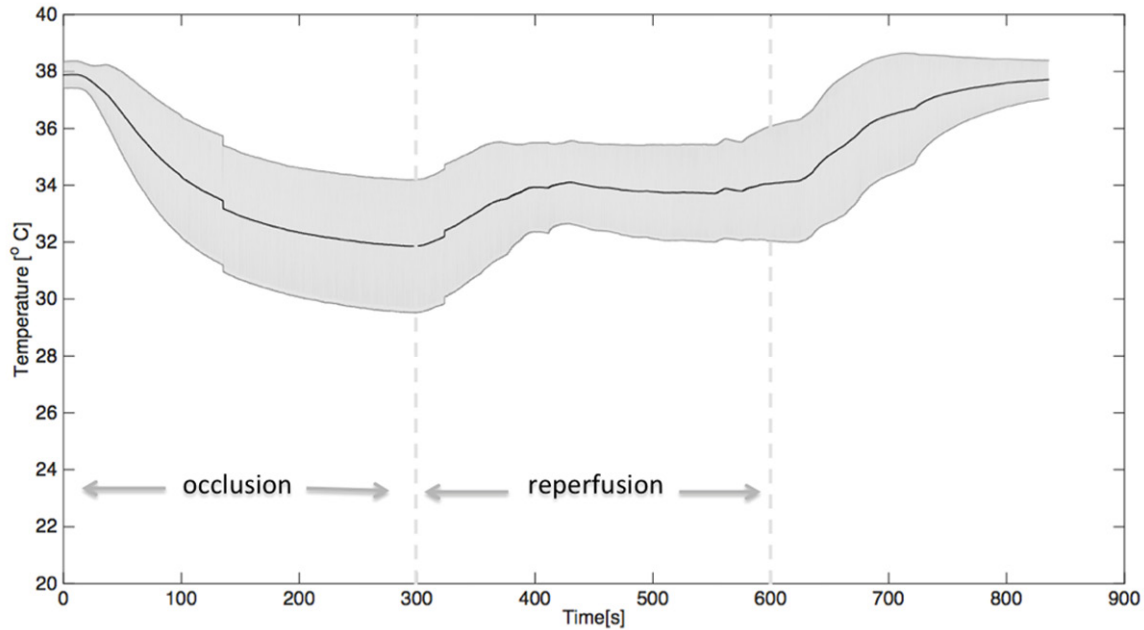
Figure 5. Graph demonstrating the mean ± SEM of the temperature in the infarct area recorded in the five experiments. At 300 seconds, the first dotted line indicates the start of reperfusion when changing the infusion temperature from 22°C to 4°C. After 600 seconds (the second dotted line) the infusion of cold saline is stopped, whereafter temperatures normalize within minutes.

this complex cascade of reperfusion-induced myocardial necrosis include oxidative stress, mitochondrial dysfunction, calcium overload, depletion of high-energy phosphates, infiltration of leucocyte aggregates with activation of cytokines and vasoconstrictors, and intra-myocardial edema with no-reflow [6, 7, 44-47]. Therefore, the pathophysiology of reperfusion injury offers numerous therapeutic windows of opportunity, but all clinical studies focusing on a single part of the cascade (such as the use of anti-oxidants, calcium-channel blockers, anti-inflammatory agents and cyclosporine) have not been able to prove a clear benefit.

This absence of effect may be due to several factors including treating an epiphenomenon instead of a true pathophysiologic part of the cascade, intolerable delay in door-to-balloon time, or maybe because the therapeutic agents only reach the target tissue (i.e. infarcted myocardium) after reperfusion has occurred.

Hypothermia has proven to affect almost every pathway involved in reperfusion injury and has proven to be effective in a number of experimental studies, but is limited by the time needed to reach the target temperature and unde-

sirable side-effects (such as shivering, enhanced adrenergic state, and iatrogenic volume overload) when using whole body cooling. Moreover, it is unclear to what extent the endangered myocardium behind an occluded coronary artery is truly cooled.

Our study is the first to overcome these limitations, enabling fast and selective cooling, and reaching specifically the target tissue before onset of reperfusion, while being able to monitor the temperature in the distal coronary artery.

Unique strengths of the present study

The methodology used in the present study has some unique advantages compared to earlier studies in this field. For safe and effective application of selective hypothermia to protect endangered myocardium from reperfusion injury in humans, three conditions are paramount. First, the endangered myocardium should be cooled to the target temperature quickly enough to avoid delay in PCI and opening of the vessel. With our protocol, a decrease by 4°C below body temperature was achieved within minutes (**Figures 4** and **5**).

Second, the cooling must be limited to the infarct area and not affect the adjacent healthy myocardium. Using the infrared camera and thermistors in the control area, we confirmed that hypothermia was limited to the perfusion territory of the infarct-related artery (as expected because cold saline is administered only distal to the occlusion).

Third, and maybe most importantly, to apply such a technique safely in humans it is paramount to monitor and control the temperature in the distal coronary artery and the myocardium. This was achieved in our study by the presence of a commercially available pressure/temperature sensor-tipped guidewire in the distal coronary artery and by showing a reproducible relationship between distal coronary artery temperature and temperature in the endangered myocardium.

Thus in contrast to earlier studies, the paramount conditions for clinical application of intracoronary hypothermia were fulfilled in the present study.

In addition, the total volume of saline is limited in our protocol to 300-600 ml, thereby avoiding volume overload.

Relation between coronary and myocardial temperature during cooling

Of special interest is the relationship between distal coronary temperature and myocardial temperature during saline infusion throughout the occlusion and the reperfusion phases, as demonstrated in **Figures 4** and **5**.

During the occlusion phase, coronary blood flow is interrupted and cooling of the myocardium occurs mainly by the physical phenomenon of heat-conduction. Consequently, there is a temperature gradient between the coronary artery and the myocardium.

During the reperfusion phase, distal coronary blood flow is present again and cooling of the dependent myocardium occurs mainly by heat-convection. Consequently, only a minimal temperature gradient is present between the coronary artery and the surrounding myocardium. These phenomena also explain why the adjacent, non-infarcted myocardium does not cool down, as it is the convection heat from the non-infarcted coronary artery that maintains

normal temperature in the reference area. Understanding of these phenomena and a reproducible relation between distal coronary and myocardial temperature is important for safe application in humans, when the rate of cold saline infusion needs to be guided by the achieved coronary temperature.

Study limitations

Our study has several limitations.

First, although the isolated beating pig heart is an elegant way of replacing classical in vivo animal studies of simulating acute myocardial infarction, ex vivo testing is not equivalent to in vivo testing. Not all physiologic feedback mechanisms remain intact in the isolated heart and unknown differences may be present [48].

Second, there is a difference between an artificial LAD occlusion in an isolated beating pig heart and a myocardial infarction in humans. The absence of noticeable side effects in the pig heart, which is usually more vulnerable to ischemic arrhythmia than a human heart, is reassuring.

Third, it has not been firmly established that a myocardial temperature of 4°C below body temperature, is the most effective temperature for preventing reperfusion injury. The choice of 4°C is therefore somewhat arbitrary. Nevertheless, because of other studies with hypothermia both in animal and in human organs, like the brain, and based upon data from cardiac surgery, we selected a decrease of 4°C as the target for this study [39, 49].

Fourth, it is unclear when hypothermia should be started and how long it should be continued. From a theoretical point of view, it seems plausible to assume that cooling time before opening the occluded coronary artery can be short and that the major condition here is that the temperature has decreased sufficiently at the moment when reperfusion occurs. It is unknown how long after reperfusion hypothermia should be continued. The post-reperfusion hypothermia period of 5 minutes in our study was chosen because of practical considerations and is arbitrary.

Fifth, it should be emphasized that this study focuses only on the method of applying selective intracoronary hypothermia and did not

evaluate outcome effects on infarct size compared to normothermia or standard therapy. The logical next step is assessing of safety and feasibility of this method in human subjects with acute myocardial infarction. Such study is currently performed, using exactly the same interventional methodology as in the present study (ClinicalTrials.gov. Identifier NCT02753478). Only thereafter, a larger randomized controlled outcome study comes into perspective as proof-of-principle. The present study is a first but mandatory step in such promising development and justifies further investigations.

Conclusion

In the isolated beating pig heart model of acute myocardial infarction, controlled and selective myocardial hypothermia can be achieved rapidly, safely, stable, and effectively. Our technique adds the advantage of minimal volume load and uses standard PCI equipment.

This study justifies further investigation on the use of intracoronary hypothermia in patients with acute myocardial infarction to reduce reperfusion injury and infarct size.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. Temperature of the coronary blood and the infarct area (proximal, mid and distal) measured during the occlusion (200 seconds) and reperfusion phases (600 seconds)

	Blood	Proximal	Mid	Distal
Exp1	26.13	35.02	36.25	36.77
Δ%	-	-	-	-
Exp2a	25.45	30.19	32.36	34.17
Exp2b	25.72	30.58	32.39	34.45
Δ%	-1.1	-1.3	-0.1	-0.8
Exp3a	26.91	30.76	30.54	31.83
Exp3b	25.87	29.64	29.21	30.18
Δ%	3.9	3.6	4.4	5.2
Exp4a	28.23	33.03	33.59	33.62
Exp4b	26.6	32.43	33.33	33.36
Δ%	5.8	1.8	0.8	0.8
Exp5a	25.97	36.92	35.43	30.32
Exp5b	25.23	37.02	35.94	30.55
Δ%	2.8	-0.3	-1.4	-0.8

Temperatures measured at 200 seconds from the start of the protocol.

	Blood	Proximal	Mid	Distal
Exp1	33.73	33.45	33.7	33.73
Δ%	-	-	-	-
Exp2a	33.9	33.72	33.73	34
Exp2b	33.02	33.82	33.65	34.13
Δ%	2.6	-0.3	0.2	-0.4
Exp3aa	33.88	33.61	33.44	33.56
Exp3b	33.71	34.15	34.17	34.01
Δ%	0.5	-1.6	-2.2	-1.3
Exp4a	28.33	30.19	30.9	31.09
Exp4b	31.75	33.46	33.9	34.32
Δ%	-12.1	-10.8	-9.7	-10.4
Exp5a	29.9	37.62	36.73	31.99
Exp5b	29.91	37.57	36.72	31.48
Δ%	-0.03	0.1	0.03	1.6

Temperatures measured at 600 seconds from the start of the protocol. Repeatability is expressed as the relative difference between two consecutive measurements (delta%). In the first heart no repeatability experiment was performed.

Supplementary Video. Infrared camera recordings showing excellent delineation of the infarct area. There is no myocardial temperature decrease observed outside the infarct area.