The small chill: mild hypothermia for cardioprotection?

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1- Introduction

Therapeutic whole body hypothermia has been considered for centuries.[1] For example, the Russian approach to resuscitation of a patient in cardiac arrest in 1803 consisted of covering him with snow and then hoping for the resumption of a spontaneous circulation.[1] A century and a half later, the beneficial effect of therapeutic hypothermia during cardiac surgery was proven in canine models.[2, 3] Outside of the operating room, hypothermia has been demonstrated to protect the brain following cardiac arrest in both animal models and humans.[4, 5] The American Heart Association and the European Resuscitation Council both recommend the use of hypothermia in comatose patients resuscitated from cardiac arrest to improve the subsequent neurological recovery.[6, 7]

Beside hypothermia's beneficial effect during cardiac surgery or following cardiac arrest, it has also been clearly demonstrated that even very mild hypothermia can greatly increase the heart's tolerance to myocardial ischemia resulting in decreases of infarct size in animal models of coronary artery occlusion. Mild hypothermia (body temperature down to 32 ˚C) has the advantage that the heart continues to pump normally and no extracorporeal support is needed. However, despite the encouraging results in animals, clinical trials of mild hypothermia in patients being revascularized for acute myocardial infarction have yielded surprisingly disappointing effects on infarct size.[8-10] One goal of the present review is to consider why past trials have failed and propose what might be done to make cooling-induced cardioprotection more effective. We will review the literature to see if an optimal target temperature and cooling method can be recommended. Finally, the mechanism of mild cooling's protection will be revisited. While deep hypothermia stops the heart and clearly preserves ATP during ischemia, cooling to 34°C has only a modest effect on ATP depletion during ischemia [11] but it is as protective to the rabbit heart as is ischemic preconditioning. [12] That has caused some to suspect that the mechanism of the

cardioprotective effect of mild hypothermia might be more complex than energy preservation alone.[13]

2. Physiological effects of mild hypothermia

Hypothermia has distinct physiological effects. Hypothermia can be classified as mild (32-35°C), moderate (28-32°C), severe (20-28°C) or profound (<20°C). Mild hypothermia is the only one to be used for a whole-body therapeutic purpose as lower temperatures are increasingly life-threatening. Below 28°C, the heart spontaneously fibrillates in most mammalian species. Conversely, mild whole-body hypothermia is remarkably well tolerated by both animals and humans. The physiological effects of mild hypothermia include a decrease in heart rate and a subsequent fall in cardiac output (-7% for each °C),[3, 14] while stroke volume and mean arterial pressure remain unchanged. Cooling of the skin provokes an increase in systemic vascular resistance. With respect to regional hemodynamics, cerebral blood flow and intracranial pressure decrease,[3, 14] whereas renal blood flow and subsequent diuresis are increased.[15] Mild hypothermia also attenuates the cerebral metabolic rate (-6 to -7% for each °C).[16] Intestinal motility is also depressed by mild hypothermia. Blood pH changes by +0.016 unit for each decrease of 1°C, and serum potassium decreases as a result of its enhanced cellular uptake.[17] Hypothermia-induced hypokalemia should be corrected with caution during hypothermia since raising serum K^+ usually leads to hyperkalemia during subsequent rewarming. Other adverse effects include increased infection risk, a mild coagulopathy[17] and hyperglycemia caused by decreased plasma insulin levels.[18]

3. The infarct limiting property of cooling: experimental *in vivo* investigations

3.1. Proof of concept of the myocardial infarct-limiting property of hypothermia

Heart temperature is known to be a major determinant of infarct size in animal models of acute myocardial ischemia.[19-21] As an example, infarct size resulting from 30 min of regional myocardial ischemia in *in situ* rabbit hearts decreases by 8% of the risk zone for each °C decrement.[21] The infarct-limiting property of mild hypothermia has been confirmed in several species including dogs,[19] rabbits,[12, 21-25] pigs[20, 26-28] and rats.[29]

3.2. What is the optimal target temperature?

Importantly, there is no threshold temperature for the cardioprotective effect of hypothermia.[19-21] In fact, warming the heart above normothermia extends infarct size by the same degree as cooling reduces it.[21] For example, Hamamoto et al.[30] demonstrated in sheep submitted to 60 min of coronary artery occlusion that infarct size was progressively reduced when cardiac temperature during ischemia and reperfusion was lowered by 1°C decrements from 39.5 to 35.5°C. As shown in Table I, temperature decrement during ischemia within the mild hypothermic range has demonstrated a powerful anti-infarct effect in many studies. Figure 1 illustrates cooling to only 35°C at the onset of ischemia elicits ~70% decrease in infarct size following 30 min of regional ischemia in the open-chest rabbit.[12] And protection is still realized if the onset of cooling is delayed for 10 min. Cooling the heart to 32°C at the onset of ischemia essentially prevented infarction, and a protective effect was still evident when cooling was initiated as long as 20 min after the onset of ischemia and, therefore, 10 min before reperfusion.[12] Furthermore, even when the ischemic period was extended to 60-120 min and cooling to 30-32°C was initiated 20-30 min after the onset of ischemia, the infarction process appeared to be halted when cooling was started and significant myocardial salvage was realized.[12, 22] And the heart continued to beat strongly and support the rabbit's circulation.

3.3. What is the window of protection?

Cooling the heart during the ischemic period reduces infarct size. Very early studies from the Corday laboratory[31, 32] clearly showed that if the dog heart were retrogradely

perfused with cooled arterial blood through the coronary sinus beginning 30 min after coronary occlusion and persisting for the remaining 2½-3 hr of the ischemic period, infarct size was decreased by 65-90%. Table 1 reveals that the degree of protection afforded by cooling is not only determined by the depth of cooling but also by its duration during the ischemic period. Miki et al.[12] demonstrated, for example, that the magnitude of infarct size reduction decreased when the onset of hypothermia was delayed from the beginning to the 20th min of a 30-min period of ischemia in rabbits. As illustrated in Figure 1, cooling during just the last 10 minutes of ischemia still afforded significant cardioprotection when the target temperature was 32°C but not when it was 35°C. Figure 2 merges the results of several studies investigating the effect of hypothermia to 32°C initiated at different times during a 30-min period of ischemia in rabbits, again illustrating that cardioprotection is attenuated when the onset of cooling is delayed. This raises a major logistical problem in the clinical setting since these patients with acute myocardial infarction present with ischemia already in progress. To significantly shorten the normothermic ischemic time cooling would have to be accomplished very soon after arrival in the hospital and efforts to effect early cooling must not come at the expense of increasing the time to reperfusion. That schedule has been difficult to implement.

Cooling the body at the time of reperfusion or beyond seems to be protective in the central nervous system.[3, 33] That has led investigators to ask whether mild hypothermia might be cardioprotective when instituted only at the onset of the reperfusion phase, i.e., a postconditioning maneuver. Cooling to 10˚C at the onset of reoxygenation and glucose resupply of chick cardiomyocytes subjected to simulated ischemia and reperfusion improved cell viability, and initiation of cooling towards the end of the simulated ischemia further diminished cell necrosis.[34] These observations strongly suggested that cooling during the peri-reperfusion period might be successful as well. Table 2 shows the results of several intact animal studies that have investigated this possibility.[25, 28, 35-39] Unfortunately, all but

one[37] failed to see any effect on infarct size and in the one positive study cooling still included that last 5 min of a 30 min ischemic period. Either the much lower temperature or the non-mammalian cell type was likely responsible for the protection in the chick cardiomyocyte study.[34] Of course, cooling a patient to 10˚C is not feasible because that would stop the heart. However, it was found that hypothermia during reperfusion prevents microvascular damage and thereby offers some protection against the no-reflow phenomenon.[24, 35] One might, therefore, speculate that hypothermia at reperfusion protects against vascular alteration and subsequent microvascular obstruction[35] but cannot protect the cardiomyocytes. This could mean that one should maintain cooling during the first hours of reperfusion even if cooling had been instituted early in the ischemic period. Surprisingly, the question of whether prolonging hypothermia after reperfusion measurably adds to the anti-infarct effect has yet to be addressed. It should be noted that most patients spend approximately 90 minutes in hospital prior to revascularization because of delays associated with admitting, diagnosis, and preparation for intervention.[40] Whole-body cooling during that period could produce significant reduction of the resulting infarct.

3.4. How can myocardium be cooled?

As emphasized earlier, the sooner hypothermia is achieved following the onset of ischemia, the more cardioprotective it would be. Experimentally, some studies have been performed using topical epicardial cooling to quickly lower cardiac temperature, but this method would be difficult to implement in patients. In patients with acute myocardial infarction, the critical parameters determining benefit would be the time when cooling could be started and also the rate at which cooling could be achieved. As explained earlier, the benefit would be expected to be small or absent if the target temperature is only reached far into the reperfusion phase. Obviously, the least invasive strategy for implementing therapeutic hypothermia in patients is external cooling. This can be done using ice packs or with specific medical devices designed to promote heat exchange by a more efficient

129 contact between the skin and the cooling medium.[2, 41, 42] Unfortunately the cooling rate
130 afforded by these strategies is rather low, averaging only 1-2°C/h in humans.[43] That is
131 because the cutaneous microcirculation tends to constrict when cold in an attempt to
132 thermally insulate the body from the environment. Small laboratory animals cool faster with
133 skin cooling than humans since their ratio of body mass to surface area is much smaller than
134 in man. The time required to cool a rabbit weighing 2.0-3.0 kg to 32°C is still ~45 min, and
135 this does not significantly limit infarct size after 60 min of ischemia.[44] While it is unlikely
136 that patients with acute myocardial infarction would experience any anti-infarct benefit from
137 conventional external cooling, this strategy is reported to be beneficial following cardiac
138 arrest when even delayed hypothermia improves overall survival and neurological recovery
139 after resuscitation.[2, 41, 42]

140 Other strategies have been proposed to induce therapeutic hypothermia. Some
141 examples include endovascular cooling with intravenous thermodes, infusion of cold
142 intravenous fluids, gastric lavage with cold fluid through a nasogastric catheter and even
143 urinary bladder lavage.[42] Most of those techniques have been investigated in the clinics for
144 their neuroprotective abilities.[42] The one that has been investigated for a cardioprotective
145 therapy is endovascular cooling.[26, 45, 46] In human-sized pigs, this strategy afforded
146 promising results when cooling was initiated early during ischemia.[26, 45] A clinical trial in
147 patients with ST-segment elevation myocardial infarction (STEMI) clearly demonstrated the
148 feasibility of that strategy,[46] but disappointingly did not show a significant cardioprotective
149 benefit,[8] probably because of an insufficient cooling rate that resulted in normothermia
150 during most of the ischemic period.[47]

151 *3.5. Ultra-fast cooling*

152 Accordingly other strategies have been proposed that elicit a much faster rate of
153 cooling which should increase the degree of cardioprotection in the clinical setting.
154 Examples include extracorporeal blood cooling[12] or total liquid ventilation with

155 temperature-controlled perfluorocarbons.[25, 48] These techniques can decrease cardiac
156 temperature to 32°C within 3-5 min in rabbits. A pericardial perfusion circuit has also been
157 proposed.[49] Unfortunately all these strategies would be challenging to implement in patients
158 presenting with myocardial infarction since they are very invasive and would significantly
159 delay reperfusion by angioplasty or thrombus extraction. A promising technique could be
160 peritoneal lavage, which, although still invasive, should be easier to institute than any of the
161 above-mentioned methods. As seen in patients with malignant hyperthermia it can cool a
162 patient very quickly.[50] Whether any of these ultrafast cooling strategies would be beneficial
163 in humans with STEMI remains to be investigated.
164
165 **4- Mechanism of cooling-induced cardioprotection**
166 The mechanism of hypothermia-induced cardioprotection has mostly been
167 investigated in experimental models receiving cold cardioplegia. The temperature of the
168 heart is reduced to very low levels which, among other things, arrests it.[13, 51] However,
169 mechanisms are likely to be quite different for mild hypothermia of an *in vivo* beating heart
170 and an arrested heart exposed to cold cardioplegia. In beating hearts, one could, for example,
171 argue that the cardioprotection might be related to the bradycardia elicited during ischemia.
172 However, this is unlikely because the relationship between infarct size and temperature was
173 unchanged when normothermic heart rate was restored by pacing in hypothermic rabbits.[21,
174 52] It is therefore commonly assumed that hypothermia protects the heart, at least in part,
175 through reduced cardiac metabolism. This assumption has been amply supported by studies
176 performed during cold cardioplegia (<20°C).[51, 53-60] Most enzymes have a Q_{10} of about 2,
177 which means the reaction rate doubles for every 10°C increase in temperature. Reducing the
178 cardiac temperature by 20°C should therefore decrease metabolism by a factor of 4. Mild
179 hypothermia (>30°C) also decreases the rate of high energy phosphate[11, 61, 62] and glucose[63]
180 utilization as well as lactate accumulation[63] but to a lesser extent than deep hypothermia.

Deep hypothermia may also alter ion exchange because it inhibits Na^+/Ca^{2+} and Na^+/K^+ sarcoplasmic exchangers, although it paradoxically activates the Na^+/H^+ exchanger.[64] Interestingly, hibernating hypothermic frogs increase their resistance to hypoxia through a decreased demand for ATP by reduced Na^+/K^+-ATPase activity.[65] An NMR study in isolated newborn rabbit hearts further confirmed that deep cooling (12°C) with cold crystalloid cardioplegia limited acidosis and calcium and sodium cellular overload during ischemia/reperfusion.[13] Deep hypothermia (17°C) also limited mitochondrial calcium overload in Langendorff guinea pig hearts undergoing ischemia.[66] While deep hypothermia tends to increase baseline reactive oxygen species formation during normoxia, it limits the burst following ischemia-reperfusion.[66] Using electron spin resonance spectroscopy in isolated reperfused rat heart investigators have observed reduced free-radical generation at reperfusion following ischemia at 4°C.[67]

Deep hypothermia reduces several modulators of the mitochondrial permeability transition pore (mPTP) *i.e.*, ATP depletion, calcium overload, and generation of reactive oxygen species. Mild hypothermia (32°C) inhibited calcium-induced mPTP opening in ventricular samples from rabbit hearts subjected to ischemia alone or to ischemia followed by 10 min of reperfusion.[48] Suppression of mPTP formation at reperfusion is thought to be the mode of action of ischemic preconditioning[68] and ischemic postconditioning.[69] Thus it is reasonable to speculate that hypothermia acts, at least in part, through inhibition of MPTP formation. However, the manner of modulation of mPTP opening is probably different than that afforded by pre- and postconditioning since the latter strategies are believed to trigger signal transduction pathways that determine the fate of the previously ischemic myocardium during the first minutes of reperfusion,[69-73] while hypothermia seems to exert its protection during ischemia.[48] Opening of mPTP at reperfusion only occurs if the heart has been injured by a period of prolonged ischemia. The nature of that injury is poorly understood, but Honda

and colleagues[74] referred to it as "priming". Mild hypothermia may act to lessen that ischemic injury.

The protection from mild hypothermia is proportional to the decrease in temperature which argues against any off/on type of mechanism. A direct effect of mild hypothermia on enzyme kinetics seems unlikely to be the protective mechanism since most enzymes are not so temperature-dependent. Hearts function quite well over the entire range of mild hypothermia (32-38°C). An intriguing possibility is that mild hypothermia might somehow activate cardioprotective signal transduction pathways. Ning et al.[75] demonstrated that cold cardioplegia (30°C) preserves mitochondrial protein gene expression during hypoxia, including genes coding for HSP70, ANT_1, and β-F_1-ATPase.[76] They observed that neither ATP levels nor anaerobic metabolism are linked to mRNA expression of these latter proteins.[77] Ning et al.[77] also showed that moderate hypothermia (30°C) promotes expression of proteins involved in cell survival, while it inhibits induction of p53 protein. It is, therefore, reasonable to hypothesize that mild hypothermia triggers its protection through thermal sensors. This hypothesis is supported by studies from Halestrap's group which reported that a short period of perfusion at 26°C in isolated rat hearts induced a preconditioning-like protection which increased protein kinase C-ε translocation to the particulate fraction (an index of its activation) and phosphorylated AMP-activated protein kinase (AMPK).[78] In one recent study in chick cardiomyocytes undergoing simulated ischemia, cooling to 25°C just before reoxygenation protected the cells.[34] More importantly that protection could be blocked by either a PKC or a nitric oxide synthase (NOS) blocker indicating signal transduction. Protection in the latter study, however, seemed to mimic that of postconditioning rather than that of mild hypothermia since the critical time for cooling was during reoxygenation rather than simulated ischemia, and PKC and NOS are well known components of the signal transduction pathways of pre- and postconditioning. Also

cooling was much more severe. All chemical reactions are at some point temperature-dependent, but the temperature coefficients (Q_{10}) for the various mammalian enzymes vary widely. The possibility that one particular enzyme might be very sensitive to temperature and could serve as a sensor to implement the cardioprotective effect of mild hypothermia through cell signaling is an attractive hypothesis, but of course that enzyme could be very difficult to find.

Hypothermia seems to protect against injury during ischemia, while ischemic preconditioning protects against injury during reperfusion. Because of the different mechanisms it is possible to add the two together to produce additive protection.[12] It is currently possible to pharmacologically postcondition a heart[79, 80] and such an intervention is thought to protect by a mechanism identical to that of preconditioning. Thus, it should be possible to combine mild hypothermia during ischemia with a postconditioning agent at reperfusion. The primary impediment to using mild hypothermia clinically has been the logistics of implementing it quickly after the onset of ischemia. However, if signaling pathways turn out to be responsible for mild hypothermia's protection, then pharmacological activation of those pathways would likely be simpler to implement and such an agent could possibly even be given by EMS personnel.

5- Cooling and cardioprotection: STEMI clinical data

Soon after the turn of the 21st century the promising experimental results regarding the cardioprotective effect of cooling inspired several clinical trials testing mild hypothermia's feasibility and safety in STEMI patients.[46, 81, 82] As shown in Table 3, two large-scale clinical trials (COOL-MI[8] and ICE-IT[10]) were conducted using endovascular cooling. Both studies assessed infarct size using single photon emission computed tomography (SPECT). The results of these studies have not yet been published in peer-reviewed journals, but they have been summarized in several reviews.[41, 83, 84]

In the COOL-MI study (*COOLing as an adjunctive therapy to percutaneous intervention in patients with acute Myocardial Infarction*), 392 STEMI patients were enrolled within 6 hours following the onset of symptoms.[8] Patients were assigned to either treatment with a percutaneous coronary intervention alone or to cooling with an endovascular cooling device (Reprieve Temperature Therapy System, Radiant Medical, Redwood City, CA, USA) prior to revascularization. The percutaneous coronary intervention was accomplished a mean of 18 min after cooling was instituted, with a mean temperature of 35.0°C at the moment of reperfusion. As shown in Table 3, cooling did not provide an overall significant reduction in infarct size, except in a sub-group of patients with anterior myocardial infarction that were cooled to less than 35°C at the time of revascularization.[8] The overall negative result of that study might have been biased since the average door-to-balloon time in cooled patients was 18 minutes longer than in control patients (110 vs 92 min).

In the ICE-IT study (Intravascular Cooling Adjunctive to Primary Coronary Intervention), the design was similar to COOL-MI with the inclusion of 228 patients randomized to either normothermic revascularization or to revascularization with mild hypothermia using another endovascular device (Innercool by Celsius Control System, San Diego, CA, USA).[10] Hypothermia again did not provide a significant benefit regarding infarct size in the whole population (Table 3). A trend for a benefit was observed in the sub-group with anterior infarction with a body temperature of less than 35°C at the time of reperfusion. Interestingly, a subanalysis of the 6 sites with the best protocol compliance out of the 22 participating sites also demonstrated a significant reduction in infarct size with cooling compared to conventional revascularization (-44%). These data suggest, as emphasized above, that the overall negative result of the COOL-MI and ICE-IT trials was related to a delay in institution of hypothermia and/or to an insufficient cooling rate which resulted in little shortening of the normothermic ischemic period. A further study (COOL-

MI II) was accordingly recently conducted with an earlier, deeper and faster cooling protocol, with cooling started in the emergency room rather than in the catheterization laboratory. The results of this last trial are not yet available.

It has conversely been suggested that hyperthermia may worsen the clinical outcome in patients with myocardial infarction.[85] In experimental conditions, Chien et al.[21] found not only that hypothermia could diminish infarct size but that hyperthermia could increase it. One would, therefore, speculate that therapeutic hypothermia would also be indicated to reverse the adverse effects of hyperthermia. A general relationship between clinical outcome and body temperature may well exist.

Cardiac Resuscitation

Another setting in which the protective effect of cooling would be relevant is cardiopulmonary resuscitation. Cooling after the heart is restarted can indeed protect the central nervous system, as has been previously shown,[5]but it may also protect the heart since myocardial ischemia is a common cause of cardiac arrest.[41, 84] Theoretically, the cardiac benefit would even be greater for those patients than for "typical" STEMI as the time before revascularization is prolonged by the resuscitation time. There is controversy as to whether resuscitated patients should be immediately submitted to a coronary intervention[41, 84] since the use of hypothermia is basically recommended for comatose survivors after out-of-hospital cardiac arrest with ventricular fibrillation.[6, 7] Importantly, the combination of cooling and percutaneous intervention is at least feasible and should be safe in those patients.[41, 84]

6. Conclusion

In conclusion, cooling the myocardium with whole body mild hypothermia is a very potent cardioprotective maneuver, at least in the experimental setting. The benefit depends upon the rapidity with which cooling is instituted and by how much it shortens the

normothermic ischemic time. To afford a clinical benefit, a cooling strategy should accordingly be designed to achieve the target temperature well before the time of revascularization. The depth of cooling is also important. Temperatures in the range of 32-35˚C are considered safe. Yet these temperatures exert a powerful anti-infarct effect in animal studies. Finally, more studies of mild hypothermia are needed to determine its actual mechanism, as compared to deep hypothermia that acts through energy preservation.

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Legend of Figures

Figure 1

Infarct sizes (expressed as % of the area at risk) in different groups of anesthetized rabbits submitted to 30 min of coronary artery occlusion and 3 h of reperfusion. In the different groups, rabbits were either subjected to a normothermic protocol (Control group) or to extracorporeal blood cooling to 35°C or 32°C from the onset (0') or from the 10^{th} or the 20^{th} minute of ischemia (0', 10' and 20' ischemia). Open circles represent the individual infarct size of each animal and closed circles represent the mean±SEM of each group.

Data adapted from Miki et al. [12] ** $p<0.05$ vs Control.*

Figure 2

Infarct sizes (expressed as % of the area at risk) obtained from several studies in anesthetized rabbits subjected to 30 min of coronary artery occlusion and cooled to 32°C starting at different times after initiation of ischemia. Closed circles represent the mean±SEM of the cooled groups for each study.

Numbers next to the data points are reference citations.

Figure 1

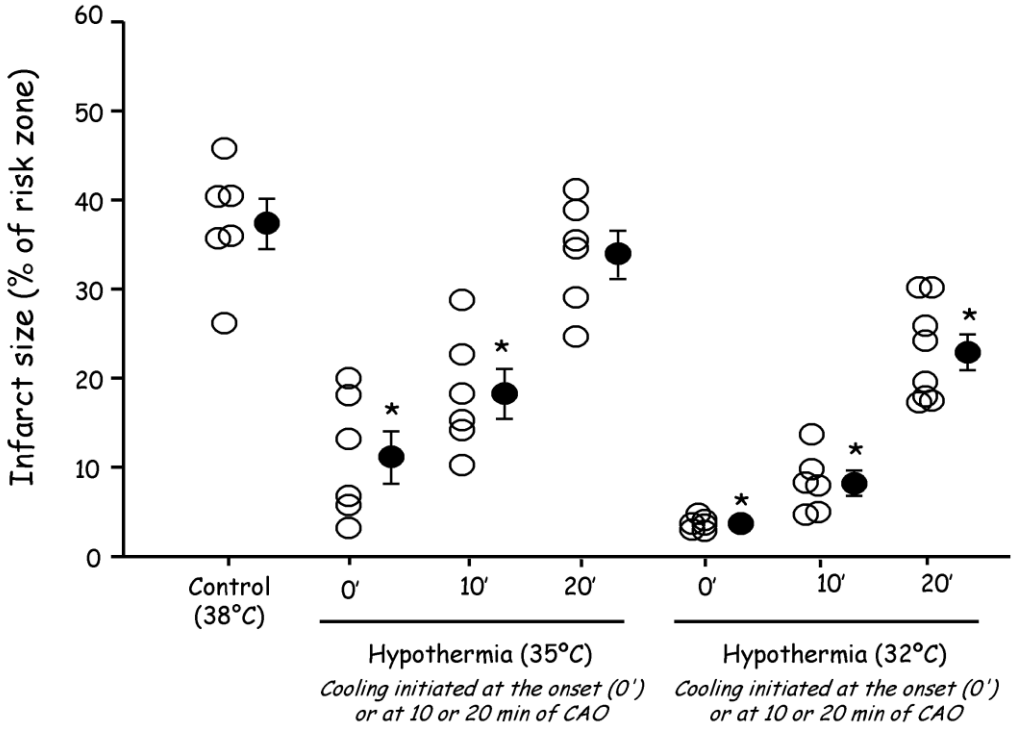
Infarct size (% of risk zone)
60
50
40
30
20
10
0
Control (38°C)
0′
10′
20′
0′
10′
20′
Hypothermia (35°C)
Cooling initiated at the onset (0') or at 10 or 20 min of CAO
Hypothermia (32°C)
Cooling initiated at the onset (0') or at 10 or 20 min of CAO

Figure 2

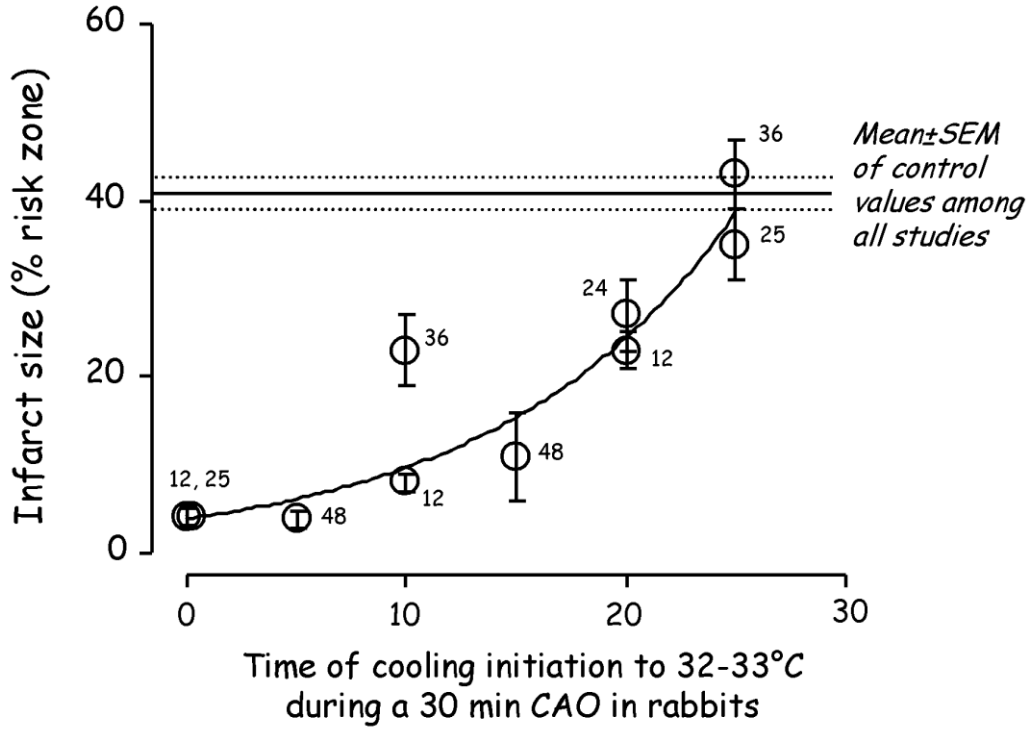
60
40
20
0
Infarct size (% risk zone)
36
25
24
12
36
48
12
12, 25
48
Mean±SEM
of control
values among
all studies
0
10
20
30
Time of cooling initiation to 32-33°C
during a 30 min CAO in rabbits

Table 1: Summary of *in vivo* experimental studies investigating the infarct-limiting effect of myocardial cooling during ischemia, i.e., with cooling initiated before ischemia or at least 5 min before the end of coronary artery occlusion

Species	Ref.	Cooling procedure	Duration of CAO (min) / CAR (h)	Target heart temperature	Time of cooling*	IS with Cooling vs Control groups *(% decrease)*
Rabbit	[36]	Topical epicardial cooling	30 min / 3 h	~33°C	10 min CAO → 15 min CAR	23±4 vs 44±4 (-48%)
Rabbit	[22]	Topical epicardial cooling	120 min / 3 h	~30°C	30 min CAO → 15 min CAR	59±3 vs 72±3 (-18%)
Rabbit	[49]	Closed pericardioperfusion circuit	30 min / 3 h	~34°C	-30 → 25 min CAO	18±3 vs 35±6 (-49%)
Rabbit	[24]	Topical epicardial cooling	30 min / 3 h	~32°C	20 min CAO → 120 min CAR	27±4 vs 51±5 (-47%)
Rabbit	[12]	Blood cooling through heat exchanger	30 min / 3 h	~35°C	0 → 30 min CAO	11±3 vs 37±3 (-70%)
					10 → 30 min CAO	18±3 vs 37±3 (-51%)
					20 → 30 min CAO	34±2 vs 37±3 (NS)
				~32°C	0 → 30 min CAO	4±1 vs 37±3 (-89%)
					10 → 30 min CAO	8±1 vs 37±3 (-78%)
					20 → 30 min CAO	23±2 vs 37±3 (-38%)
Rabbit	[25]	Total liquid ventilation	30 min / 3 h	~32°C	0 → 30 min CAO	4±1 vs 38±1 (-89%)
Rabbit	[48]	Total liquid ventilation	30 min / 72 h	~32°C	5 → 30 min CAO	4±1 vs 39±2% (-90%)
					15 → 30 min CAO	11±5 vs 39±2% (-72%)
Rabbit	[37]	Surface cooling (water blankets)	30 min / 3 h	*~37.0°C*	Before CAO→ 180 min CAR	30±5 vs 59±1% (-48%)
					0 min CAO → 180 min CAR	33±5 vs 59±1% (-43%)
					15 min CAO→ 180 minCAR	42±1 vs 59±1% (-28%)
Rabbit	[52]	Topical epicardial cooling	30 min / 3 h	*~35°C*	-20 min before CAO → 15 min CAR	16±3 vs 46±4 (-65%)
Rabbit	[44]	Surface cooling	60 min / 4 h	*~32°C*	5 → 30 min CAO	78±10 vs 82±7 (NS)
		Total liquid ventilation			5 → 30 min CAO	45±18 vs 82±7 (-45%)
					20 → 30 min CAO	58±5 vs 82±7 (-29%)
Pig	[26]	Endovascular cooling	60 min / 3 h	*~34°C*	20 min CAO → 15 min CAR	9±6 vs 45±8 *(-80%)*
Pig	[27]	Topical epicardial cooling	40 min / 3 h	*~29°C*	0 → 40 min CAO	25±2 vs 62±5 *(-60%)*
Pig	[28]	Intracoronary cold saline infusion	60 min / 3 h	*~33°C*	15 min CAO → 15 min CAR	9±2 vs 36±4 *(-75%)*
Pig	[35]	I.V. cold saline + endovascular cooling	40 min / ~4.3h	*~33°C*	25 min CAO → 25 min CAR	46±8 vs 75±5 (-39%)
Sheep	[30]	Surface cooling (ice bags)	60 min / 3 h	*~38.5°C*	0 min CA0 → 180 min CAR *Control Group at 39.5°C*	63±2 vs 72±3 (-12%)
				~37.5°C		49±1 vs 72±3 (-31%)
				~36.5°C		39±1 vs 72±3 (-46%)
				~35.5°C		22±2 vs 72±3 (-70%)
Dog	[32]	Hypothermic retroperfusion of autologous blood	210 min / 3 h	*~28-30°C*	30 → 210 min CAO	6±3 vs 24±7† (-75%)

CAO, coronary artery occlusion; CAR, coronary artery reperfusion; IS, infarct size (expressed as % of area at risk); ref, reference number

**represents the time of the application of the cooling strategy and not the actual time at which the target temperature was reached. In several studies a delay was inevitable between the onset of the cooling protocol and the time of achievement of the target temperature (e.g., with low rate cooling strategy such as surface cooling).*

† the Control value corresponds to infarct sizes observed with normothermic retroperfusion.

Table II: Summary of *in vivo* experimental studies investigating the infarct-limiting effect of myocardial cooling during the reperfusion phase, i.e., with cooling started only 5 min before reperfusion or later yielding normothermic ischemia and hypothermic reperfusion

Species	Ref.	Cooling procedure	Duration of CAO (min) / CAR (h)	Target heart temperature	Time of cooling*	IS with Cooling vs Control groups *(% decrease)*
Rabbit	[36]	Topical epicardial cooling	30 min / 3 h	*~33°C*	25 min CAO → 15 min CAR	43±4 vs 44±4 *(NS)*
Rabbit	[25]	Total liquid ventilation	30 min / 3 h	*~32°C*	25 min CAO → 30 min CAR	35±4 vs 38±1 *(NS)*
Rabbit	[37]	Surface cooling (water blankets)	30 min / 3 h	*~37.0°C*	25 min CAO → 180 min CAR	44±2 vs 59±1% (-25%)
					30 min CAO → 180 min CAR	51±2 vs 59±1% (NS)
Pig	[38]	Regional blood cooling through heat exchanger	45 min / 3 h	*~33°C*	43 min CAO → 120 min CAR	71±8 vs 68±1 *(NS)*
Pig	[28]	Intracoronary cold saline infusion	60 min / 3 h	*~33°C*	0 min CAR→ 30 min CAR	33±2 vs 45±5 *(NS)*
Pig	[35]	Intravenous infusion of cold saline + endovascular cooling	40 min / ~4.3h	*~33°C*	0 min CAR → 30 min CAR	80±6 vs 75±5 (NS)

See Table 1 for legend.

Table III: Infarct size assessed by single photon emission computed tomography in patients presenting with ST-segment elevation myocardial infarction and treated by revascularization alone or in conjunction with endovascular cooling in the COOL-MI [8] and the ICE-IT [10] studies. Data were obtained from ref [83], [41] and [84].

	Infarct size (% left ventricle)		**P value**
	Cooled group	Normothermic group	
COOL-MI (n=392 patients)			
Overall population	13.8%	14.1%	0.86
Subgroup with anterior STEMI cooled to <35° at the time of revascularization	9.3%	18.2%	0.05
ICE-IT (n=228 patients)			
Overall population	10.2%	13.2%	0.14
Subgroup with anterior STEMI cooled to <35° at the time of revascularization	12.9%	22.7%	0.09

STEMI, ST-segment elevation myocardial infarction