Myocardial temperature reduction attenuates necrosis after prolonged ischemia in rabbits

Sharon L. Hale\textsuperscript{a, *}, Robert A. Kloner\textsuperscript{b}

\textsuperscript{a}The Heart Institute, Good Samaritan Hospital, 1225 Wilshire Blvd, Los Angeles, CA 90017, USA
\textsuperscript{b}University of Southern California, Division of Cardiology, Los Angeles, CA 90017, USA

Received 15 December 1997; accepted 17 April 1998

Abstract

Objective: Previously we observed that a large reduction in infarct size was attained by cooling the risk region of the heart, either before or early after the onset of a 30-min coronary artery occlusion. While this is a standard duration of ischemia used in the rabbit model of infarction, it may not reflect the situation of patients who are reperfused late. The effects of regional hypothermia with a longer duration of ischemia, and when the intervention is applied later, are unknown. This study tests the hypothesis that a local reduction in cardiac temperature protects myocardium during prolonged ischemia (2 h) even if begun well after coronary artery occlusion. Methods: Anesthetized rabbits received 2 h of coronary artery occlusion and 3 h of reperfusion. Rabbits were randomly assigned to a treated group: topical myocardial cooling starting 30 min after coronary occlusion ($n=14$), or control group, no intervention ($n=12$). Myocardial temperature in the risk zone, hemodynamics and regional myocardial blood flow were measured. Results: Ischemic zone temperature was similar in both groups at 30 min post occlusion, but the cooling maneuver produced a reduction in temperature in the risk region of the treated group such that myocardial temperature was reduced an average of 10.8°C between 30 and 60 min of coronary artery occlusion. Myocardial temperature in the control group remained within 0.3°C of baseline during coronary artery occlusion and into reperfusion. Core temperatures were similar in both groups. Hemodynamic parameters and collateral blood flow during occlusion were also equivalent in both groups. After 120 min of coronary occlusion, necrosis in the control group comprised 72±3% of the ischemic risk region. However, in cooled hearts, infarct size, expressed as a fraction of the risk region was significantly lower. Infarct size in this group averaged 59±3% of the risk region ($p<0.004$ vs. controls), and thus cooling resulted in a salvage of approximately 18% of the risk region. Conclusion: These results show that reducing myocardial temperature protects ischemic myocardium during a long duration of ischemia even if initiated after coronary artery occlusion. © 1998 Published by Elsevier Science B.V. All rights reserved.

Keywords: Infarct size; Temperature; Hypothermia; Rabbit heart

1. Introduction

Previously we observed that a profound reduction in infarct size was achieved by cooling the risk region of the heart, either before or early after the onset of a short, 30 min coronary artery occlusion [1,2]. Potentially this approach could be developed for use in the clinical realm as an adjunct to reperfusion therapy or to protect potentially jeopardized myocardium during minimally invasive cardiac surgery. However, it is not known how effective myocardial cooling would be against more prolonged coronary artery occlusions. The time to reperfusion in patients may take 2–3 h after the onset of chest pain. It is important to determine whether hypothermia can still reduce infarct size when the duration of ischemia is prolonged to 2 h. It has been shown that not all interventions that reduce myocardial infarct size are beneficial during prolonged episodes of ischemia. Ischemic preconditioning, for example, does not reduce experimental...
myocardial infarct size when the duration of coronary artery occlusion is prolonged beyond 90–120 min, at least in the canine model [3,4].

The significance of temperature on the progression of myocardial necrosis has been demonstrated by other investigators showing that the amount of the ischemic risk zone that advances to necrosis correlates with body temperature at the time of coronary artery occlusion. Studies have shown this association between core temperature and infarct size in several different species [5–7].

The purpose of this study was to test the hypothesis that regional cooling of the myocardium would be beneficial in the setting of prolonged ischemia and when hypothermia is produced after coronary artery occlusion. Rabbits received 2 h of coronary occlusion followed by 3 h of reperfusion. In the treatment group cooling was initiated 30 min into the coronary artery occlusion. Control hearts received no intervention. We used a technique to induce regional hypothermia of ischemic myocardium which involved cooling the anterior surface of the heart by the application of an ice- and water-filled bag.

2. Methods

The rabbits used in this study were maintained in accordance with the policies and guidelines of the Position of the American Heart Association on research animal use [8] 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.

2.1. Preparation

Male New Zealand white rabbits weighing 2.2–3.0 kg were anesthetized using a mixture of ketamine (400 mg per rabbit) and xylazine (200 mg per rabbit), administered intramuscularly in two doses, approximately 10 min apart. Throughout the study, a level of deep anesthesia was maintained using sodium pentobarbital given intraperitoneally at a dose of approximately 50 mg/h. The rabbits were intubated and mechanically ventilated with room air supplemented with oxygen. Fluid filled catheters were placed into the jugular vein to administer fluids and into the left carotid artery to measure hemodynamics and to obtain reference blood samples during regional myocardial blood flow measurements. The chest was opened through the left fourth intercostal space, the pericardium was incised and the heart was exposed. A large anterolateral branch of the circumflex artery, or the circumflex artery itself, was identified and encircled with a 4-0 silk suture. The ends of the suture were threaded through a piece of flanged tubing, forming a snare, which was later used to occlude the artery. A catheter was placed into the left atrial appendage to inject radioactive microspheres and blue pigment at the end of the procedure. A thermocouple probe was inserted into the myocardium in the region expected to become ischemic during coronary occlusion. Heart rate, blood pressure, myocardial and core body temperature were recorded throughout the study protocol. Body temperature was maintained using a heating pad.

2.2. Experimental protocol

After surgical preparation, baseline hemodynamics, myocardial and core body temperature were recorded. Next, the artery was occluded for 120 min. After 30 min of occlusion, the rabbits were randomly assigned to either a treated group: topical myocardial cooling (n=14), or control group, no intervention (n=12). In animals assigned to receive cooling, a bag filled with ice and water was placed on the anterior surface of the heart (risk region). The bag remained in place for the remaining 90 min of coronary occlusion and for 15 min into the reperfusion period. The bag was then removed and the hearts allowed to warm. After 120 min of occlusion, the hearts were reperfused for 3 h. Heart rate and arterial blood pressure were recorded every 30 min during occlusion and during reperfusion at a 25 mm/s paper speed. Myocardial and core body temperatures were measured throughout the experiment. Regional myocardial blood flow was determined at 90 min of coronary occlusion to confirm that the risk zone was ischemic and at 30 min of reperfusion to confirm reflow in the same zone. At the end of 3 h of reperfusion, the coronary artery was reoccluded and four ml of 50% Unisperse blue (Ciba-Geigy, Hawthorne, NY, USA) were infused through the left atrial catheter and allowed to circulate throughout the vascular system. The location of the tip of the thermistor probe in the ischemic risk area was confirmed. The rabbit was then euthanized by an overdose of xylazine (300 mg, IV) followed by 12 mequiv. of potassium chloride given into the left atrium. Prospective exclusion criteria included an ischemic risk zone of less than 10% of the left ventricular weight, a regional blood flow of more than 0.2 ml/min/g in the risk zone during coronary artery occlusion (lack of ischemia), or a regional blood flow of less than 0.4 ml/min/g in the risk zone at 30 min of reperfusion (failure to reperfuse).

2.3. Analysis of infarct size

The right ventricle was trimmed off and the left ventricle was sliced transversely into seven or eight sections, approximately 2 mm in thickness. These slices were photographed to identify the ischemic risk regions (uncolored by the blue pigment) and the non-ischemic regions (colored by the blue pigment). The slices were then incubated in a 1% solution of triphenyltetrazolium chloride pre-heated to 37°C for 10 min and rephotographed for
analysis of the area of necrosis. All sections were later fixed in formalin. The photographic slides were projected and areas of risk (AR) and areas of necrosis (AN) were traced by planimetry. The planimetered areas of each slice were multiplied by the weight of the slice and then summed.

2.4. Measurement of regional myocardial blood flow

Regional myocardial blood flow was measured with 11 µm radioactive microspheres labeled with $^{141}$Ce, $^{95}$Nb or $^{103}$Ru (New England Nuclear, North Billerica, MA, USA), using approximately 500 000 per injection. These microspheres were injected into the left atrial catheter. At the same time, a reference blood sample was obtained from the carotid artery at 2.06 ml/min. At the end of the protocol, myocardial samples were obtained from the center of the non-ischemic and the ischemic regions, weighed and counted with the reference blood samples in a well gamma counter. Blood flows at each interval, for ischemic and non-ischemic tissues, were then computed and expressed in ml/min/g [9].

2.5. End points and data analyses

The following end points were measured: core body temperature, myocardial risk zone temperature, mean arterial blood pressure, heart rate, regional myocardial blood flow, area at risk and infarct size. All data summary and statistical analyses were performed using SAS (Version 6.04, Cary, NC, USA). Left ventricular weight, infarct size, and area at risk were compared using group t-test. Regional myocardial blood flow data were analyzed by analysis of variance. Temperature, heart rate and blood pressure were analyzed by repeated measures analysis of variance. Analysis of covariance was used to test for a group effect on the regression model of myocardium at risk and necrotic myocardium. Data are expressed as mean±standard error of the mean.

3. Results

3.1. Animal population

Thirty-eight rabbits entered the protocol. Three rabbits died of hypotension. Data from an additional nine rabbits were excluded based on prospective exclusion criteria. One heart had an ischemic risk zone of less than 10% of the left ventricle. One heart (cooled) failed to reperfuse, indicated by a regional myocardial blood flow of less than 0.4 ml/min/g at 30 min after reperfusion. Seven rabbits exhibited regional blood flow in the ischemic zone after 90 min of occlusion of greater than 0.2 ml/min/g (three cooled hearts and four controls). Although the rabbit heart has low collateral blood flow, it is possible that either the clamp occluding the artery loosened during the occlusion period causing premature reperfusion or that in some hearts collateral vessels opened. Data is reported on 14 control rabbits and 12 treated rabbits.

3.2. Myocardial temperature

Myocardial temperatures were similar in both groups at baseline and after 30 min of occlusion, before the start of cooling (38.8±0.2°C in control hearts and 38.6±0.2°C in hearts randomized to cooling). Topical cooling successfully reduced temperature so that 30 min later (60 min of occlusion) average myocardial temperature in the cooled hearts was 29.9±0.5°C. Myocardial temperature remained near this level for the entire cooling period (Fig. 1). After topical cooling was ended at 15 min of reperfusion, the hearts in this group began to rewarm, and by 1 h of reperfusion myocardial temperature was similar in both groups. In control hearts, myocardial temperature remained within 0.3°C of baseline. There were no significant differences in rectal temperature between the groups during the protocol. (Fig. 1).

3.3. Risk region and infarct size

Mean body weight, left ventricular weight, and the volume of the risk region (expressed either in weight or as a percentage of the left ventricle), were comparable in both groups (Table 1). After 120 min of coronary occlusion, necrosis in the control group had extended to comprise 72±3% of the ischemic risk region. However, in cooled hearts, infarct size, expressed as a fraction of the risk region was significantly lower. Infarct size in this group averaged 59±3% of the risk region ($p<0.004$ versus controls), and thus cooling resulted in a salvage of approximately 18% of the risk region. Analysis of co-
variance testing for a group effect on the relationship between risk and necrotic regions revealed a significant group effect (p=0.001). The slope of the regression line of the treated group was lower than that of the control group, showing that for equivalent risk sizes, the treated hearts developed a smaller infarct than controls.

3.4. Regional myocardial blood flow

Myocardial blood flow was measured after 90 min of coronary artery occlusion. At this time point, the risk zones of both groups were equally ischemic (0.05±0.01 ml/min/g in control hearts and 0.04±0.01 in cooled hearts). In the normal (not ischemic) region, average blood flow was lower in the cooled hearts (1.10±0.11 ml/min/g) than in control hearts (1.44±0.12, p<0.04).

After 30 min of reperfusion, RMBF was again measured. Flow to the nonischemic regions was comparable in both groups (1.22±0.07 and 1.37±0.14, respectively). In the previously ischemic region, RMBF was significantly higher in cooled hearts than in control hearts (p<0.02). Mean RMBF in cooled hearts was 1.50±0.30 ml/min/g (range 0.42–3.65), and the level of blood flow in this region was negatively correlated with the amount of necrosis expressed in grams, i.e., the smaller the extent of necrosis the greater the blood flow (r=0.66, p<0.02). In control hearts mean RMBF was 0.64±0.09 (range 0.30–1.66) and the level of blood flow in this region was also negatively correlated with the extent of necrosis (r=0.61, p<0.02).

3.5. Heart rate and arterial pressure

Heart rates were similar in both groups at baseline. Heart rate was lower in the treated groups during the cooling phase; however, overall there were no significant differences between the two groups. (Fig. 2). Mean arterial pressures were also similar at baseline and although tending to decrease over time, there were no significant differences between groups.

4. Discussion

The main finding in our study is that topical cooling of ischemic myocardium reduces infarct size in long duration ischemia with reperfusion, even when initiated well after the onset of coronary artery occlusion. These results indicate the important role of myocardial temperature level during the later phase of an evolving myocardial infarction. The longer duration of ischemia resulted in an extension in the amount of necrosis compared with previous studies in our laboratory of 30 [1,2] to 60 min (unpublished data) of ischemia, such that after 2 h of ischemia infarct size in controls comprised about three quarters of the risk area. However, in this study, even when cooling was begun 30 min after the start of ischemia, it still significantly salvaged ischemic tissue by 18%. Although previous studies [1,5–7] have shown the importance of myocardial temperature during ischemia of shorter duration (30–45 min), this is the first study to show that hypothermia is effective in reducing the amount of necrosis resulting from a lengthy coronary occlusion even when therapy was initiated well into the ischemic period. Thus, the advantage of this therapy over ischemic preconditioning or preconditioning-mimetic drugs is that the intervention can be started after coronary artery occlusion and is effective with longer coronary occlusions.

Earlier studies have shown that blood, pericardial or myocardial temperature at the time of coronary occlusion correlates with the eventual myocardial infarct size [1,5–7]. For example, data from a previous study in our laboratory [1] showed that myocardial infarct size expressed as a percentage of the area at risk correlated strongly with myocardial temperature at the time of occlusion (r=0.85). A correlation between temperature and infarct size has also been noted in the open-chest dog model of coronary artery occlusion [7]. Duncker et al. [6] emphasized the importance of controlling for temperature in studies testing interventions aimed at reducing infarct size. These investigators studied the effect of temperature...
on infarct size in pigs. Again, a strong correlation was noted between body core temperature and the proportion of the ischemic risk zone that became necrotic. Chien et al. [5] studied the importance of blood temperatures in the normothermic range (35–42°C) on infarct size development in rabbits. They altered body temperature by heating or by application of ice to the ears and groin of rabbits and blood temperature in the right jugular vein was monitored. Infarct size was found to be closely correlated with temperature in both paced and unpaced hearts [5].

A recent interesting study by Miki et al. [10] indicates that hypothermia enhances the protection provided by ischemic preconditioning. Rabbit hearts received ischemic preconditioning, then whole body cooling was initiated 20 min after the onset of a 45-min coronary artery occlusion. Ischemic preconditioning alone reduced infarct size by approximately 46%. However, when the two interventions were combined, infarct size was reduced by 88% compared with control hearts.

In our study, differences in hemodynamics during the protocol probably cannot explain the differences in mean infarct sizes. Mean heart rates and mean arterial blood pressures did not differ significantly between groups. Both groups had comparably sized ischemic risk areas, and the degree of ischemia during coronary occlusion was similar. Body core temperatures were also comparable. Blood flow in early reperfusion was higher in the previously ischemic region of cooled hearts, probably reflecting better perfusion due to a smaller extent of necrosis.

In the non-working heart, hypothermia, often combined with cardioplegia, is thought to protect against anoxia due to its ability to slow cellular metabolism and lower myocardial oxygen demand. We have shown that cold cardioplegia can protect canine myocardium for at least 3 h of global ischemia [11]. However, the use of cold cardioplegia alone in the arrested heart has become controversial. Some studies have indicated that warm cardioplegia is as protective as cold and results in similar metabolic changes in lactate production and creatine kinase release [12]. In addition, warm cardioplegia may result in improved recovery of left ventricular function [13].

In our model, the heart is not arrested but is beating and supporting cardiac output. The mechanism for the protective effect of regional hypothermia in the ischemic, beating heart remains to be determined. Although our data shows that the beneficial effects are independent of changes in heart rate or blood pressure, a possible protective effect of hypothermia is a reduction in high energy phosphate utilization in the myocardium. This may occur in the risk region itself, or be due to reduced contractility (and metabolic demand) in the border zone. It has been shown that lowering the temperature from 40 to 28°C slows the rate of high energy phosphate utilization in dog hearts [14]. Also, Ning et al. studied isolated, perfused rabbit hearts, given either normothermic (37°C) or hypothermic (31°C) perfusion for 20 min prior to 120 min of ischemia followed by reperfusion [15]. They reported that hypothermic perfusion resulted in increased myocardial ATP preservation during both ischemia and reperfusion, compared to normothermic perfusion. Recovery of indices of left ventricular function such as LV dP/dt during reperfusion were also better in hypothermic hearts.

A second potential mechanism could be the induction by hypothermia of heat shock proteins, which have been shown to confer protection to the myocardium in the setting of ischemia and reperfusion. [16,17]. In addition to hyperthermia, these proteins are expressed in response to cold stress [18,19]. Ning et al. in the study discussed above [15] found that levels of heat shock protein (Hsp70-1) mRNA were three times higher in hypothermic vs. normothermic hearts.

Our data suggest the potential use of hypothermia as a therapeutic maneuver to protect regionally ischemic myocardium. In humans, the development of infarction occurs more slowly, as humans with coronary artery disease generally have more collateral blood flow than the rabbit, which has very little. It is known that the rate of development of infarction is directly correlated with the level of collateral flow [20]. It is probable that in humans the application of hypothermia would be beneficial even if initiated later in the ischemic process. However, a less invasive technique than that used in this study must be developed. Ongoing studies in our laboratory are exploring methods of cooling the myocardium by cooling the pericardial space.

In the present study, hypothermia was initiated 30 min into a 2-h period of ischemia, and we observed a reduction in infarct size of about 18%. It is probable that the timing of the initiation of cooling, the duration of cooling and the level of temperature reduction are all important elements in the reduction of necrosis, and by adjusting any or all of this factors, an improvement in the level of infarct size reduction might be obtained.

In summary, the results obtained in our study support the important role of myocardial temperature level in the progression of necrosis. We have shown that cooling the heart well after the onset of occlusion, protects ischemic myocardium. This effect does not arise from changes in hemodynamics or in regional myocardial blood flow. It does not require a systemic effect, because mean body temperature was similar in all groups.

References

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