Hypothermia during reperfusion limits ‘no-reflow’ injury in a rabbit model of acute myocardial infarction

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Abstract

Objective: Reflow following coronary artery occlusion is an important predictor of clinical outcome. This study tests the effects of regional hypothermia, initiated late during ischemia and maintained for 2 h of reperfusion, on the no-reflow phenomenon. Methods: Anesthetized, open-chest New Zealand White rabbits received 30 min of coronary artery occlusion and 3 h reperfusion. Regional myocardial hypothermia (H, n=14), starting 10 min before reperfusion and continuing for 2 h of reperfusion, was compared with normothermia (N, n=14). Regional myocardial blood flow (microspheres) was measured during occlusion and at the end of reperfusion. The anatomic zone of no-reflow (thioflavin S in vivo injection) and infarct size were measured in the ischemic risk region at the end of the study. Results: Myocardial temperature in H rabbits was decreased by 5.0±0.4°C from baseline (37.1±0.2°C) and remained about 32°C during the cooling phase, returning to 36.0±0.3°C at 3 h. N hearts remained within 0.2°C of baseline (37.3±0.1°C) throughout. Both groups were equally ischemic during occlusion, but at the end of reperfusion reflow to the previously ischemic zone was significantly higher in H, 77±6% of normal blood flow versus 36±4% in N (P=0.0001). The zone of anatomic no-reflow was significantly smaller in H, 11±3% of the ischemic risk zone versus 37±3% in N (P=0.0001), and was proportionally smaller when represented as a percent of the necrotic zone 36±6% compared with 75±5% in N. Infarct size, expressed as a percent of the ischemic risk zone, was significantly smaller in H versus N (27±4 and 51±5%, P=0.0000). Conclusion: This study shows that hypothermic therapy initiated late during ischemia and continuing for several hours of reperfusion significantly improves reflow and reduces macroscopic zones of no-reflow and necrosis in this model. The improvement in reflow was greater than would be expected in the H group compared with N, based on the extent of necrosis. As reflow is a predictor of outcome, this intervention may have clinical implications.

Keywords: Blood flow; Coronary circulation; Infarction; Ischemia; Regional blood flow

1. Introduction

In addition to killing myocytes, myocardial ischemia and reperfusion damage the microvasculature, decreasing or preventing reflow after coronary artery occlusion and/or reperfusion. The ‘no-reflow’ phenomenon, observed after transient coronary artery occlusion, is characterized by an anatomic area of hypoperfusion and by decreased regional myocardial blood flow [1,2]. This damage might further injure myocytes and their contractile capability, as well as impede the healing process. Despite thrombolytic agents and PTCA treatment for acute myocardial infarction, microvascular dysfunction occurs in a large proportion of patients [3,4]. Impaired microvascular function in AMI patients is associated with poor outcome [5,6]; thus it is important to develop adjunctive therapeutic strategies for its treatment.

No-reflow has been shown to expand in size over time [2,7]. Early after the reperfusion of a major epicardial coronary artery, zones demonstrating a microvascular perfusion defect are small; however over time there is a
progressive increase in no-reflow in areas that initially were perfused. Thus there is a delayed component of no-reflow. It is likely that this delayed component represents true microvascular reperfusion injury.

Temperature has a strong influence on the development of necrosis after coronary artery occlusion and reperfusion. Moderate cooling of only 3–5 °C during ischemia has been shown to dramatically reduce myocardial infarct size in many animal models [8–12]. Although it has been established that cooling exerts a protective effect on myocytes during ischemia, its effects on the microvasculature remain unknown. It is possible that cooling provides additional protection to the microvasculature above and beyond reducing myocyte necrosis, including reducing the delayed component of no-reflow. The purpose of this study was to test the effects of myocardial hypothermia, instituted late in the ischemic period (at 20 min of a 30-min coronary artery occlusion) and continued for 2 h of reperfusion, on the development of anatomic areas of no-reflow and on regional myocardial blood flow.

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 [13] 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 American Association for Accreditation of Laboratory Animal Care accredits Good Samaritan Hospital.

2.1. Surgical preparation

Male New Zealand White rabbits, weighing 2.2–3.2 kg, were anesthetized with an intramuscular injection of a mixture of ketamine (approximately 100 mg/kg) and xylazine (30 mg/kg). Pentobarbital anesthesia was given during the study as required to maintain a deep level of anesthesia. The rabbits were intubated and mechanically ventilated with oxygen-enriched air. Fluid-filled catheters were inserted into the left jugular vein to administer fluids and into the left carotid artery to measure systemic pressure and to take reference blood samples during regional myocardial blood flow (RMBF) measurement. The chest was opened through the left fourth intercostal space, the pericardium incised, and the heart exposed. Near the base of the heart the first large antero-lateral branch of the circumflex artery, or the circumflex artery itself, was encircled with a 4-O silk suture. Coronary occlusion in this region normally results in ischemia of a large territory of the antero-lateral and apical ventricular wall. The ends of the suture were threaded through a piece of tubing, forming a snare that was tightened to occlude the artery. A hypodermic (30 gauge) thermocouple probe (Omega, Stamford, CT, USA) was inserted into the free wall of the heart in the area that was expected to become ischemic after coronary artery occlusion.

2.2. Experimental protocol

After surgical preparation and a 15-min stabilization period, baseline hemodynamics and temperatures were obtained; then the coronary artery was occluded. The rabbit was randomized to one of two groups: normothermia or hypothermia. At 15 min of coronary artery occlusion (CAO), a RMBF measurement was obtained. Treatments were initiated 20 min after CAO. In rabbits randomized to hypothermia, the hearts were cooled by bathing the heart in a small amount of saline and placing an ice-filled rubber-glove finger against the anterior surface of the heart in the region supplied by the occluded artery. Cooling was thus primarily localized over the risk region, however the entire heart was included to an extent. The thermocouple probe was placed in the midmyocardium of the risk region, and the temperatures given represent those from this area. A target temperature of 32 °C was chosen based on previous studies [8,9]. In rabbits randomized to the control group no intervention was performed.

At 30 min of CAO, the clamp was released and the artery was reperfused. Cooling was continued for 120 min after reperfusion in the hypothermia group. The hearts were reperfused for 3 h. Regional myocardial blood flow was measured again at the end of reperfusion. Heart rate and systemic arterial pressure and myocardial temperature were monitored and recorded at baseline, 15 and 29 min of occlusion, and at 30, 60, 90, 120 and 175 min of reperfusion.

At the end of the study (after 3 h reperfusion), 1 ml/kg of a 4% solution of thioflavin S was injected into the heart via the left atrial catheter to define the region of no-reflow. Thioflavin S, a fluorescent green-yellow dye, stains endothelium, serves as a marker of perfusion, and is used as a standard marker for identifying zones of no-reflow. The coronary artery was reoccluded and the ischemic risk region was delineated with 4 ml of a 50% solution of Unisperse blue dye (Ciba-Geigy, Hawthorne, NY, USA) injected into the left atrium. The deeply anesthetized rabbit was killed by an injection of KCl (12 mequiv.) into the left atrium, and the heart was excised.

2.3. Myocardial temperature

Myocardial temperature was monitored with a hypodermic thermocouple probe connected to an ADI (Advanced Digital Instruments, Grand Junction, CO, USA) system. The probe was inserted transversely into the myocardial wall. To ensure that the tip of the probe was located within the ventricular wall and not in the left ventricular cavity, cool saline was injected on the surface
of the heart overlaying the probe. An immediate reduction in temperature indicated proper placement.

2.4. Regional myocardial blood flow

Regional myocardial blood flow (RMBF) was measured using approximately 500,000 radioactive microspheres (Perkin-Elmer Life Sciences, Boston, MA, USA), 15 μm, labeled with 141Ce or 103Ru. Microspheres were injected into the left atrium through the left atrial catheter, and a reference blood sample was obtained from the carotid artery at the rate of 2.06 ml/min. At the end of the protocol, samples were cut from the risk region (determined by the absence of the blue dye) and from nonischemic regions (containing blue dye). The samples were weighed and counted together with the reference blood samples in a computerized gamma well counter (Canberra, System S100, Meriden, CT, USA). After appropriate subtraction of backgrounds and correction for overlapping radioactivity between isotopes, RMBF was computed and the results were expressed as ml/min/g [14].

2.5. Hemodynamic measurements

Heart rate and arterial pressures were measured using a fluid-filled catheter inserted into the carotid artery. Data were digitized and recorded using an ADI system.

2.6. Analysis of risk zone, no-reflow zone and necrosis

The heart was sliced transversely into 6–8 sections and photographed. The slices were photographed under ultraviolet light to identify the region of no-reflow and under standard lighting to identify the area at risk. The slices were then incubated in a 1% solution of triphenyltetrazolium chloride (TTC) for 15 min, immersed in formalin, and rephotographed. The photographic slides were later projected and traced. The areas of no-reflow, ischemic and normally perfused regions, and the areas of necrotic and non-necrotic regions in each slice were determined by planimetry. These areas in each slice were multiplied by the weight of the slice and the results summed to obtain the weights of the no-reflow, risk and infarcted areas.

2.7. Protocol 2—extended reperfusion

To assure that the reduction observed in infarct size with hypothermic reperfusion was not an artifact due to a failure of TTC to delineate necrosis, an additional nonrandomized hypothermia study was performed. In this limb four rabbits received 120 min of hypothermic reperfusion followed by an additional 3 h of reperfusion with no cooling (5 h total reperfusion). RMBF was measured in these hearts at 3 and 5 h of reperfusion.

2.8. Exclusion criteria

Prospective exclusion criteria included hearts with very small risk zones of <10% of the left ventricle or hearts with a regional myocardial blood flow of >0.2 ml/min/g measured in the risk zone during occlusion (not sufficiently ischemic).

2.9. Data analyses

All data summary and statistical analyses were performed using SAS (Version 6.04, Cary, NC, USA). Left ventricular weight, infarct size, area at risk, area of no-reflow and RMBF were compared using Student’s t test. Heart rate and blood pressure were analyzed by repeated measures analysis of variance. If an F value of <0.05 was obtained for the model, differences among means were determined by the method of contrasts. Analysis of covariance (ANCOVA) was used to test for a group effect on the regression models of necrotic myocardium with risk zone, no-reflow zone and collateral blood flow. Data are expressed as mean±S.E.M.

2.10. End points

The following end-points were analyzed: anatomic area of no-reflow, area at risk, area of necrosis, regional myocardial blood flow (RMBF), heart rate and mean arterial pressure, and myocardial risk-zone temperature. Relative reflow to the risk zone at the end of the reperfusion period, ‘reflow’, was calculated as RMBF in the risk zone/RMBF in the nonischemic zone.

3. Results

3.1. Rabbit population

A total of 39 rabbits were entered into the study. One rabbit died from ventricular fibrillation at 12 min of occlusion (prior to randomization). One rabbit (hypothermia) died of hypotension during reperfusion. Data from an additional four animals (hypothermia) were excluded because of a risk zone <10% of the left ventricle or regional myocardial blood flow in the risk zone during occlusion greater than 0.2 ml/min/g (prospective exclusion criteria). Data from one rabbit (normothermic) was excluded due to a technical difficulty with the myocardial temperature probe resulting in no temperature data. The results are reported on the remaining 32 rabbits: 14 in the hypothermic group (H), 14 in the normothermic group (N) and 4 in the extended reperfusion group. Mean body weights and mean left ventricle weights (3.15±0.10 g in H and 3.16±0.09 g in N) were similar in both groups.
3.2. Myocardial temperature

Average myocardial temperatures were similar in both groups at baseline (37.1±0.2 °C in H and 37.3±0.1 °C in N) and at 15 min CAO (Table 1). Cooling reduced myocardial temperature in the hypothermic group by an average of 5.5 °C from baseline by 29 min of CAO (just before reperfusion) to 31.7±0.3 °C. Mean myocardial temperatures remained at 31.8±0.3 °C during the cooling phase in H, and remained slightly below the normothermic level at the end of reperfusion (36.0±0.3 °C in H versus 37.2±0.2 °C in N, \( P < 0.0002 \)). Temperatures in N hearts remained within 0.2 °C of baseline throughout the duration of the protocol.

3.3. Heart rate and blood pressure

Mean heart rates and mean arterial pressures (Table 1) were similar in both groups at baseline and at 15 min of CAO (before treatment). With cooling, heart rates in H had decreased significantly by 29 min of occlusion compared with N, and they remained lower for the duration of the study. Mean arterial pressure decreased after CAO and further decreased during reperfusion in both groups, but there were no significant differences between groups.

3.4. Anatomic area of no-reflow (ANR)

The ANR was significantly smaller in H (11±3%) than in N (37±3%, \( P = 0.0000 \)), expressed as a percent of the risk zone (Fig. 1, top). It might be expected that the ANR was smaller in H because there was less overall necrosis. However, analyses indicated that the reduction in ANR that occurred in H was not simply due to a reduction in necrosis but was of greater magnitude than in N. ANR comprised only 36±6% of the necrotic region in H compared with 75±5% in N (Fig. 1, bottom). Fig. 2 is a scattergram representing the ANR as a function of the necrotic region (both expressed in grams). Analysis of covariance showed a significant effect of group on this relationship (\( r^2 = 0.86, P < 0.001 \)). It can be seen that on average for any given amount of necrosis, less no-reflow developed in the H group.

Previous studies have shown that the major predictors of the size of the no-reflow zone are a function of: the extent of the risk zone (AR), the extent of the necrotic zone (AN) and amount of collateral blood flow during occlusion (RMBF) [15,16]. In control rabbits in the present study there was a close correlation between ANR and these variables, which fit a multiple linear regression equation.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Base</th>
<th>Occlusion</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
<td>29 min</td>
<td>30 min</td>
</tr>
<tr>
<td>HR Hypo</td>
<td>195±5</td>
<td>193±5</td>
<td>163±4*</td>
</tr>
<tr>
<td>Normo</td>
<td>192±8</td>
<td>196±7</td>
<td>194±8</td>
</tr>
<tr>
<td>MAP Hypo</td>
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<td>68±2</td>
</tr>
<tr>
<td>Normo</td>
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<td>76±1</td>
<td>77±2</td>
</tr>
<tr>
<td>MT Hypo</td>
<td>37.1±0.2</td>
<td>36.8±0.2</td>
<td>31.7±0.3*</td>
</tr>
<tr>
<td>Normo</td>
<td>37.3±0.1</td>
<td>37.1±0.2</td>
<td>37.1±0.2</td>
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</tbody>
</table>

* \( P < 0.0002 \) versus normothermic; † \( P < 0.02 \) versus normothermic; HR, heart rate (beats/min); MAP, mean arterial pressure (mmHg); MT, myocardial temperature (°C); hyp0, hypothermic group (n=14); normo, normothermic group (n=14).
expressed by the formula: $ANR = -0.035 + 0.14 \times (AR) + 0.561 \times (AN) + 0.003 \times (RMBF)$, (where $ANR$, $AR$ and $AN$ are expressed as a fraction of the left ventricle and $RMBF$ as ml/min/g) and with a correlation coefficient of $r=0.97$. When the equation coefficients obtained from control rabbits were used to calculate the expected no-reflow areas in hypothermic rabbits, the actual observed no-reflow areas in hypothermic hearts were significantly smaller (Fig. 3). Animals in the treated group were mostly distributed below the regression line obtained for controls. Thus on average for any given expected no-reflow zone the actual size was smaller in hypothermic hearts.

3.5. Regional myocardial blood flow (RMBF)

Both groups were equally and severely ischemic during coronary artery occlusion, and blood flow to the nonischemic regions was comparable (Table 2). At the end of 3 h of reperfusion, absolute blood flow in H to the previously ischemic region was significantly higher than in N. Relative reflow to the risk zone (calculated as $RMBF$ in the risk zone/$RMBF$ in the nonischemic zone) was $77 \pm 5\%$ in H compared with $36 \pm 4\%$ in the control group.

3.6. Risk zone and infarct size

The ischemic risk zones were similar in both groups comprising $30 \pm 2\%$ of the left ventricle in N and $28 \pm 2\%$ in H ($P=ns$). An unexpected finding from the study was that infarct size, expressed as a percent of the risk zone, was significantly reduced in H and was $27 \pm 4\%$ of the risk zone compared with $51 \pm 5\%$ in N ($P=0.0012$).

Fig. 4 is a scattergram representing the relationship between the risk region and the necrotic region (both expressed in grams). Analysis of covariance showed a significant effect of group on this relationship ($P=0.0018$).
so that on average for any given size of risk zone, less necrosis developed in the H group.

3.7. Is the reduction in infarct size observed with hypothermia therapy due to differences in heart rate between groups?

Both groups had similar baseline temperatures, arterial pressures, and collateral blood flow during occlusion. However, heart rates were lower in the H group, due to cooling the heart. We examined the relationship between average heart rate during the time of cooling with infarct size in both groups. There was no significant correlation in either N (r = 0.36, P = 0.21) or H (r = 0.18, P = 0.52). It must be noted that the analysis was performed on a limited number of animals and thus does not necessarily negate the hypothesis that a decrease in heart rate is the mechanism for a reduction in infarct size. However, these results support a previous study from our group \([8]\) and others \([10]\) in which pacing hypothermic hearts to baseline did not prevent a reduction in infarct size.

3.8. Was improvement in no-reflow due to prolonging hypothermia during reperfusion?

In a previous study from this laboratory \([9]\) we reported that initiating hypothermia 5 min before reperfusion and continuing for 15 min of reperfusion failed to reduce infarct size compared with normothermic hearts. In addition, reflow at the end of the reperfusion period was similar in both groups. In contrast, in the present study when cooling was initiated at 10 min before reperfusion and continued for 2 h of reperfusion, return of flow to the risk region was significantly improved compared with normothermia and infarct size was reduced. A retrospective analysis comparing the correlation between infarct size (expressed as a percentage of the risk zone) and reflow in these two studies is depicted in Fig. 5. On average, reflow was better in the group with prolonged hypothermia regardless of infarct size (P = 0.058 for group effect by ANCOVA).

3.9. Protocol 2—5 h of reperfusion

Since it is conceivable that 2 h of hypothermic reperfusion in some way altered the ability of TTC to delineate necrosis, we did an additional four studies of hypothermic treatment in which reperfusion was extended to 5 h. Thus these hearts had 3 h of reperfusion with no cooling, comparable to the normothermic group. Myocardial temperature returned to baseline by at least 4 h of reperfusion. The ischemic risk zone in this subgroup was similar in size to the other groups, averaging 30% of the left ventricle. Infarct size, expressed as a percentage of the risk region, was 22 ± 4%, similar to the primary hypothermic group (27 ± 4%). These results demonstrate that the smaller

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Table 2
Regional myocardial blood flow (ml/min/g)

<table>
<thead>
<tr>
<th></th>
<th>Hypothermia</th>
<th>Normothermia</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min occlusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk zone</td>
<td>0.04±0.01</td>
<td>0.03±0.01</td>
<td>0.61</td>
</tr>
<tr>
<td>Nonischemic zone</td>
<td>3.04±0.16</td>
<td>2.73±0.28</td>
<td>0.35</td>
</tr>
<tr>
<td>180 min reperfusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk zone</td>
<td>1.21±0.15</td>
<td>0.65±0.11</td>
<td>0.0048</td>
</tr>
<tr>
<td>Nonischemic zone</td>
<td>1.64±0.19</td>
<td>1.82±0.14</td>
<td>0.43</td>
</tr>
<tr>
<td>Reflow (%)</td>
<td>77±5</td>
<td>36±4</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

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Fig. 4. Relationship between necrosis and risk region in the two groups. Infarct size was reduced by hypothermia treatment.

Fig. 5. Retrospective analysis comparing the correlation between infarct size (expressed as a fraction of the risk zone) and reflow in the present study of 2 h of hypothermic reperfusion with a previous study of 15 min only of hypothermic reperfusion. On average, reflow was better in the group with prolonged hypothermia regardless of infarct size (P = 0.058 for group effect by ANCOVA).
infarcts in hypothermic hearts were not due to a failure of the TTC method of detecting necrosis.

4. Discussion

The main findings of the present study show that hypothermia protects against impaired reflow even when established late in the ischemic period, if cooling is maintained for 2 h of reperfusion. In hypothermic hearts, regional myocardial blood flow after 3 h of reperfusion was significantly higher, relative reflow to the previously ischemic region was significantly higher, and infarct size was reduced. Better reflow was not due to differences in baseline temperature, collateral blood flow during CAO or differences in hemodynamics in hypothermic hearts.

The anatomic region of no-reflow in the risk area was significantly smaller in hypothermic hearts, and, in addition, was proportionally smaller than in normothermic hearts when expressed as a percentage of the necrotic tissue. Thus the improvement in reflow was not due solely to a reduction in necrosis. Analysis of covariance showed that on average, for any given amount of necrosis, the extent of no-reflow was less with hypothermia treatment. To our knowledge, this is the first study to show a dissociation between a reduction in necrosis and improved reflow. This suggests that hypothermia during reperfusion may be decreasing microvascular reperfusion injury.

We cannot determine from our study whether we modified a component of ischemic damage or whether we reduced a component of reperfusion injury, or both. In a previous study in this laboratory, we initiated cooling at 20 min of a 60 min coronary artery occlusion and continued for only the first 15 min of reperfusion [9]. No difference in infarct size was observed comparing hypothermic with normothermic hearts. In addition, in that study relative reflow of blood to the previously ischemic region was similar in both groups (approximately 50%). A retrospective analysis comparing the correlation between infarct size and reflow in that study compared with the present study indicated that on average, reflow was better in the group with prolonged hypothermia regardless of infarct size. Thus if hypothermia is to be used clinically, it may need to be extended well into the reperfusion phase.

Few studies have examined the effects of hypothermia during ischemia and reperfusion on reflow. In one recent study, Dae et al. [12] tested hypothermia in a pig model of myocardial ischemia–reperfusion. In treated animals cooling was initiated at 20 min of a 60 min coronary artery occlusion followed with reperfusion. Microvascular perfusion was assessed at 3 h and 5 days of reperfusion using sestamibi uptake followed by autoradiography. Necrosis was significantly less in hypothermic hearts, and importantly the distribution of sestamibi uptake was correlated with that of necrosis, suggesting better microvascular perfusion in hearts treated with hypothermia after both 3 h and 5 days of reperfusion.

There is a progressive deterioration in reflow after ischemia and reperfusion [2,7]. However, the mechanisms responsible for no reflow are incompletely understood. It is possible that different mechanisms come into play at reperfusion versus later in the time course of reperfusion [7]. The present study was not designed to determine the mechanisms for the protective effect of hypothermia on the microvasculature. However, hypothermia might be beneficial either directly by reducing vessel damage such as preventing endothelial swelling or blebs that often appear to obstruct the lumen of microvessels, or indirectly by reducing the release of cytokines or other inflammatory factors.

A potential mechanism of no-reflow is endothelial injury from reactive oxygen species, which are released at reperfusion. Hypothermia may protect the microvasculature by reducing postischemic endothelial injury due to oxidative stress. Zar et al. [17] have examined the effects of mild hypothermia in an isolated rat liver model of ischemia and reperfusion. Data from their studies show that hypothermic perfusion decreased the formation of reactive oxygen species and reduced postischemic vascular resistance compared with normothermic perfusion.

4.1. Clinical implications

No-reflow occurs in patients after both thrombolysis and PTCA for acute myocardial infarction [4,5], and the extent of no-reflow after acute myocardial infarction has been shown to be a predictor of long-term outcome in patients [5,18]. Thus protecting the microvasculature after opening the occluded epicardial vessel must be a clinical goal (see recent reviews by Rezkalla and Kloner [19] and R effelmann and Kloner [20]). Zones of no-reflow occur primarily in regions that are already necrotic. However, treating no-reflow may enhance delivery of blood to necrotic areas, improving scar healing by decreasing infarct expansion and left ventricular remodeling [19]. In addition, protected vessels may allow delivery of pharmacologic agents to the myocardium.

Recently, pilot studies have been initiated to test the potential of hypothermia to decrease ischemic damage in patients undergoing acute myocardial infarction. Data from our study indicate that hypothermia may protect microvessels in addition to reducing necrotic injury. This may result in improvements in function and in long-term survival.

4.2. Limitations

The heart wall in the rabbit is thin and we were able to achieve rapid temperature reduction (within 1–2 min). This may not be possible in larger mammals including man. In addition, the method used to reduce heart temperature is not clinically applicable. However, in humans the develop-
ment of infarction occurs more slowly, and it is probable that the application of hypothermia is beneficial even when initiated later in the ischemic period and more slowly. New heat-exchange catheter technologies are being developed that are able to cool humans in a relatively short time (30 min).

5. Summary

Data from this investigation show, that compared with normothermic controls, when the rabbit heart is cooled to approximately 32 °C starting during the last one third of the ischemic period and for 2 h of reperfusion: (1) the anatomic region of no-reflow was significantly smaller when expressed as a percent of the risk region, and in addition was smaller when expressed as a proportion of the necrotic tissue compared to normothermic hearts, (2) regional myocardial blood flow after 3 h of reperfusion was significantly higher compared to normothermic hearts, (3) relative reflow to the previously ischemic region was significantly higher compared to normothermic hearts, and (4) infarct size was reduced by about 47%. Improvements in these variables were not due to differences in baseline temperature, collateral blood flow during CAO or differences in hemodynamics. Thus myocardial cooling, introduced during the last third of the ischemic phase and continued for 2 h after reperfusion, reduces microvascular damage, assessed by anatomic measurement of no-reflow and regional myocardial blood flow measurements, and reduces necrosis.

Acknowledgements

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References