Reperfusion Adequacy and Functional Recovery*

Thomas J. Rohs, Jr, MD; Mary B. Benedict, MD; and Steven F. Bolling, MD, FCCP

Ischemic myocardium undergoes many physiologic changes, including alterations in contractile function, histologic condition, and oxygen utilization. Reperfusion of ischemic myocardium may reverse some of these changes; however, the level of reperfusion needed for adequate functional recovery, as opposed to myocyte salvage, remains controversial. This study examines the effects of varying levels of reperfusion following ischemia on functional parameters of recovery. Isolated rabbit hearts were subjected to global ischemia at 37°C for 10, 20, or 45 min. The hearts were reperfused at either mean normal baseline pressure (80 mm Hg), one half baseline (40 mm Hg), or one fourth baseline (20 mm Hg). With reperfusion, although all hearts retained the compensatory metabolic ability to upregulate oxygen extraction, reduction of reperfusion pressure resulted in depression of contractility and myocardial oxygen consumption, especially with low-pressure reperfusion. These findings suggest that all levels of reperfusion are not equal for optimal myocardial functional recovery. As minimal reperfusion may result in persistent stunning of myocardium, other means of enhancing reperfusion, such as percutaneous transluminal coronary angioplasty or coronary artery bypass grafting, should be considered. (CHEST 1997; 112:1075-78)

Key words: myocardial ischemia; recovery; reperfusion

Abbreviations: CF=coronary flow; DP=developed pressure; dP/dt=first derivative of LV pressure; EDP=end-diastolic pressure; LV=left ventricular; MVO₂=myocardial oxygen consumption; O₂ ext=oxygen extraction; TIMI=thrombolysis in myocardial infarction

The effects of myocardial ischemia have been investigated extensively.1-3 However, with the advent of salvage therapy via thrombolysis, attention has focused on reperfusion of ischemic myocardium. Reibel and Rovetto1 have demonstrated that post-ischemic myocardium is poorly contractile and has loss of nucleotides, depressed subcellular function, and prolonged histologic damage.1 Alterations in damaged tissue may persist for days.4 Although reperfusion restores oxygen delivery, contractile function continues to be downregulated, as even minutes of ischemia result in loss of systolic wall thickening for hours.2,3 Whether to strive to reestablish full reperfusion following coronary occlusion remains controversial. Some investigators have suggested that any level of reperfusion is adequate for "myocardial salvage," ie, avoidance of myocyte death; however, few previous experiments have examined the significance of reperfusion level on return of function, as opposed to myocyte necrosis following global ischemia. Conversely however, diminishing an existing stenosis by percutaneous transluminal coronary angioplasty or complete revascularization by coronary artery bypass grafting has known risks and morbidity. Therefore, this study investigated the effects of varying levels of reperfusion following ischemia on functional recovery in a rabbit model.

Materials and Methods

Studies were conducted using 54 New Zealand white rabbits (male or female, 1.9 to 2.7 kg) that were anesthetized with pentobarbital sodium (45 mg/kg, IV) and heparinized (700 U/kg, IV). Hearts were rapidly excised and immersed in ice-cold physiologic salt solution containing 118.0 mM NaCl, 4.0 mM KCl, 22.3 mM NaHCO₃, 11.1 mM glucose, 0.66 mM KH₂PO₄, 1.23 mM MgCl₂, and 2.38 mM CaCl₂. The aorta was cannulated in the Langendorff mode and perfused with physiologic salt solution, equilibrated with 95% O₂/5% CO₂ at 37°C, and a pH of 7.4. Perfusion pressure was maintained at 80 mm Hg. An incision was made in the left atrium and a fluid-filled latex balloon was passed through the mitral orifice and placed in the left ventricle. The balloon was connected to a pressure transducer for contin-

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uous measurement of left ventricular (LV) pressure and its first derivative (dp/dt). The superior and inferior vena cava andazygous veins were ligated. The pulmonary artery was cannulated to enable timed collection measurements of coronary flow, and the cannula was connected to an oxygen meter (Chemical microsensor; Diamond Electro-Tech Inc, Ann Arbor, Mich) for continuous measurement of the partial pressure of oxygen in the coronary effluent.

Analog signals were continuously recorded on a pressurized ink chart recorder (model 2500S; Gould, Inc, Cleveland) and on an online computer (AST Premium/386; AST Research Inc, Irvine, Calif). To characterize cardiac function, developed pressure (DP) was defined as peak systolic pressure minus end-systolic pressure (EDP). Myocardial oxygen consumption (MVO₂) was calculated as MVO₂=CF×([PaO₂-PvO₂]×[v/760]), where CF is coronary flow (mL/min/g), PaO₂-PvO₂ is the difference in the partial pressure of oxygen (PpvO₂, mm Hg) between perfusate and coronary effluent flow, and v is the Bunsen solubility coefficient of O₂ in perfusate at 37°C (22.7 μL O₂/atm/mL perfusate). The PaO₂ of the perfusate was 663 mm Hg. CF was measured by timed collections of the pulmonary effluent flow with a graduated cylinder. Oxygen extraction (O₂ ext) was calculated as O₂ ext=MVO₂/oxygen content in the perfusate.

**Experimental Protocol**

After completing instrumentation and performing calibrations, LV balloon volumes were varied to construct modified LV function curves. Ventricular systolic function was evaluated isovolumically by introducing the same volume into the LV balloon during reperfusion. Hearts characterized by developed pressures <90 mm Hg or >140 mm Hg were not used. Baseline data were obtained after hearts were allowed to stabilize for a period of 30 min at normothermia. During this period, normal sinus rhythm was regained and all hearts had a rate of 180 beats/min. A thermistor needle was inserted into the midmyocardium to record myocardial temperature. After the 30-min stabilization period, baseline control measurements of EDP, DP (peak systolic pressure minus EDP), dp/dt, MVO₂, O₂ ext, and CFs were made in each heart. Myocardial oxygen utilization efficiency ([DP/MVO₂, mm Hg]/mL O₂/min/g) was calculated from these measurements. Hearts were randomized into three groups of ischemic intervals of 10, 20, or 45 min (n=18 each). The hearts were rendered globally ischemic for the assigned time interval. The intraventricular balloon remained deflated during ischemia. All hearts were reperfused at 37°C by means of a circulating water jacket during ischemia.

Following the ischemic interval, hearts were again randomized within each of the three groups to be reperfused at either baseline (80 mm Hg), one half baseline (40 mm Hg), or one fourth baseline (20 mm Hg) pressure (n=6). All hearts were maintained at 37°C during reperfusion. Defibrillation was performed as needed. During the initial 15 min of reperfusion, the intraventricular balloon was kept deflated to allow for recovery. After the initial 15 min of reperfusion, the balloon was refilled to the preischemic control volume and remained inflated for the remainder of reperfusion. Isovolumic measurements of EDP, DP, dp/dt, O₂ ext, MVO₂, and CFs were obtained throughout reperfusion. After 30 min of reperfusion and completion of the protocol, the hearts were removed from the perfusion column and water content determinations were made. Wet weight of the heart was determined after trimming the great vessels and fat and blot drying with eight-layer cotton gauze. The myocardium was then desiccated for 48 h at 80°C and reweighed. Water content was determined using the following formula: (1−dry weight/wet weight)×100=percent water. The experimental design for this model is well established and has been published previously.4,5 Values reported in the text and in Table 1 are mean±SE. A computer program (Statview; Brain Power Inc; Calabasas, Calif) was used for statistical analysis. Data were evaluated with repeated measures analysis of variance within groups and single-factor analysis of variance across groups. When significant F values were obtained, the Scheffé test was used to distinguish which time periods or groups differed from one another significantly. Differences were considered significant when p<0.05. All animals received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research.

**RESULTS**

Results are summarized in Table 1. There were no significant differences in baseline data, among developed pressure (DP), dp/dt, CF, MVO₂, O₂ ext, or heart rate at preischemic baseline in all 54 hearts. Specifically, DP for all groups was 97±5 mm Hg, MVO₂ was 68±5 mL O₂/g, O₂ ext was 72±7 mL O₂/100 mL O₂. For comparative purposes, myocardial oxygen utilization (DP/MVO₂, percent mm Hg/percent mL O₂/mg) for nonstressed, nonischemic tissue at baseline was defined as 100%.

<table>
<thead>
<tr>
<th>Ischemic Interval, min</th>
<th>No.</th>
<th>RP, mm Hg</th>
<th>DP, mm Hg</th>
<th>DP/MVO₂, mL O₂/min/g</th>
<th>O₂ ext, mL O₂/min</th>
<th>MVO₂, mL O₂/min/g</th>
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<td>10</td>
<td>6</td>
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*RP=reperfusion pressure; DP/MVO₂=oxygen utilization efficiency. Data (mean±SEM) are shown for each group over time following ischemia and reperfusion (all values differed significantly from nonischemic baseline).

p<0.05 vs full−80 mm Hg reflow for each ischemic length.
Following ischemia and reperfusion, all hearts exhibited significantly (p<0.05) diminished isovolumic systolic function, as reflected by DP measurements, at all time intervals compared to baseline. Moreover, the decrease in function was proportional to the decrease in reperfusion pressure, ie, the lower the reperfusion pressure, the lower the DP. This finding was consistent regardless of the length of ischemia; however, DP was most reduced following the longer intervals of ischemia. This decrement in DP was statistically significant at one fourth baseline reperfusion for all ischemic times and at one half baseline reperfusion for the hearts undergoing 45 min of ischemia. Changes in +dP/dt closely followed those for DP.

LV EDP, an estimate of diastolic function in this model, was measured following reperfusion. After 10 min of ischemia, hearts recovered EDP to 9±4, 11±4, and 12±6 mm Hg with 80, 40, or 20 mm Hg of reperfusion, respectively. After 20 min, hearts recovered EDP to 10±5, 16±9, and 14±4 mm Hg with 80, 40, or 20 mm Hg of reperfusion, respectively. After 45 min, hearts recovered EDP to 13±7, 18±4, and 18±5 mm Hg with 80, 40, or 20 mm Hg of reperfusion, respectively.

Similar to DP, recovery of MVo2 decreased with decreasing perfusion pressure. The preischemia value was 68±5 mL O2/mg/min. Reflow at 80 mm Hg after any length of ischemia allowed full recovery of MVo2. However, after 20 and 45 min of ischemia, reflow reperfusion at 40 mm Hg decreased MVo2 to 52±8 and 26±8, respectively. Reflow at one fourth flow (20 mm Hg) resulted in MVo2 of 36±10 and 26±8. Compensatory O2 ext increased significantly at one fourth reflow to 96±3%, 96±7%, and 92±16% for ischemia of 10, 20, and 45 min significantly above O2 ext values at full reflow of 83±8%, 85±9%, and 74±3%.

Myocardial efficiency (DP/MVo2) was reduced from baseline following all periods of ischemia. Even at full reperfusion pressure (80 mm Hg), efficiency was reduced by 32%, 27%, and 50% after 10, 20, and 45 min of ischemia, respectively. However, efficiency was not significantly different between the various reperfusion levels. There were no differences before or after ischemia in the water content of the hearts. Finally, myocardial tri-phenyl triazolium chloride (TTC) staining, in this model, consistently demonstrated no evidence for myocyte necrosis.

**DISCUSSION**

Myocardium subjected to ischemia undergoes alterations in contractile function, histologic condition, and oxygen utilization.1-3 Reperfusion of ischemic myocardium may reverse some of these changes; however, the level of reperfusion for adequate functional recovery has not been defined. In this model of isolated rabbit hearts, differing levels of reperfusion pressure following ischemia demonstrated differing functional recovery. Postischemic contractile dysfunction was present at all levels of reperfusion, including reperfusion with pressures at 100% of baseline. However, higher levels of reperfusion pressure correlated with better isovolumic systolic function (DP), contractility (dP/dt), and MVo2 than lower pressures. As reperfusion pressures were reduced, function, contractility, and MVo2 were diminished. These differences became more significant with increasing ischemic time. It was noted that O2 ext increased with lower reperfusion pressures following ischemia. This confirms the finding of others that with ischemia, O2 ext is upregulated. However, despite upregulation of O2 ext, in this model, the myocardium could not compensate and oxygen consumption dropped with diminished perfusion, resulting in overall diminished contractile function.

Previous studies have demonstrated that oxygen consumption and extraction may recover near normal following global ischemia, but as in this study, contractility remains depressed.6-8 As reperfusion pressures were lowered, however, MVo2 decreased as well as contractility. Stahl et al9 noted an increase in oxygen consumption as well as extraction in ischemic myocardium despite a marked decrease in contractile function. Their results implicated an inefficient use of oxygen for myocyte contraction or increased use of oxygen for noncontractile activities in ischemic myocardium.

Postischemic flow limitation leads to especially poor contractile function and potentially limits cellular reparative functions, such as structural repair of free radical damage, high-energy phosphate repletion, and maintenance of electrochemical gradients, all of which may be intimately involved with and exacerbate the effects of ischemia.9-12 Under these conditions, functional recovery and even continued myocyte survival may be suboptimal. Although there is little information regarding the natural history and prognosis of poorly reperfused myocardium, it has been postulated that such tissue evolves to hibernating myocardium with minimal metabolism and contractile dysfunction. This progression to hibernating myocardium is theoretically due to inadequate reperfusion with continued ischemic-mimetic signaling, resulting ultimately in energetic downregulation.

Clinically, patients following coronary artery occlusion frequently undergo reperfusion via thrombolyis. The level of reperfusion pressure to achieve optimal functional recovery has not been well de-
fined. However, it may be essential that eventual reperfusion of this ischemic myocardium be optimal to achieve adequate functional myocardial recovery. Mere reestablishment of perfusion to ischemic myocardium, regardless of level, does not guarantee adequate recovery of contractility. As outlined by this study, the level of reperfusion may be relevant and possibly correlated to functional recovery. Angiographic correlation of suboptimal TIMI (Thrombolysis in Myocardial Infarction) flows after thrombolytic therapy is suggestive of poor reflow on the order of that produced in this study. Although no quantitative correlation is known between TIMI grades and reflow pressures, poorer grades of reflow could result, at the very least, in prolonged contractile dysfunction, and perhaps in permanent dysfunction. This postulate correlates well with a clinical study by Galli et al.\textsuperscript{13} in postinfarct patients. These patients were divided into two groups consisting of those with scintigraphic evidence of reperfusion and those without any evidence of reperfusion. The average TIMI flow grade that had been achieved in the reperfusion group was 1.8—denoting suboptimal reflow. At 8 months of follow-up, there were no global or regional differences between groups in terms of improvement of LV dysfunction despite “reflow” in one group and no reflow in one group.\textsuperscript{13} These data suggest that in terms of contractility, suboptimal reflow is no better than no reestablishment of flow. Although other authors\textsuperscript{14-16} have suggested that perhaps early, short-term “low” or “gentle” postischemic reperfusion may be beneficial following cardioplegic ischemia or acute coronary occlusion in regards to myocardial salvage, these reports all then had quick resumption of normal pressure reperfusion. This finding correlates with the present study, as continued low-pressure reflow without quick resumption of normal pressure perfusion resulted in continued poor function.

In conclusion, our laboratory findings support maximally achievable reperfusion to ensure adequate functional recovery of ischemic myocardium. Although this rabbit model has limitations (global, ischemia, crystalloid perfusion, etc), the scenario is analogous to thrombolysis with ineffective antegrade flow or continued significant gradient across the coronary obstruction. Further clinical studies to evaluate the correlation of adequacy of reperfusion and subsequent contractile recovery after ischemia are well warranted. Importantly, these data lead to potential consideration of percutaneous transluminal coronary angioplasty or even full surgical revascularization when less invasive interventions to “fully” reverse ischemia are not optimally successful.

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