

Review

Mild Hypothermic Cross Adaptation Resists Hypoxic Injury in Hearts : A Brief Review

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Abstract

Severe cardiac hypoxia is responsible for significant morbidity and mortality in an emergency setting. Most cardiac hypoxia relates to ischemia and surgical events. Although the ischemic mortality rate and the risks of cardiac surgery have significantly decreased in past decades, myocardial protection still plays a major role in survival of hypoxic injury. Cross adaptation as a physiological regulation for homeostasis can resist injury caused by harmful environmental effects and diseases, including hypothermic adaptation. Treatment with hypothermia has been used for fifty years as a protective mechanism to avoid hypoxic injury. Since cold temperatures can cause damage, it is important to gather physiological data to distinguish protective from injurious temperatures. Although results of temperature trials in clinical practice vary, a critical temperature to resist hypoxic/ischemic injury in heart was found to be around 30°C, suggesting a hypothermia protective threshold. Pretreatment with mild hypothermia can resist subsequent hypoxia/ischemia, implying involvement of cross adaptation in protection. Safeguard hypothermia can directly reduce the build up of harmful metabolites and energy demand in hypoxic tissues, as well as preserve mitochondrial membrane specific proteins beta subunit of F₁-ATPase and adenine nucleotide translocase isoform 1. Mechanisms of preservation include inactivation of the p53 related pathways, representing anti-apoptosis, and modification of the mRNA level of succinodehydrogenase, indicating a beneficial effect on the aerobic pathway. Stress proteins are also induced. Resultant cellular adaptations serve to maintain myocardial integrity and improve functional recovery during reoxygenation or reperfusion.

Key Words: ANT₁, apoptosis, β F₁-ATPase, energy status, reoxygenation, reperfusion, stress proteins

Introduction

A stressor can turn on adaptive reactions to resist subsequent injury induced by other harmful stressors (21, 32). This kind of cross adaptation not only exists in systemic homeostasis but also in the isolated organs, tissues and cells. Changes in homeostasis status, such as oxygen content or temperature, may induce injury, adaptation or cross adaptation. Since the 1950s, induced hypothermia has been used in surgery to resist ischemic or hypoxic insults. The basic objective was to reduce the

metabolic rate in order to diminish the difference between oxygen demand and supply during hypoxic conditions. However, myocardial cooling with infusion of 15°C physiological solution in the un-arrested state resulted in significant myocardial damage in isolated rabbit hearts (49). *Pre-ischemic* hypothermia at 17°C had a deleterious effect on post-ischemic recovery of hearts in neonatal lambs (2). This could be problematic in emergency situations. To optimize benefits, it is necessary to identify the optimal hypothermic protective temperature and define injury temperature, as well as the relative

pathways.

Protective or harmful effects probably depend on the specific hypothermia temperatures, and whether there is a linear relationship between the protection and the decrease in temperature or whether the relationship is described by a sigmoid curve. The first relationship would be similar a temperature-dependent chemical reaction. The second phenomenon would represent thresholds: a critical temperature can induce tissue injury, representing an injury threshold; a different critical temperature may induce protective effect, representing a protective threshold.

Since the 1990s, mild-hypothermia-protection has been reported by several laboratories. After arrest with cardioplegia, functional recovery depends on two main factors; cardioplegia temperature and its composition, such as glucose, calcium etc. (31). In addition, the beneficial effects of glucose are also dependent on temperature (31, 34). We have defined a threshold temperature of 30°C for hypothermia protection in hearts (35, 56). During hypothermia protection, some pathways are activated and others are inactivated by the critical temperature (36, 41). These data may shed light on the underlying mechanisms of the hypothermia cross adaptation.

Hypothermia Protective Effects on Heart

Hypoxia is a common cause for many disorders, especially cardiac and pulmonary diseases. Incidents such as suffocation and cardiac arrest may induce severe systemic hypoxia. Many lives are lost from systemic hypoxia before the patient arrives at the hospital for treatment.

During the 1950s investigators studied the beneficial effects of low body temperature to decrease metabolism in animal models in many laboratories around the world in parallel with human surgical studies in Russia and the United States. One of the pioneers, Bigelow reported that reduction of dog body temperature to 20°C or lower could produce better recovery than normal physiologic temperature during intracardiac surgery (5). A decrease in oxygen consumption was considered to be the mechanism. Others reported that cold treatment of around 15°C also showed a protective effect during heart ischemia (22). However, Rebeyka indicated that rapid myocardial cooling with infusion of a 15°C physiological solution in the un-arrested state resulted in significant myocardial damage in isolated rabbit hearts (49). Aoki *et al.* (2) observed *pre*-ischemic hypothermia at 17°C had a deleterious effect on post-ischemic recovery of hearts in neonatal lambs. Those studies indicate that *pre*-arrest cardiac cooling may induce myocardial contracture and a sustained elevation of intracellular calcium level. Williams *et*

al. (55) recommended the avoidance of rapid myocardial cooling on the bypass. Experiments completed in different laboratories showed mild hypothermia have the potential to reduce infarct size (23, 28). These observations raised an important question: how can hypothermia treatment induce protective effects without injurious effects?

Temperature Thresholds for Protective and Injury

There are three levels of hypothermia-induced heart protection. Tissue or organs can be preserved under extreme cold condition (cryobiology) in liquid nitrogen for subsequent biochemical or molecular studies. An organ donated for transplant is immersed in a 4°C physiological solution to await transplantation (50, 51). An *in vivo* heart under hypoxic /ischemic conditions requires protection from injury with hypothermia temperatures higher than 4°C.

Cardioplegic Hearts

Temperature protection studies were conducted in the Thoracic Surgery Laboratory at the University of Michigan in the isolated rabbit heart in 1994. When the infused temperature was reduced to 30°C for 30 min *prior to* two hours of cardioplegic ischemia, a better functional recovery was observed during reperfusion than that in the hearts without hypothermic pretreatment. Besides hypothermic treatment *prior to* ischemia, a series of experiments were then conducted at 4, 18, 26, 30, 34 and 37°C for two hours of cardioplegic ischemia. The functional recoveries were markedly improved, although not statistically different at temperatures between 30°C to 4°C, contrasting with poor recovery at a temperature of 34°C and above (Fig. 1). The results indicate that there exists a temperature threshold for myocardial protection around 30°C in the cardioplegia arrested hearts (32, 35, 56). It is suggestive that the tissue storage temperature should be below 30°C (50).

Isolated Beating Hearts

In the beating heart, an infusion temperature around 28°C can increase the left ventricular end diastolic pressure, and is often accompanied by arrhythmia. This is indicative of myocardial stiffness and cardiac conductive system injury. At *pre*-ischemic hypothermia of 15°C, an elevation of intracellular calcium level is observed, accompanied by myocardial contracture and deleterious post-ischemic recovery of hearts (49). The beating isolated heart studies indicate that *pre*-arrest cardiac cooling may induce injury at temperatures of 28°C and below, that has been confirmed in many laboratories including ours.

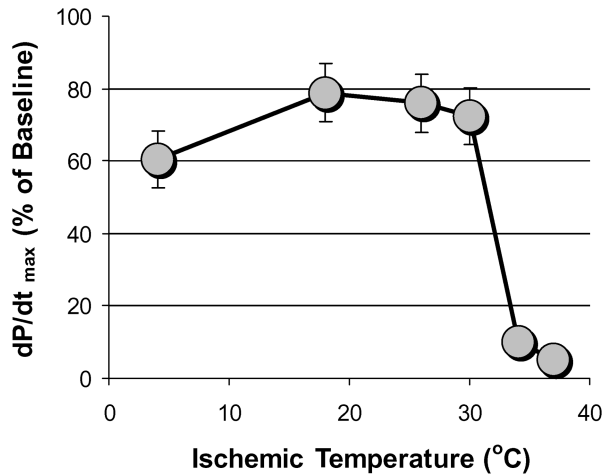


Fig. 1. Hypothermia protective threshold. The myocardial contractility is represented by dP/dt_{max} . The recovery of dP/dt_{max} is shown as % of the baseline value. The curve from 4°C to 30°C as a plateau shows good recoveries. A decreasing steep slope is between 30-34°C. It suggests that around 30°C is the protective threshold. Above 34°C would offer less protection. This figure data are cited partially from Fig. 1A of *Cryobiology* 36:7, 1998 with permission from Elsevier.

Hearts In Vivo

Cardiopulmonary bypass (CPB) was performed in pigs under propofol anesthesia (44). The esophageal temperature was decreased from 35°C to 20°C by using a heart-lung machine. A decrease in heart rate paralleled the reduction of temperature. The cardiac contractility (dP/dt_{max}) and performance (power, cardiac output etc.) showed a sigmoid curve change. There was a high plateau at 35-29°C; a low plateau at 24-20°C; and a steep slope between 28-25°C (Fig. 2). Arrhythmia and shivering occurred at the steep part of the curve. The pigs were treated within these 3 temperature range for 40 min *prior to* and 60 min *during* cardioplegic ischemia. After stopping the heart-lung machine, the heart function totally recovered in the 29-31.5°C groups and was significantly better than that in the 34-35°C and 20°C groups (44).

In another study, the pigs were treated at 30°C and 33°C. The hearts appeared significantly improved as evidenced by defibrillation success and resuscitation outcome (7).

The data from both isolated hearts and the hearts *in vivo* indicate that the mild hypothermia injury threshold is about 28°C in the beating hearts and the optimal protective temperature is $30.5 \pm 1^\circ\text{C}$ to resist hypoxia/ischemia.

Additive Effects of Combined Hypothermic Protocols

As mentioned above, treatment with 31°C *prior*

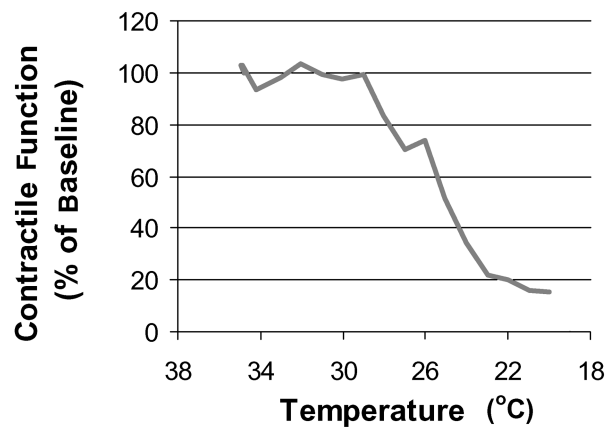


Fig. 2. Conceptual diagram of sigmoid curve. Between a high plateau and a low plateau there is a steep slope (28-25°C). The injury threshold of contractile function is around 28°C in the beating heart.

to ischemia could resist subsequent ischemia (32, 36). The study was performed in isolated perfused rabbit hearts ($n = 77$). The hypothermic group was treated by decreasing infusate temperature to 31°C from 37°C for 20 min, while a control group was maintained at 37°C. Subsequent ischemia was at 34°C for 120 min followed by reperfusion at 37°C in both groups. During reperfusion, recoveries of left ventricular developed pressure, maximum first derivative of left ventricular pressure, and product of heart rate and pressure were significantly better in 31°C treated hearts ($P < 0.01$). Thus 31°C hypothermia could induce cross adaptation to resist subsequent ischemia.

Presumably hypothermia plus thermal cross adaptation would have additive effects on myocardial recovery. The experiment proved this hypothesis. Hypothermia treatment *prior to* and *during* ischemia showed a synergistic response on protection. The functional recovery of these hearts was much better than in animals receiving hypothermia only during or before the hypoxic event (37). Fig. 3 summarizes the results from several published data (35, 36, 41, 42).

Direct Effects of Hypothermia on Metabolism

The basis of hypothermic protection is widely believed to be related to the decreased metabolic rate and reduced oxygen consumption associated with lower temperatures. Even when oxygen is insufficient, a physiologic decrease in oxygen uptake diminishes injury.

Hypothermia Reduces Imbalance between Oxygen Demand and Supply

When myocardial temperature decreases to 31°C

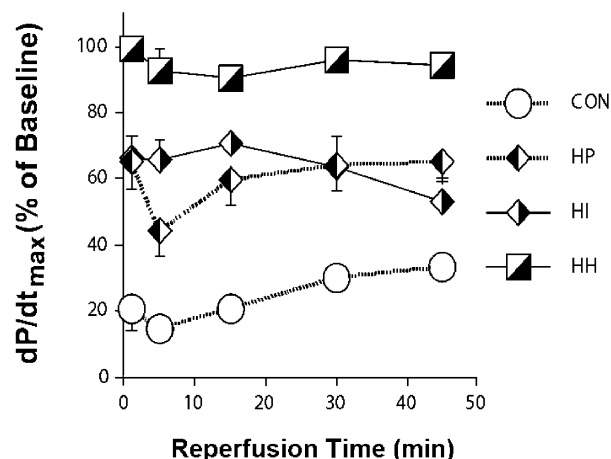


Fig. 3. Functional recovery in different hypothermia treatment. A synergistic beneficial response occurs with the combining treatments of hypothermia *prior to* ischemia (HP) plus *during* ischemia (HI). During 5 min of reperfusion, the “reperfusion injury” appears in the control (CON) and hypothermia-pretreatment groups, but does not in the combining treatment group (HH). This data are partially cited from an abstract in *FASEB J.* 13: A1064, 1999 (37).

from 37°C, the myocardial oxygen consumption is reduced to 2.5 ± 0.2 from 3.9 ± 0.3 $\mu\text{mol/min/g}$ (35, 36). Fig. 4 illustrates the difference between the hypothermic heart and control heart in the imbalance for oxygen demand and supply. During hypothermia *prior to* ischemia, the oxygen demand is less than in the control, indicating a lower metabolic rate at the start of ischemia.

During earlier ischemia, oxygen supply is equal to the oxygen remaining in the capillary beds and tissue spaces. The higher the metabolic rate and faster the consumption of oxygen result in much oxygen insufficiency in the control than in the hypothermic myocardium. The lower the metabolic rate and lower oxygen demand result in a smaller oxygen deficit in hypothermic conditions, as measured by oxygen consumption, CO_2 , and lactate. The CO_2 production represents aerobic metabolism and lactate accumulation represents anaerobic metabolism. Both CO_2 and lactate production are less in the hypothermic heart than in the control (35, 36). The less metabolic rate implicates less oxygen demand under a similar oxygen supply condition.

The Injury Threshold Can Be Modified by Energy Status

Glucose supply during hypothermia also effects organ recovery. St. Thoms cardioplegic solution containing 5-88 mM glucose significantly improves functional recovery over cardioplegic solution containing 0-1 mM glucose in warm (34°C) cardioplegic

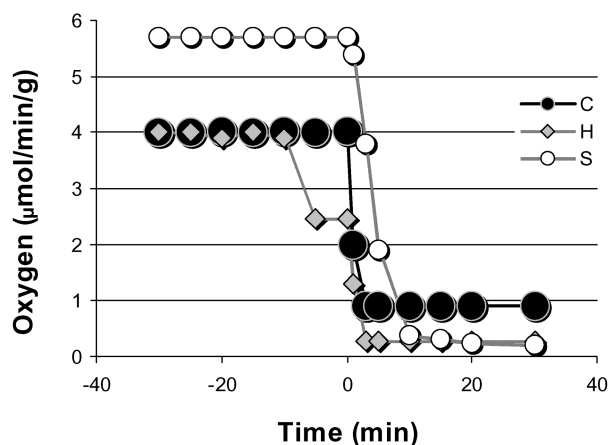


Fig. 4. Hypothermia reduces the un-balance between oxygen demands and supply. The abbreviations used are C = O_2 demand in control; H = O_2 demand in hypothermia heart; S = O_2 supply of control; Time 0 = ischemia starting time. During ischemia, the decrease in S curve depends on metabolic rate, here only shows a curve of control; the S curve of hypothermia should be less decrease than the control due to lower metabolic rate. After starting ischemia oxygen supply quickly decreases. Thus oxygen demand is over the supply in the control hearts and the difference between oxygen demand and supply is much bigger than that in the hypothermic hearts.

ischemic hearts. Optimal recovery, as measured by increased levels of high-energy phosphate was observed with 22 mM glucose (34).

Paradoxically, glucose administration increases production of lactate, which has been suggested as an injury factor in ischemic myocardium (35, 38). An optimal temperature may provide a balance between glucose utility and lactate accumulation.

Energy demand significantly increases at 34°C and above, making the organ more susceptible to injury from hypoxia than it would be at 30°C. Susceptibility to injury, however, can be modified by provision of glucose in cardioplegia solution (35, 41). These processes may be linked to reduced ATP depletion.

Hypothermia Modifies Aerobic and Energy Related Pathways

If hypothermia acts only by decreasing metabolic rate, there should be a parallel relationship between the hypothermia intensity and the injury reduction. The threshold phenomenon shows a trigger /gate like mechanism, however, implying that signaling, and receptor pathways are also involved in the reactions.

Aerobic Pathway Response

Aerobic metabolic efficiency is 18 times higher

for glucose than anaerobic metabolism. During myocardial ischemia, aerobic pathways are broken due to insufficient oxygen supply (35). Succinohydrogenase (SD), a respiratory chain enzyme important in aerobic metabolism was stimulated to over express mRNA as a feedback response to ischemia. Since hypothermia reduces oxygen demand, the signal that insufficient oxygen provides would be abated. The over expression of SD mRNA represents cell injury caused by ischemia (39, 42). Thus the SD mRNA level is also a marker representing the status of aerobic pathways during ischemia and hypoxia.

Mitochondrial Specific Proteins

The nuclear encoded mitochondrial proteins, beta subunit F_1 -ATPase (βF_1 -ATPase) and adenine nucleotide translocase isoform 1 (ANT_1), mediate ATP synthesis and transports ADP to pass mitochondrial membrane, respectively. Ischemia induces impairment of mitochondrial function including decreasing expressions of βF_1 -ATPase and ANT_1 . Treatment with 4°-30°C hypothermia can preserve the process at a normal level (41). Treatment with 31°C hypothermia for 20 min *prior* to ischemia showed 3-fold mRNA levels of βF_1 -ATPase and ANT_1 than warmer temperatures (Fig. 5), and was accompanied with higher ATP levels during reperfusion (36). It is logical to conclude that hypothermia can preserve ANT_1 and βF_1 -ATPase to maintain ATP synthesis.

An experiment was designed with 91 isolated hearts to observe the effects of substrate supply and hypothermia separately, and combined. Warm ischemic controls (n = 16) had the lowest levels of ATP, and decreased expression of ANT_1 and βF_1 -ATPase mRNAs. This indicates energy-pathways injury. The hearts treated with hypothermia prior to ischemia (n = 12) had significantly better functional recovery and higher levels of ATP, ANT_1 and βF_1 -ATPase mRNAs. This indicates that ANT_1 and βF_1 -ATPase are preserved by hypothermia. Glucose administration without hypothermia (n = 16) showed better functional recovery and higher ATP level as well as the highest lactate production, suggesting the use of substrate. In this group a low level of ANT_1 and βF_1 -ATPase mRNA were observed, implying that warm temperature alone did not adequately preserve them. The combination of hypothermia and glucose administration (n = 10) showed the best functional recovery and highest levels of ATP, ANT_1 and βF_1 -ATPase mRNAs, but lower lactate production. This indicates that hypothermia preserves ANT_1 and βF_1 -ATPase function and activity, protects energy pathways, saves energy use, and further improves functional recovery. Combination of hypothermia and glucose seems to be a better process

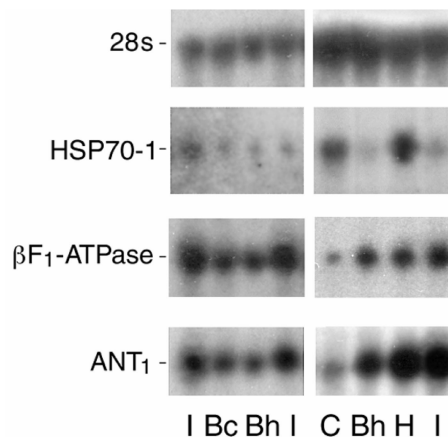


Fig. 5. A representative Northern blot. Each lane was loaded with 15 mg total RNA from ventricular myocardium and probed specifically for 28s, adenine nucleotide translocase isoform 1 (ANT_1), β -subunit of F_1 -adenosinetriphosphatase (βF_1 -ATPase), and inducible heat shock protein (HSP70-1). Samples were taken from hearts *in situ* (I), hearts at control baseline (Bc) and hearts after 20 min of hypothermia (Bh), and hearts after 2 hours of ischemia and 45 min of reperfusion in the ischemic control without hypothermia-treated group (C) and hypothermia treated-ischemia group (H). This figure is cited from Fig. 3 of *Am. J. Physiol.* 274 (Heart Circ. Physiol 43): H790, 1998 with permission from The American Physiological Society.

to combat oxygen insufficiency (38).

ANT_1 and βF_1 -ATPase proteins can continue to operate in the early ischemia period. The time period before cellular oxygen is depleted depends on the metabolic rate of the tissue. Hypothermia can prolong the time period that a tissue can remain hypoxic and enable functional recovery.

Observation post heart transplantation has shown preservation to be dependent on mitochondrial function as well (51).

The Effect of Hypothermia on Adaptive Response

Hypothermia induced adaptive response relates to changes in gene expression, such as self-antigen, melatonin, encephalin, and so on. The genes that are affected by hypothermia are those related to endogenous defense pathways and stress response.

Endogenous Defense Pathways

Mild hypothermia may induce up-regulation of endogenous defense pathways (12). Such as gene array analysis has indicated that hypoxia induced factor-1 α (HIF- α) increased in hypothermia treated hearts (42) with a characteristic adaptive response. HIF-1 may act by trans-activation to increase glucose-

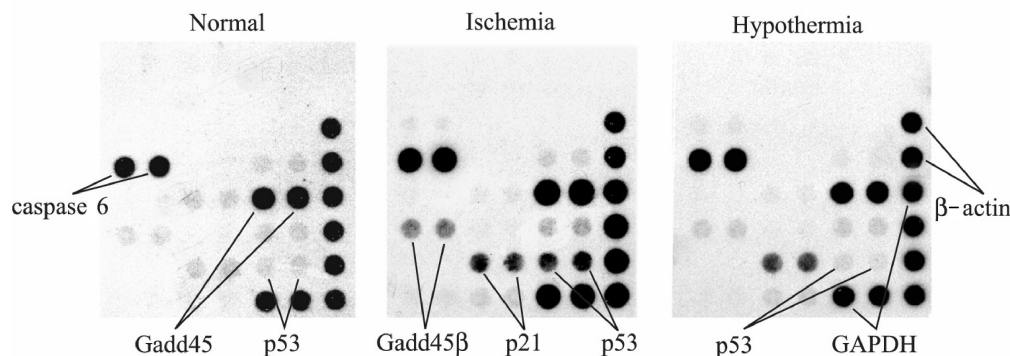


Fig. 6. A representative cDNA array of 40 selected genes including p53. The intensities of p53 are much higher in the ischemic heart than in the normal hearts. Hypothermia treatment reverses the ischemia condition. The intensities of β -actin are the same in the three groups. This figure is cited from Fig. 5 of *J. Appl. Physiol.* 92: 2205, 2002 with permission from The American Physiological Society.

regulated proteins Grp94 mRNA that can be induced by exposure to hypoxia (33). Grp94 can also be induced by change in temperature (12). The array analysis also suggested that cytochrome P-450 might be involved in hypothermia protection. Although the multiple mechanisms by which changes occur are not clear, many studies implicate intra-protective factors in various tissues that might be activated by specific mechanisms (4, 15, 19, 24, 27, 29).

Heat Shock Proteins

Substantial evidence has confirmed that HSPs play an essential role in tissue protection, including hypoxia and ischemia (46, 57). Both low and high temperature can induce HSP (1, 27). A study on isolated heart showed that cold ischemia induced HSP70-1, but there was no difference between 4°, 18°, and 30°C during 2 hours of cardioplegic ischemia (41). Ischemia itself or hypoxia without hypothermia can also increase HSP70-1 mRNA. Combined 30°C hypothermia with ischemia when followed by 2 hours of re-warming at 37°C showed no further enhancement of the mRNA level than two hours of 30°C hypothermia only. This implies that the mRNA elevation may be a trigger that is turned on by a strong stressor. Once the trigger is turned on, the mRNA level may not have a linear relationship with the changes in intensity or duration of the stimulation.

Hypothermia Inactivates Apoptosis Pathways

Apoptosis pathway activation has been considered as an index for hypoxic-ischemic status (10, 17, 26, 30, 54). An analysis was conducted in the isolated perfused rabbit hearts (42). The ischemic group was infused at 37°C before and after ischemia and at 34°C during 120 min of ischemia. A hypothermic-adapted

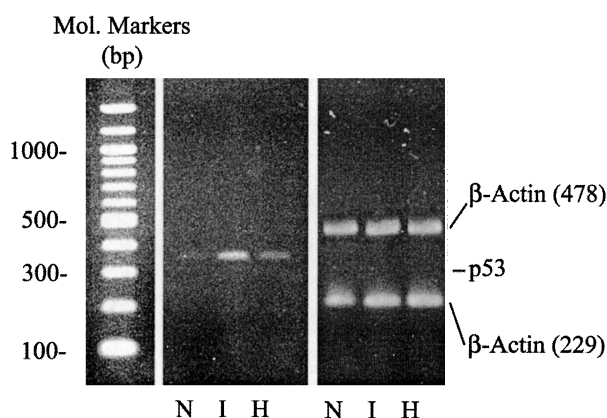


Fig. 7. RT-PCR of a p53 fragment (349 bp) after 35 cycles of amplification. The abbreviations used are N = normal heart; I = ischemic heart; H = hypothermia-treated ischemic heart; mol. = molecular. Densitometry analysis of average ratio of p53 are 4.0 (I/N) and 0.3 (H/I) $P < 0.05$; however, there are no significant difference in the ratio of β -actin between the groups (I/N, H/I, $P > 0.5$). This figure is cited from Fig. 6 of *J. Appl. Physiol.* 92: 2206, 2002 with permission from The American Physiological Society.

group was treated with 30°C for 30 min prior to ischemia and for 120 min during ischemia. A group of *in vivo* hearts was designed as a normal control. The functional recovery was consistently higher in the hypothermia treated hearts than the ischemic hearts. For example, the dP/dt_{\max} recoveries were $95 \pm 4\%$ and $33 \pm 4\%$ of baseline values in the hypothermia treated and control hearts, respectively. An analysis of the Super cDNA array showed that hypothermic adapted hearts decreased p53 (Fig. 6 & Fig. 7), gadd45, and bak, and increased the bcl-x. The normalized intensity of caspase 10 and I κ B α were also increased. The results suggest that hypothermia protection may relate to the proteins that

suppress apoptosis. Reduction of apoptosis is also an important protective mechanism seen with cold cardioplegia in the human atrial appendage (53).

An anti-rabbit p53 monoclonal antibody was used to test apoptosis in the isolated rabbit hearts for 2 hours ischemia followed by 3 hours of reperfusion. The histo-immunolabeling study revealed that ischemia induced the apoptosis marker p53 in the hearts, however, 29.5°C of mild hypothermia reversed the expression of p53 level to normal. The results confirm hypothermia protects myocardium from ischemic apoptosis (39).

Besides hypothermia protection in the myocardium, a recent study was completed in spinal cord ischemia. Increase in p53 expression and decrease in bcl-2 expression has been shown in the rabbit with spinal cord ischemia. Hypothermia treatment at 26-28°C mitigated these changes (54).

Relationship between Functional and Histological Status

To observe the relationship between functional and histological status, an experimental model of normothermic and hypothermic treatments (29.5°C) in isolated rabbit hearts was explored with one hour of pretreatment, two hours of cardioplegic arrest and three hours of reperfusion to examine effects of the hypothermia on histological status. During reperfusion there was marked improvement ($P < 0.05$) in cardiac function in the hypothermic rabbit hearts. Oxygen consumption and pressure-rate production were improved, developed pressure, dP/dt_{max} improved, and aerobic metabolism was maintained in a stable condition without lactate accumulation during ischemia. Histological evidence showed that the hearts treated with 34°C ischemia experienced a hydropic swelling; severe damage to the myocardium and accumulation of collagen was obvious in the connective tissue. The hypothermia treated hearts showed less swelling and damage to the myocardium, as well as less collagen in the connective tissue. The results indicated that mild hypothermia preserved myocardial stability (39). This relates to preservation of metabolic pathways, activation of molecular adaptive responses, and moderation of the apoptosis process to eliminating ischemic injury, resulting in improvement of functional recovery.

Lower Body Temperature

Hybridization-Related Materials

Low body temperature is one of the characteristics seen in mammalian hibernation. Cellular and molecular responses to hibernation are hot topics (12). There is differential gene expression between the active and

torpid states. These differences are crucial to understanding the mechanisms that may lead to improve medical application. For example, α_2 -macroglobulin is up-regulated during hibernation and also potentially beneficial in the hypothermic rats; it can prevent blood clotting during cardiac arrest. In addition, D-Ala2-Leu5-enkephalin has been used to mimic natural hibernation for cardiac storage (9). More research is necessary, however, before application in humans.

Chemical-Induced Hypothermia

Hypo-metabolism can be induced by inhibition of oxidative phosphorylation. Hydrogen sulfide (H_2S) has been reported to inhibit cytochrome c oxidase, which is the terminal enzyme complex in the electron transport chain. Inhalation of H_2S at 80 ppm is capable of decreasing metabolic rate and core body temperature in mice (6). Chemically induced hypothermia has potential for therapy.

Regional Hypothermia

Topical hypothermia is simple to apply during surgery, as shown by a regional hypothermia study conducted in rabbits (23). The investigators put an ice-water bag directly on the region of risk heart surface after 30 min of coronary occlusion and maintained 15 min of reperfusion. The hypothermia treatment significantly reduced infarct size. Although the actual myocardial temperature was not clear, the protection was similar to that observed previously with topical hypothermia at 30-32°C. Hypothermia started after occlusion mimics patient reperfusion therapy after delay, as could be seen clinically.

Hypothermia for Therapeutics and Prevention

Because of harm reduction, hypothermia has been used in the clinical arena for more than 5 decades. The clinical application parallels physiological study and has been ongoing prior to understanding of the underlying mechanism. Therefore, mild hypothermia as a therapeutic intervention is well documented (7, 14, 45, 47, 48). Studies where no benefit was seen from hypothermia pretreatment may reflect lack of sufficient physiological information, or the use of a sub-optimal temperature.

Animal Experiments In Situ

An intra-coronary infusion was performed with hypothermic Ringer's solution to decrease the local ischemic temperature in pigs (25). The myocardial temperature was decreased to 29.5~31.5°C. Results showed obvious protection, indicating that mild

hypothermia reached the protective threshold necessary to play a beneficial role.

Cardiopulmonary bypass is frequently used in cardiac surgery. After cardiac arrest, a group of dogs was treated by cardiopulmonary bypass reperfusion with 27°C or 34°C hypothermia for 4 hours, and were then maintained at 34°C to 12 hours. Hypothermia preserved viability of cerebral and myocardial organs compared with a normothermic control group (47).

Decreasing core temperature is an important step for hypothermia treatment in the heart. A design has been tested in large pigs with an endovascular heat-exchange catheter to lower core body temperature. The infarct size with 60 min of ischemia was significantly lower at 34°C treatment than at 38°C (16). Although 34°C offers less protection than the optimal range of $30.5 \pm 1.0^\circ\text{C}$, it is better than 38°C. The significance of this design suggests a direction for hypothermia therapeutics in a clinic setting.

Cross Adaptation

A stressor can induce adaptation to resist another stressor, which is termed cross adaptation (21). Myocardial ischemia induced arrhythmia and a decrease in contractility in rat hearts. After treatment with alternating exercise and cold, the disorders of cardiac rhythm and myocardial contractile state could be prevented in acute ischemia. (3, 8). How and why cross adaptation promotes a beneficial response rather than deteriorative remains a puzzle.

A stressor can induce two kinds of responses. One is a non-specific response such as increased heart rate and cardiac output that shows similar effects to different stressors, such as heat, cold, hypoxia, and physical loading. Another is a specific response to the stressor, such as the increase in heart rate and myocardial contractility seen in response to heat. Hypoxia shows a decrease in contractility and lower heart rate. Hypothermia lowers heart rate and increases contractility initially and then decreases it with extended times at low temperatures. Non-specific responses are mainly controlled by neurohumoral regulation. Specific responses are intracellular. At high altitude and in an acute hypoxic environment, non-hypoxic stress has been treated to test adaptive capacity in mountaineering performance (43). Heat stress could improve myocardial function after reperfusion if applied at an appropriate time prior to ischemia (15). It is reasonable to suppose that hypothermia induces cross adaptation, and would be a potential way to improve tolerance to injury from hypoxia and ischemia.

During Emergency

Many patients suffering a heart attack die on the

way to the hospital. According to the reports, an estimated 95% of patients are outside hospitals when they have a cardiac arrest. Cardiac arrest could induce severe brain hypoxia and damage in 6-9 min or less. In the 1950s, many groups studied how to delay brain death with cold-treatment. When the dog esophageal temperature was decreased to 25-28 degrees and a circulating ice-water cooling cap was put on the head, the brain function as evaluated by EEG, blink reflex, light reflex etc. could tolerate a complete occlusion of the carotid artery for as long as 90 min without post-operative damage. The results suggest that under appropriate low-temperature conditions, heart surgery with an arrested heart could be prolonged to longer than 1 hour without brain damage (13).

Treatment by lowering core body temperature to 32-34°C (52) or 33°C (11) has been used in patients and can reduce the mortality rate. Up to now, systemic hypothermia has become a therapy widely applied in the world (48). Side effects do appear in the applied hypothermic studies, however, indicating the need for further research into physiology and patho-physiology of hypothermia. Protective and injury temperature thresholds need to be established for different age ranges, and the optimal protocols established for different organs as well as the underlying mechanisms.

Endovascular Cooling

Patients with acute myocardial infarction were treated with endovascular cooling to $33.2 \pm 0.9^\circ\text{C}$ for 3 hours during reperfusion. The technique was found to be safe, but the results did not show a significant decrease in infarct size (18). Many factors might affect the results. A 34°C temperature might not reach the protective threshold. A temperature above 34°C would offer less protection, as was observed in the isolated heart model (35, 36, 40, 41) and pig hearts *in vivo* (44).

During Surgery

When the temperature decreases to 25°C, severe heart arrhythmia may begin. The selective perfusion of the upper (29°C) and lower body (23°C) respectively has been used to perform surgery in a coronary artery disease patient (20). The surgery was successful for a thoracoabdominal aortic aneurysm and an abdominal paraanastomotic pseudoaneurysm with good postoperative outcome.

It is obvious that the temperature of 29°C reaches the hypothermic protective threshold (35, 36) and is in the optimal temperature range without reaching the threshold for injury of 28°C (44). This successful result at least partially relates the physiological threshold principle. Basic investigation is the benchmark for

“bedside” in the myocardial protection that has been called the New Holy Grail of contemporary cardiology (14).

Conclusion

Mild hypothermia can play an important role in myocardial preservation and *via* cross adaptation to resist injury caused by hypoxia and ischemia. The protective threshold is $30.5 \pm 1.0^\circ\text{C}$. In the cardioplegic ischemic hearts, the protection is not significantly different between 4° and 30°C . In the beating hearts *in vivo*, temperature below 28°C induces injury. The myocardial temperatures above 34°C or below 28°C would result in less protective *in vivo*. Modification in the balance of oxygen demand and supply, preservation of energy and its relative pathways, up-regulation of endogenous defense pathways, down-regulation of apoptosis pathways, and maintenance of cell integrity are involved in the protection. Hypothermia treatment must consider both the brain and heart. When the gap between clinical practice and physiological research is filled, we expect mild hypothermia will be a powerful therapeutic tool for prevention of hypoxic cardiac injury.

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