Does mild hypothermia protect against reperfusion injury? The debate continues

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Abstract Mild hypothermia (32–35°C) salvages ischemic myocardium and reduces infarct size in hearts undergoing ischemia/reperfusion. It is clear that a cardioprotective effect is evident when the heart is cooled during ischemia, and the protection is greater as the duration of normothermic ischemia is increasingly limited. The effect of cooling just before and at reperfusion is more controversial. Multiple experimental studies have revealed no effect of mild hypothermia on myocardial infarction when cooling was initiated in the waning minutes of ischemia. But Götberg et al. have demonstrated a small effect in pigs cooled with cold intravenous saline and a venous thermode, although the effect of cooling during ischemia continued to be more prominent. Clinical studies have been disappointing, and possible explanations are offered. Götberg's new data are encouraging, but it is questioned whether this is the correct time to conduct a new large-scale clinical trial.

Keywords Cardioprotection · Hypothermia · Ischemia · Myocardial infarction · Reperfusion

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In animal models of coronary artery occlusion/reperfusion cardiac temperature is known to be a major determinant of infarct extension, along with collateral blood flow and risk zone size [2, 4, 23]. Cooling to 4°C (deep hypothermia) has long been used to protect the heart during open heart surgery and transplantation. In those cases, the heart is quiescent and metabolism is greatly curtailed. What is surprising is that mild hypothermia (32–35°C) is also quite protective. Mild hypothermia has the advantage that, under those conditions, the heart beats normally which allows simple whole body cooling without the need for cardiovascular support. Chien et al. [2] reported that for each degree C decrement of the core temperature in rabbits undergoing 30 min of ischemia followed by reperfusion infarct size was reduced by an average of 8% of the risk zone. When induced during the ischemic process, mild hypothermia is, therefore, one of the most potent cardioprotective strategies yet to be identified.

Studies of therapeutic whole body cooling have limited the range of cooling to be no lower than 32°C because lowering the temperature below 30°C is life-threatening. But 32°C is already quite protective [18]. This protective effect of whole body cooling against infarction has been reported for many experimental animal species (pigs [3, 4, 21, 22], rats [28], rabbits [2, 9, 12, 13, 18, 27], dogs [23]). Mild hypothermia also attenuates no-reflow [1, 5, 9], post-ischemic left ventricular dysfunction [26], and remodeling [14]. As emphasized by two recent reviews [11, 25], the evidence for a cardioprotective effect of therapeutic mild hypothermia against ischemic injury is very strong.

An important consideration in therapeutic hypothermia is not only its dose (depth of cooling) but also its schedule. All investigators would probably agree that hypothermia is most protective when present during ischemia. We have found that the magnitude of the protection depends upon how much of

Fig. 1 The effect of schedule on the cardioprotective effect of mild hypothermia. In several studies, mild hypothermia (32–33°C) was instituted at various times after a 30-min period of ischemia had begun in rabbits. The reduction in infarct size in each study is plotted against the time at which the target temperature was reached for that group. The number in brackets is the reference number for that study. Hypothermia became less effective the later it was instituted, suggesting that hypothermia prevents an ischemic injury rather than a reperfusion injury

the ischemic period is hypothermic and that the tissue-sparing effect progressively decreases the later into the ischemic period that hypothermia is instituted [18]. This effect is illustrated in Fig. 1. The infarct size reduction elicited by cooling to 32°C decreases as the delay between the onset of a 30-min period of ischemia and the time when target cooling is finally achieved increases [9, 10, 18, 26, 27]. When hypothermia began at the 25th min of ischemia, reduction in infarct size was no longer significant [27]. In all of the studies noted in Fig. 1, target hypothermia was already present at reperfusion. A lack of protection of mild hypothermia when initiated only at reperfusion has been shown in many other experimental reports [5, 10, 17, 21, 27] and would suggest that hypothermia has no effect on reperfusion injury per se. This is an important point because it is not easy to cool the human body quickly. As a result, past technology has not been able to significantly reduce the normothermic ischemic time in patients undergoing primary percutaneous coronary intervention (PCI) for acute myocardial infarction (AMI), but it is still possible to reach an adequate level of hypothermia at the time of reperfusion. If hypothermia can attenuate a component of injury associated with reperfusion, then such a protocol would still be of therapeutic value.

Not all studies failed to find a positive effect of hypothermia on reperfusion injury. Kanemoto et al. [16] induced very mild total-body hypothermia (temperature reduction $\leq 2.5^\circ\text{C}$) in rabbits 5 min prior to reperfusion

following 30 min of ischemia. But unlike earlier studies, they did not allow the animal to start rewarming until the heart had been reperfused for 3 h. In this protocol, the authors observed a 25% decrease in infarct size. This would suggest that protection against a reperfusion injury might be achieved if the hypothermia were prolonged several hours into the reperfusion period.

Hale et al. [9, 10] looked at reperfusion injury in two studies in which rabbits underwent 30 min of myocardial ischemia. In the first study [10], they quickly cooled the heart to 32°C by holding an ice cube next to it. While cooling during ischemia reduced infarct size, cooling starting 5 min prior to reperfusion did not. In a second study [9], they started cooling 10 min prior to reperfusion and extended the cooling into the first 2 h of reperfusion. In that study, infarct size was reduced. It was not determined whether it was the additional 5 min of hypothermic ischemia or the additional hour of hypothermic reperfusion that caused the salvage. Cultured chick cardiomyocytes undergoing simulated ischemia were cooled to 25°C just before reoxygenation and were protected [24]. However, in that study cooling only to temperatures below the range of mild hypothermia was evaluated, and such a degree of cooling could not be tolerated in patients without external circulatory support. Whether mild hypothermia limits reperfusion injury in vivo, therefore, remained unsettled.

Two large-scale clinical trials in patients with AMI (COOL-MI [19] and ICE-IT [8]) have already been conducted, but both yielded negative results. One might hypothesize that these negative results were related to slow rates of cooling. Although there was some hypothermia at reperfusion after PCI in most patients, the target temperature was never achieved until far into the reperfusion phase.

In the present issue of *Basic Research in Cardiology*, Göteborg et al. [7] investigated the effect of cooling against reperfusion injury in pigs subjected to 45 min of coronary artery occlusion. In one group, rapid cooling was started 5 min prior to reperfusion using endovascular cooling with a venous thermode and intravenous infusion of ice-cold saline. The target core temperature was 33°C. In the normothermic control group, the ischemic period was 5 min shorter (40 vs. 45 min) to account for the period during ischemia when the hypothermic animals were being cooled. Both groups were, therefore, subjected to a similar period of 40 min of normothermic ischemia. Under these conditions, any benefit of hypothermia should be related to a specific effect on reperfusion injury rather than any anti-ischemic effect. Infarct size was 18% smaller in the animals with hypothermic reperfusion than in normothermic controls. Interestingly, starting the cooling period after only 25 min of ischemia and extending the cooling period to 1 h during reperfusion further decreased infarct size by

an additional 21%. However, this protocol cannot attribute the additional protection specifically to either the longer period of hypothermic ischemia or the longer reperfusion hypothermia. A previous study [5] showed that cooling from 25 min of ischemia to 15 min of reperfusion elicited the same infarct-sparing effect as the current group with hypothermia extending to 60 min of reperfusion. Therefore, it seems clear that the extra protection was related to the additional hypothermic ischemia. Additionally, the authors took this to mean that the beneficial effect of hypothermia on reperfusion injury does not require a prolonged period of hypothermic reperfusion. Also in the earlier study [5], the authors failed to observe any effect of cooling in pigs when the same treatment was started at the onset of reperfusion rather than 5 min before. In this prior study the hearts were, therefore, not cooled during the first moments of reperfusion, presumably explaining the discrepancy between the conclusions of the two studies. Hence, the authors extrapolated that having the patient at the target temperature at the time of reperfusion confers benefit, whereas extending the cooling several hours into the reperfusion period is not required.

Few explanations come to mind that might explain the apparent discrepancy between the present results and the previous ones that failed to show any effect of cooling against reperfusion injury [5, 10, 17, 21, 27]. In their report, Göteborg et al. [7] induced cooling by endovascular cooling and intravenous infusion of cold saline. The large volume of saline that the pigs received could account for the difference. Previous negative studies used cooling methods not affecting the blood volume, such as topical cooling [10], extracorporeal blood cooling [18] or total liquid ventilation [27]. But infusing cold saline alone did not cool as well nor was it protective suggesting that the temperature rather than volume was the critical factor.

Of course, there is always the possibility of a species difference whereby hypothermia might target reperfusion injury in pigs but not in rabbits. What about humans? Most recently, Göteborg et al. [6] reported a pilot study in which infarct size was measured in 20 patients receiving intervention for AMI. Ten were rapidly cooled with the combination of cold saline and endovascular cooling. The two previous clinical trials using endovascular cooling [19] did not include cold saline infusion so the cooling was not nearly as rapid. There was an average reduction of infarct size normalized to the ischemic zone of 38%. It should be noted that in this small clinical pilot trial cooling included an average of 40 min of the ischemic period so it is impossible to determine how much of the benefit came from suppression of reperfusion injury. Thus the debate as to whether hypothermia can reduce reperfusion injury continues. Whatever the case, it should be noted that the degree of reperfusion injury prevented by cooling in the pig studies

was relatively small (18% of the infarct size) as compared to that observed with other modes of cardioprotection, and that raises the question as to whether such modest protection could even be detected in a clinical model.

One disturbing aspect of the paper [7] is the claim of the authors that the study was conducted to provide experimental data to support a proposed large-scale clinical trial of hypothermia as an adjunct to primary PCI in AMI patients without further attempts to resolve the conundrum of whether cooling at reperfusion indeed protects against reperfusion injury. Over the past 30 years, a great number of cardioprotective interventions have been tested in AMI patients, and so far all adequately-powered studies have failed. A critical retrospective look at these studies reveals that most of these trials were conducted despite a history of very discrepant preclinical studies. Some interventions like ischemic preconditioning work in all of the laboratories that test it and preconditioning has become the gold standard for cardioprotection. Other interventions are not so clear. An example is infusion of adenosine at reperfusion. Olafsson et al. [20] reported that such an infusion greatly reduced myocardial infarct size in open-chest dogs. Yet when Vander Heide et al. [29] tried to exactly duplicate the study with the same species and protocol, they could not find any protection. It is not known why two established laboratories using identical protocols and species could not arrive at the same conclusion, but that is not an uncommon event in this field of cardioprotection. The AMISTAD trials were started without trying to resolve the discrepant preclinical data. Both AMISTAD I and II failed to achieve their primary objectives.

The difficulties of extrapolation of pre-clinical successes to the clinical arena have previously been identified and discussed [15]. Reproducibility has been such a problem in cardioprotection that Roberto Bolli recently formed a consortium of four institutions under the aegis of the National Institutes of Health. This Consortium for pre-clinical assessment of cARdioprotective therapies (CAE-SAR) was designed to perform blinded, randomized evaluations of promising cardioprotective agents in mice, rabbits and pigs with each species being studied in at least two institutions by blinded investigators using identical protocols. Only those agents that are reproducibly effective in all of these preclinical models will be recommended for clinical testing. In our opinion, Göteborg's single study has still not established the effectiveness of cooling near the end of ischemia. But perhaps sufficient data will be generated in their clinical trial to yield an analysis comparable to that depicted in our Fig. 1 which would establish once and for all whether mild hypothermia protects against reperfusion injury in man, although this determination will be dependent on sufficient variability of pre-reperfusion start times for cooling.

In most experimental models, ischemia/reperfusion leads to large hemorrhagic infarcts and areas of no-reflow. Whereas the effect of hypothermia confined to the reperfusion period remains controversial with respect to infarct size, its ability to reduce microvascular failure and no-reflow seems to be well supported [5, 9]. There has been a debate as to whether vascular failure contributes to infarction of myocardium or visa versa. Maintaining the integrity of the microcirculation could also have beneficial effects on post infarction remodeling even if it does not reduce initial infarct size. Unfortunately, the jury is still out on whether no-reflow should be treated as well.

Conflict of interest None.

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