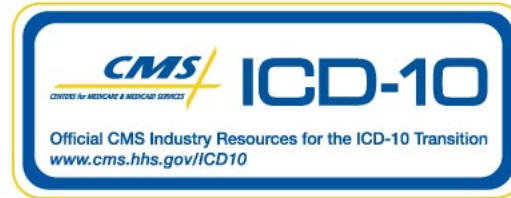


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EXPERIMENTAL STUDIES

Hypothermic, Closed Circuit Pericardioperfusion: A Potential Cardioprotective Technique in Acute Regional Ischemia

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Objectives. This study sought to determine whether infarct size can be reduced by hypothermic pericardioperfusion.

Background. We have shown that myocardial infarct size can be reduced by topical cooling of the heart. The present study tests whether myocardial cooling and protection can be produced by hypothermic pericardioperfusion using a catheter.

Methods. The catheter was sutured into the pericardial space of anesthetized rabbits. Beginning 30 min before coronary artery occlusion, the space was perfused with either chilled ($n = 10$) or body temperature ($n = 10$) fluid. The artery was occluded for 30 min and reperfused for 3 h.

Results. After 30 min of pericardioperfusion, myocardial temperature was reduced to $34.1 \pm 0.9^\circ\text{C}$ in chilled hearts compared with $38.9 \pm 0.4^\circ\text{C}$ in control hearts, $p < 0.001$, a reduction in myocardial temperature of $\sim 5^\circ\text{C}$. Risk areas were similar in both

groups ($32 \pm 4\%$ left ventricle in cooled and $31 \pm 3\%$ in control hearts, $p = \text{NS}$). However, infarct size in cooled hearts was significantly reduced by 49% ($18 \pm 3\%$ of risk area vs. $35 \pm 6\%$, $p = 0.025$). Tamponade did not develop, and there were no significant differences in heart rate, arterial pressure or body temperature between groups.

Conclusions. A significant reduction in myocardial temperature, without the development of cardiac tamponade, can be attained using a pericardial catheter to cool the pericardial space. This reduction in temperature causes a significant reduction in necrotic damage. This technique might be used to cool and protect the heart as an adjunct to thrombolysis or during minimally invasive cardiac surgery.

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Several recent animal studies have demonstrated the protective role of hypothermia in the setting of acute regional ischemia, reducing necrosis in the myocardium (1-9). Data from two studies in rabbits in our laboratory have shown that reducing myocardial temperature by direct local cooling of the surface of the heart, either before or early after coronary artery occlusion, results in a profound reduction in infarct size (8,9). Although this method was very effective in producing hypothermia and reducing infarct size, it has limited clinical application.

The pericardial space has been proposed as a site of delivery of agents to the heart. One advantage of this route of administration is an enhanced local effect with reduced systemic effects. Hypothermia induced by this technique might be beneficial in reducing necrosis and providing myocardial protection in patients undergoing acute myocardial infarction or in patients with coronary artery disease undergoing bypass surgery without extracorporeal circulation.

In the present study we investigated the ability of a specially designed pericardial catheter to deliver coolant to the pericardial space and to reduce myocardial temperature before

coronary artery occlusion in rabbits. We also studied the influence of this intervention on myocardial infarct size.

Methods

The rabbits used in the present study were maintained in accordance with the policies and guidelines of the Position of the American Heart Association on research animal use and the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (Department of Health, Education and Welfare Publication No. 85-230). The Good Samaritan Hospital is accredited by the American Association for Accreditation of Laboratory Animal Care.

A double lumen, twin port catheter was designed for delivery of fluid to the pericardial space (Kloner/Comedicus). One port served as the inlet allowing infusion; the second port continuously removed fluid so that a constantly circulating system was bathing the heart with either the chilled or the warm infusate. The lumen utilized for infusion terminated as a single outlet 3 cm before the tip. Suction of the infusate was achieved at the distal 3 cm of the tip of the catheter by an opening at the tip and multiple side holes. The catheter was prepared for introduction after flushing the two lumens with heparinized Ringer's lactate.

Animal preparation. White male New Zealand rabbits weighing 2.2 to 3.2 kg were anesthetized with intramuscularly administered xylazine (200 mg) and ketamine (400 mg), intu-

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bated and mechanically ventilated. A deep level of anesthesia was maintained during the study with pentobarbital (~50 mg/h) given intraperitoneally. Catheters were placed in the internal jugular vein for infusion of saline and in the carotid artery for hemodynamic monitoring and withdrawal of reference blood samples during regional myocardial blood flow measurements. A left lateral thoracotomy was performed and the parietal pericardium was exposed. Without opening the pericardium, the course of the left circumflex artery or its major branch was identified for future occlusion. The pericardial catheter was placed by making a small incision in the parietal pericardium near the base of the heart. The pericardium around the catheter was closed by a previously placed purse-string suture, thus preventing any leaks.

The two ports of the catheter were connected to a closed circuit tubing system filled with heparinized Ringer's lactate. This circuit was passed through a peristaltic pump that perfused the pericardial space at a rate of 20 ml/min without net accumulation of fluid.

Experimental protocol. The rabbits were then randomized to one of two treatment groups. In the chilled perfusion group the tubing was passed through an ice water bath to chill the liquid. In the warm perfusion group the tubing was passed through a 40°C water bath to warm the fluid to maintain average rabbit body temperature (39°C). Rectal temperature was measured at baseline and every 30 min. Body temperature was maintained by heating pad if necessary. The pericardium was perfused for 30 min before coronary artery occlusion. Hemodynamic variables were recorded continuously and measured every 30 min during the entire protocol to identify any early instability suggestive of tamponade. If bubbles developed in the system, the tubing was reprimed with heparinized Ringer's lactate.

A temperature probe was placed into the myocardium through the pericardial layers 5 min before occlusion. The probe tip was inserted horizontally into the myocardium with the tip in the region expected to become ischemic. The placement was typically in the anterior, apical region of the heart, within 0.5 cm of the perfusion catheter. Temperature measurement of the myocardium using the thermocouple probe is subject to some technical limitations. The thickness of the rabbit ventricular wall is ~3 mm. We attempted to insert the tip of the probe into the midmyocardium; however, the location of the probe within the wall could not be exactly determined. It is probable that the temperature measured by the probe reflected a gradient from epicardial to endocardial surfaces, because the epicardial surface was being perfused externally and the endocardial surface is warmed by blood in the ventricular cavity.

The temperature was recorded. Next, a stitch was taken through the pericardium, around the previously identified coronary artery, and the artery was occluded by forming a snare using a piece of flanged plastic tubing. In a few rabbits, a coronary vein, because of its proximity to the artery, may also have been occluded. Leakage from the pericardial cavity

around the sutured areas was prevented by occlusion of the holes by the flanged end of the plastic tubing.

The rabbits were then subjected to 30 min of coronary artery occlusion followed by 3 h of reperfusion. At 25 min of occlusion, the pericardial perfusion was terminated and the pericardium was opened. A catheter was placed into the left atrial appendage and secured with a hemostatic clip. Immediately after catheter placement, radiolabeled microspheres were injected and a reference blood sample was collected from the carotid artery at 2.06 ml/min, as described previously (10). This blood flow measurement confirmed ischemia in the risk area. After 30 min of coronary artery occlusion, the myocardium was allowed to reperfuse by releasing the occluding snare. The presence of reflow was confirmed by repeating regional myocardial blood flow measurements at 30 min in the reperfusion period. After 3 h of reperfusion the coronary artery was reoccluded and blue pigment was instilled from the left atrial catheter to delineate the risk area. Under deep anesthesia the rabbit was euthanized with 12 mEq of potassium chloride. The heart was excised from the thoracic cavity and the right ventricle was trimmed off. The left ventricle was sliced into multiple cross-sectional slices ~2 mm in thickness. These slices were photographed to demonstrate the risk zone, unstained by the blue pigment. Then these slices were incubated in triphenyltetrazolium chloride at 37°C for 10 min. The necrotic myocardium was identified as pale nonstained areas and rephotographed. The photographs of the slices demonstrating the risk and necrotic areas were enlarged and traced. Area of risk and infarct were calculated after planimetry and expressed as a proportion of the total left ventricle. Volumes of the risk and necrotic regions were calculated as described previously (8). The following end points were measured: rectal temperature, myocardial temperature, mean arterial blood pressure, heart rate, regional myocardial blood flow, area at risk and infarct size.

Statistical analysis. All data are expressed as mean value \pm SEM. Statistical analyses were performed using SAS (Version 6.04). Data on the ischemic and necrotic zones, temperatures and regional myocardial blood flow were evaluated using a two-tailed *t* test. Analysis of covariance was used to test for a group effect on the ratio between the necrotic and ischemic zones. Analysis of variance for repeated measurements was used to evaluate heart rate and mean arterial pressure.

Results

A total of 27 rabbits were studied. Data are presented here from the 20 rabbits randomized to chilled ($n = 10$) or control ($n = 10$) perfusion. Seven rabbits were excluded. Four rabbits were excluded at the start of the protocol because the coronary artery could not be visualized under the intact pericardium. Of the remaining three, one was excluded because the coronary artery failed to reperfuse (as determined by regional myocardial blood flow <0.2 ml/min per g in the previously ischemic

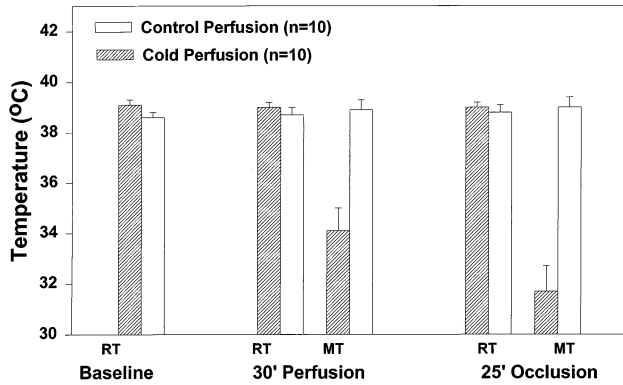


Figure 1. Average rectal temperatures (RT) and myocardial temperatures (MT) in the chilled and control groups during baseline, after 30 min of pericardial perfusion and at 25 min of occlusion.

region 30 min after reperfusion), and two died during the reperfusion period.

Temperatures. At the beginning of the experiment, the body temperature was similar in both groups ($39.1 \pm 0.2^\circ\text{C}$ in chilled and $38.6 \pm 0.2^\circ\text{C}$ in warm perfusion hearts, $p = \text{NS}$). At 30 min of perfusion the myocardial temperature was significantly reduced to $34.1 \pm 0.9^\circ\text{C}$ in the chilled group compared with $38.9 \pm 0.4^\circ\text{C}$ in the control group ($p < 0.001$, as shown in Fig. 1). Continued pericardial perfusion during occlusion further reduced the temperature in chilled hearts ($31.7 \pm 1.0^\circ\text{C}$) when compared with the control hearts ($39.0 \pm 0.4^\circ\text{C}$), $p < 0.0001$. Thus, perfusion of chilled Ringer's lactate in the pericardial space successfully lowered temperature by $\sim 5^\circ\text{C}$ after 30 min of pericardial perfusion and by $\sim 7^\circ\text{C}$ at end of occlusion.

Infarct size. The data on risk region and infarct size are shown in Table 1. The variables of mean body weight, left ventricular weight and the volume of the risk region (expressed either in weight or as a proportion of the left ventricle) were comparable between the two groups. Infarct size, expressed as a proportion of the risk region was reduced by 49% with pericardial perfusion with chilled Ringer's lactate ($18 \pm 3\%$) compared with control perfusion ($35 \pm 6\%$, $p = 0.025$). On visual inspection the salvage appeared to occur transmurally. Analysis of covariance testing for a group effect on the relation of the amount of tissue becoming necrotic to the amount of

Table 1. Weights, Risk and Infarct Size Data

Variable	Cold Perfusion	Control Perfusion
Risk zone/LV (%)	32 ± 4	31 ± 3
Necrosis/risk zone (%)	$18 \pm 3^*$	35 ± 6
Myocardium at risk (g)	1.23 ± 0.21	1.08 ± 0.09
Necrotic myocardium (g)	0.28 ± 0.10	0.41 ± 0.09
LV weight (g)	3.63 ± 0.15	3.48 ± 0.12
Body weight (kg)	2.6 ± 0.1	2.4 ± 0.1

* $p = 0.025$ cold perfusion versus control perfusion. LV = left ventricle, ventricular.

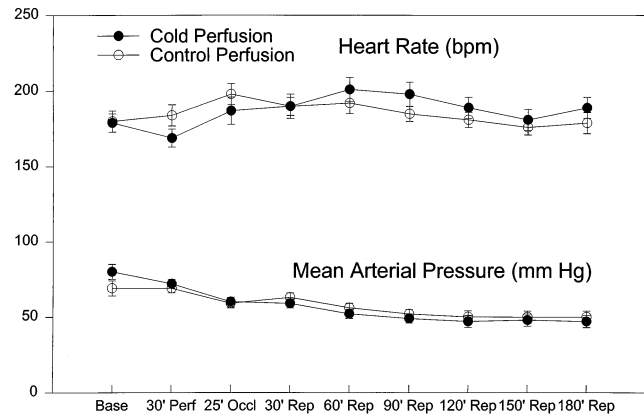


Figure 2. Changes in average heart rate and mean blood pressure over time in the chilled and control groups. There were no significant differences in hemodynamic variables between the two groups. Base = baseline; bpm = beats/min; Occl = occlusion; Perf = perfusion; Rep = reperfusion.

tissue at risk revealed a significant group effect ($p < 0.009$). Therefore on average, for any given size risk region, cooled hearts developed smaller infarcts.

Heart rate and blood pressure. Pericardial perfusion performed using a closed circuit connected to a peristaltic pump was achieved without any hemodynamic compromise as shown in Figure 2. Heart rate and mean arterial pressure were similar in both the chilled and the warm groups during the entire protocol. Thus, perfusion of chilled Ringer's lactate was safely accomplished by this technique.

Regional myocardial blood flow. Regional myocardial blood flow measurements in both groups performed during occlusion confirmed very low flow in the ischemic zone (0.05 ± 0.02 ml/min per g in chilled vs. 0.03 ± 0.01 ml/min per g in control perfusion, $p = \text{NS}$). Measurements repeated at 30 min of reperfusion demonstrated successful reflow in ischemic zones in both groups (1.64 ± 0.25 ml/min per g in chilled and 2.60 ± 0.39 ml/min per g in control hearts, $p < 0.05$). Although both of these values are within the normal range for the rabbit heart, absolute flow at 30 min of reperfusion was lower in the chilled group.

Discussion

Background. Many interventions have been tested with the aim of reducing infarct size, yet only a few maneuvers reliably and consistently reduced myocardial infarct size. These include early coronary artery reperfusion and ischemic preconditioning. Experiences with pharmacologic agents have been variable, depending on the agent used. The technique of topical regional cardiac hypothermia has led to the most striking reductions in infarct size that we have observed in the rabbit myocardial infarction model (even greater than ischemic preconditioning). The technique could be adapted for use as an adjunct to reperfusion therapy or to protect potentially jeopardized myocardium during minimally invasive cardiac surgery. However, our previous studies have been done using a tech-

nique of directly cooling the heart with an ice and water bag. For clinical application, methods must be developed to cool the heart less invasively.

Previous related studies. Evidence from recent animal studies have shown a favorable outcome in infarct size reduction from temperature reduction (1-9). Hale and Kloner (8) from our laboratory have studied the effects of topical regional hypothermia on infarct size in the rabbit model. Cooling was achieved by direct application of a bag filled with ice and water to the risk zone, beginning 20 min before occlusion and lasting 15 min into reperfusion. Reduction of myocardial temperatures by $3.6 \pm 0.4^\circ\text{C}$. was associated with a 65% reduction in infarct size when compared with control rabbits. More recently we have shown that hypothermia begun after 10 min of occlusion reduced infarct size by 50% (9).

Results from the present study. In the present study, chilled Ringer's lactate perfusion through a pericardial catheter was shown to successfully reduce myocardial temperatures by $\sim 5^\circ\text{C}$ at 30 min and by $\sim 7^\circ\text{C}$ at 55 min. At this level of temperature reduction, no deaths were observed that could be attributed to ventricular arrhythmia or bradyarrhythmia. By utilizing a peristaltic pump at a set rate accumulation of fluid in the pericardial space and subsequent tamponade was prevented. Thus, this method successfully lowered myocardial temperatures and was safe. Moreover, infarct size was significantly reduced by 49% in the chilled group when compared with the control group.

Use of the pericardial space. The pericardial space has been proposed as an alternate site of delivery of agents to the heart and coronary arteries. One of the advantages of this route of administration is an enhanced local effect with reduced systemic effects. Hypothermia induced with a technique similar to ours may be beneficial in reducing necrosis and providing myocardial protection in patients with coronary artery disease undergoing bypass surgery without extracorporeal circulation. We envision that the pericardial catheter could be inserted when the chest is first opened or even before opening the chest with subxyphoid catheter insertion. At the time the internal mammary artery is isolated and prepared, the pericardial space and myocardium could be cooled in preparation for the anastomosis. With safer techniques in implementing this method, this therapy may also have a potential role as an adjunct to angioplasty or thrombolysis. The advent of improved technology allowing safer introduction of catheters into the pericardial space will allow the study of the potential benefits of hypothermia in similar and other clinical settings where myocardium is in jeopardy from ischemia insult.

Potential therapeutic applications. Advances in techniques and improvements in technology have allowed cardiac surgeons to successfully perform minimally invasive coronary artery bypass graft surgery. The most popular approach is to perform coronary artery bypass graft surgery on a beating heart without extracorporeal circulation (11,12). The other method is to conduct anastomosis on a nonbeating heart with cardiopulmonary bypass by a percutaneous endovascular aortic occluder and cardioplegia solution delivery system (13,14).

During anastomosis of the left internal thoracic artery with the left anterior descending artery on a beating heart, reduction of cardiac motion is aided by adenosine, which causes brief asystole (15), pharmacologic bradycardia (16) or vagus nerve stimulation (17). In addition, many devices have been reported to stabilize the coronary artery and the adjoining myocardium, allowing ease of anastomosis (12). However, patients undergoing revascularization on beating hearts may have a limited tolerance to ischemia and have the potential for great risk of myocardial damage. Proposed methods of myocardial protection in such a setting are the benefits of preconditioning by adenosine (18), $\text{Na}^+\text{-H}^+$ exchange inhibitors like HOE 694 (19) and brief aortic cross clamping before initiating cardiopulmonary bypass (20). Many of these methods are currently under investigation.

Conclusions. A significant reduction in myocardial temperature, without the development of cardiac tamponade, can be attained using a pericardial catheter to cool the pericardial space. This reduction in temperature causes a significant reduction in necrotic damage. This technique might be used to cool and protect the heart during minimally invasive cardiac surgery or as an adjunct to thrombolysis.

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