Left Ventricular Unloading During Reperfusion Does Not Limit Myocardial Infarct Size

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To determine whether venting the left ventricle during coronary reperfusion limits myocardial infarct size, we studied paced (200 beats/min) Langendorff rabbit hearts, perfused with blood from a support rabbit. A left coronary artery was occluded for 60 minutes, followed by 2 hours of reperfusion. Four experimental conditions, as follows, were used: In group 1 (control), the hearts contracted isovolumetrically on a fluid-filled balloon in the left ventricle during both occlusion and reperfusion. In group 2, the balloon was present only during occlusion, and the heart was vented during reperfusion. Hearts in group 3 were vented during occlusion and developed pressure during reperfusion. In group 4, the left ventricle was vented during occlusion and reperfusion. Perfusion pressure (91.2±0.9 mm Hg) and coronary flow (0.88±0.03 ml/min/g) were not different between groups. Left ventricular pressures (mean of all groups) were 87.3±1.5 mm Hg systolic and 6.5±0.6 mm Hg diastolic. Infarcted myocardium was assessed by triphenyl tetrazolium staining and expressed as a percentage of the area at risk, as measured by fluorescent particles. Venting during both ischemia and reperfusion (n=10) did result in significantly smaller infarcts than in the unvented controls (n=10), that is, 13±5% vs. 41±6%, respectively. Venting only during reperfusion (n=10) or occlusion (n=11) did not significantly limit infarct size (57±6% and 32±5%, respectively), as compared with controls. Thus, the clinically feasible intervention of left ventricular venting during reperfusion was not cardioprotective. (Circulation 1990;81:1374–1379)

Restoration of blood flow to ischemic myocardium has become a routine method for salvage of reversibly injured myocytes.1–3 Many investigators, however, believe that some injured but viable myocytes die during the reperfusion period because of toxic components of reperfusion itself. This has led to the search for agents, interventions, or both that can be instituted at the time of reperfusion to limit this late component of cell death and, thus, maximize the salvage of ischemic myocardium.4

It has been suggested that the amount of necrosis after an ischemic insult is related to myocardial oxygen consumption, and that modification of the determinants of oxygen consumption such as ventricular developed pressure can directly affect infarct size.5–7 Pulsatile left atrial bypass and pulsatile left ventricular bypass, both of which unload the left ventricle, have been reported to limit infarct extension and reduce mortality after acute coronary ligation in dogs.8–11 Decompression of the left ventricle by venting in the presence of cardioplegic reperfusion reportedly results in improved recovery of regional function, as well.12 Finally, two studies examined unloading of the left ventricle during coronary reperfusion by percutaneous pulsatile left ventricular bypass, an intervention that could be performed during emergency reperfusion of the acute myocardial infarction patient. They both reported an improved left ventricular function and limitation of infarct size but, unfortunately, were only performed in fibrillating hearts13 or in conjunction with cardioplegia.14

It is now technically possible to use percutaneous cardiopulmonary bypass to unload the left ventricle of the acute myocardial infarction patient during reperfusion. To date, however, clear evidence that left ventricular decompression during reperfusion, without confounding variables such as cardioplegia or fibrillation, can induce salvage in that setting is unavailable. The purpose of this study, therefore, was to test, in a model of acute regional ischemia,
whether unloading the beating ventricle at the onset of reperfusion could limit myocardial infarct size. This was accomplished using an isolated blood-perfused rabbit heart.

**Methods**

**Surgical Preparation Donor Rabbit**

New Zealand White rabbits (1.6–3.3 kg) of either sex were anesthetized with 25 mg/kg sodium pentobarbital administered with a 25-gauge butterfly in a marginal ear vein. The neck was opened with a ventral midline incision, and a tracheotomy was performed. The rabbits were ventilated on a positive pressure respirator (MD Industries, Mobile, Alabama) with 100% oxygen. Ventilation rate was 30–35 breaths per minute, and tidal volume was approximately 20 ml. A catheter (PE100, Clay Adams, Parsippany, New Jersey) was inserted into the right carotid artery for measurement of arterial blood pressure. A left thoracotomy was performed, and the pericardium was opened to expose the heart. A 2-0 silk ligature on a curved taper needle was passed underneath a prominent branch of the left coronary artery, at a level approximately one third the distance from base to apex. The left main coronary artery in the rabbit gives rise to several large branches that radiate across the free wall of the left ventricle. We chose the largest of these, and its distribution corresponds closely to that of the anterior descending artery in the dog. The rabbit was given 1,000 units/kg sodium heparin, after which the heart was rapidly removed, placed in ice-cold saline, and immediately transferred to the Langendorff apparatus.

**Surgical Preparation Support Rabbit**

New Zealand White rabbits (1.95–3.55 kg) were anesthetized, and tracheotomy performed as previously described. To avoid hypoxemia, the rabbits were ventilated with 100% oxygen; ventilatory rate varied at 30–35 breaths per minute, and tidal volume was set at 20 ml. The right carotid artery was cannulated with a 13-gauge needle adapter to supply arterial blood to the isolated heart. A large-bore Tygon catheter (constructed in the laboratory from Tygon-hand tubing, 1.6 mm i.d., 3.2 mm o.d.) was placed in the left common external jugular vein for return of blood from the isolated heart chamber to the support rabbit. Aortic blood pressure was measured through a catheter (PE90, Clay Adams) that was inserted into the left femoral artery and advanced into the thoracic aorta. To maintain patency of the perfusion circuit, the support rabbit was anticoagulated with a 1,000 units/kg bolus of sodium heparin plus 500 units/kg supplements every 90 minutes.

**General Preparation**

The perfusion circuit consisted of silicon tubing running from the support rabbit’s carotid artery, through a roller pump (Harvard Apparatus 1215, Harvard Apparatus, South Natick, Massachusetts) to a stainless-steel tube encased in a heated water jacket. The isolated heart was suspended by the aorta from the distal end of the steel tube and placed in a glass water-jacketed chamber, and the chamber was covered with plastic film. The temperature of the heat exchanger was set at 37°C. The pulmonary artery was transected, allowing blood to drip into the chamber, from which the blood was returned to the support rabbit. Pressure in the perfusion circuit was measured by a side arm in the tubing connected to a Statham P23De pressure transducer (Gould Inc., Glen Burnie, Maryland). If the aortic blood pressure of the support rabbit was sufficient to perfuse the isolated heart at or higher than 75 mm Hg (measured at the juncture of the isolated heart and the perfusion apparatus), the isolated heart was perfused directly from the support rabbit. If the perfusion pressure decreased below 75 mm Hg, a roller pump was engaged to maintain perfusion pressure. Conversely, in those instances in which the aortic pressure of the support rabbit exceeded 100 mm Hg, the roller pump was engaged and set such that isolated heart perfusion pressure was 90–100 mm Hg. An electrode was placed in the right ventricular free wall for recording of a single-lead electrocardiogram. A pacing electrode was positioned in the right atrium or right ventricle, and the isolated heart was paced at 200 beats/min with pulses of 5 V and 3-msec duration. The left ventricle was then either vented or made to develop pressure. The left ventricle was vented by placing a catheter (polyethylene, 2.7 mm i.d., 16 mm in length, Intramedic, Clay Adams), open to the atmosphere, through the left atrium into the left ventricular lumen. The institution of an afterload was accomplished by placing a fluid-filled balloon into the lumen of the left ventricle. The balloon was connected to a pressure transducer (COBE, Lakewood, Colorado) by PE240 tubing (Clay Adams). Balloon volume was periodically adjusted to maintain the left ventricular systolic pressure near 90 mm Hg. To avoid overdistention of the ventricle, left ventricular diastolic pressure was never allowed to exceed 15 mm Hg. In some hearts after ischemia, the target systolic pressure could not be achieved with a diastolic pressure below 15 mm Hg, and a reduced systolic pressure was accepted. During the latter part of occlusion, left ventricular diastolic pressure increased to more than 15 mm Hg in several of the hearts. When this occurred, the balloon was also deflated to keep diastolic pressure below 15 mm Hg. In those instances, it was also not possible to maintain the target systolic pressure of 90 mm Hg. The lowest systolic pressure seen under any circumstance was 50 mm Hg.

Total coronary flow was assumed to be represented by the effluent blood that dripped out of the severed pulmonary artery. This should be composed of coronary sinus plus thebesian flow. This flow was measured by timed collection in a graduated cylinder. Epicardial surface temperature was assessed with a temperature probe (Telethermometer, Yellow Springs...
Instrument Co., Yellow Springs, Ohio). The heart was allowed to equilibrate for at least 10 minutes before the experimental protocol was begun. The epicardial surface temperature of the heart was 35.85±0.03°C (n=7), indicating 1.15°C cooling between the heat exchanger and the heart. This very mild hypothermia could have imparted some protection to the hearts but it should have affected all groups equally.

**Measurement of Infarct Size**

At the conclusion of the experiment, the heart was flushed with saline through a side port in the perfusion tubing. The coronary artery was then reclosed, and fluorescent particles (zinc cadmium-sulfide, 1–10 μm in diameter) (Duke Scientific Corp., Palo Alto, California) were infused for the subsequent determination of the risk zone. The heart was removed from the Langendorff apparatus, weighed, and frozen. The heart was then cut into transverse slices approximately 2 mm thick. The slices were incubated in triphenyl tetrazolium chloride (TTC) (1% [wt/vol] in phosphate buffer at 37°C) for 20 minutes. After staining, the slices and area of infarcted tissue were traced. The risk zone was determined by viewing the slices under ultraviolet light; the areas lacking fluorescence, which represented the perfusion defect, were also traced. The area (cm²) of the infarct and ischemic zone were determined by planimetry, and the volume (cm³) of infarcted myocardium and myocardium at risk was calculated from the planimetered areas and the slice thickness (2 mm). The volume of the planimetered areas in cubic centimeters was assumed to be equal to the weight of the tissue in grams.

**Experimental Protocol**

To test whether time of unloading (i.e., occlusion versus reperfusion) affected infarct size, four experimental groups were used. In the control group (group 1), the balloon was placed in the left ventricle for the duration of the experiment; thus, the heart developed pressure during both occlusion and reperfusion. In group 2, the balloon was present only during occlusion, and the left ventricle was vented within 2 minutes of reperfusion. In six animals, venting was accomplished within 2 minutes after the snare was released, and in four animals, the ventricle was unloaded 2 minutes before reperfusion. Hearts in group 3 were decompressed during occlusion; approximately 1–3 minutes after reperfusion, the balloon was inserted and the heart developed pressure. In group 4, the left ventricle was unloaded during both ischemia and reperfusion. Data collection was identical in all groups. After the equilibration period, control values for temperature, coronary flow, mean perfusion pressure, left ventricular systolic pressure, and left ventricular diastolic pressure were recorded. Immediately after occlusion of the coronary artery, mean perfusion pressure and left ventricular systolic and diastolic pressures were noted. These variables were monitored every 10 minutes during the 60-minute period of occlusion. Total coronary flow and temperature were also measured at 30 and 60 minutes of occlusion. At the time of reperfusion, mean perfusion pressure, left ventricular systolic pressure, and left ventricular diastolic pressure were noted. These variables were checked every 10 minutes for the first 30 minutes of reperfusion. Flow and temperature were measured at 30, 60, 90, and 120 minutes of reperfusion. Differences between group 1 and the other groups were assessed using one-way analysis of variance. Analysis of variance was also used to screen for differences in all measured variables between all groups. Data are reported as mean±1 SEM.

**Results**

Hemodynamic variables (i.e., heart rate, coronary perfusion pressure, and coronary flow) were not significantly different between groups. Left ventricular systolic and diastolic pressures obtained during loaded conditions were also similar between groups at each time period, with the exception of left ventricular systolic pressure during the preischemia phase. At this time, left ventricular systolic pressure for group 1 was 86±4 mm Hg as compared with 98±2 mm Hg for group 3 (p<0.005). (Isolated hearts of groups 2 and 4 were vented.) These data are presented in Table 1.

Risk zone and infarct size data for each group are shown in Table 2. The risk zone, whether analyzed simply as number of grams of tissue at risk or as the percentage of myocardium at risk, was not significantly different between groups.

Absolute infarct size, expressed in grams of tissue infarcted, was similar when the left ventricle was not vented at all or vented only during part of the protocol (groups 1, 2, and 3, 0.52±0.15, 0.64±0.14, and 0.31±0.05 g, respectively). There was a modest but nonsignificant reduction in absolute infarct size when the left ventricle was vented during both occlusion and reperfusion (0.18±0.10 g). The data are easier to interpret after the infarct size is normalized as a percentage of the region at risk. Although the average percentage of infarction in group 2 (unloaded during reperfusion) was significantly larger than in groups 3 and 4 (p<0.04), this difference did not reach statistical significance when compared with the control hearts in group 1 (p=0.29). Similarly, the percentage of infarction in the hearts in group 3 experiencing unloading only during reperfusion was not different from the controls. The percentage of infarction in those hearts in which the left ventricle was decompressed during both occlusion and reperfusion (group 4), however, was significantly smaller than in the group 1 controls (p<0.02). The infarct size of the individual isolated hearts in each group (expressed as percentage of risk zone infarcted) is plotted in Figure 1. As previously described, six of the rabbits in group 2 were unloaded shortly after reperfusion was started, whereas four were unloaded several minutes before reperfusion. The percentage
of the risk zone infarcted was not significantly different between those two groups (53.6±9% vented "before," and 58.5±9% vented "after").

Was the present model sensitive enough to detect a beneficial effect of unloading if one had existed? To address that question, we calculated the 95% confidence limit between the control group and group 2, which was unloaded only during reperfusion. That analysis indicated that a difference of 18.4% or more could have been resolved in this model.

**Discussion**

These data indicate that in isolated blood-perfused rabbit hearts, left ventricular unloading during occlusion only or reperfusion only does not limit infarct size. Decompression of the left ventricle during both occlusion and reperfusion, however, did result in limitation of myocardial infarct size at 2 hours of reperfusion. Unfortunately, the procedure of ventricular unloading during both ischemia and reperfusion is not feasible as a therapeutic intervention.

The lack of benefit from unloading of the ventricle at reperfusion suggests that the simple reduction of myocardial oxygen demand, secondary to the abolition of pressure-work at the time of reperfusion, is not by itself cardioprotective. In fact, the data showed a strong trend for an increase in infarct size in this group. How unloading during both periods promoted myocardial salvage is not evident. It seems that the component of injury that was prevented must have been present during both periods because unloading only during the ischemic period was also not protective.

If cardiac work was a major determinant of infarct size, then unloading the heart only during the ischemic period should have reduced infarct size. Muller
and colleagues suggested that the work load of the heart during the first minutes of ischemia was a major determinant of infarct size. On the other hand, Miura et al. failed to see any correlation between the rate-pressure product, an index of cardiac metabolism, and infarct size in a dog model of acute myocardial infarction. In our past experience, although quite variable, infarct size also does not correlate with the rate-pressure product at occlusion. Moreover, reduction of myocardial oxygen demand with β-blockers has not altered infarct size in most of the animal models, either. Thus, it seems that the extent of necrosis might not be as dependent on the metabolic conditions during the first few minutes of ischemia as was originally believed. Whether unloading can be instituted at some critical time period after the onset of ischemia and still achieve protection was not addressed in this study but is an intriguing possibility.

It is likely that unloading throughout the ischemia-reperfusion period limited infarct size by a mechanism totally unrelated to metabolic changes. The protection might be because mechanical disruption of the ischemic zone resulting from passive stretch during pressure development is prevented by unloading. That zone would be akinetic during both the ischemic and the reperfusion period in this model and, therefore, vulnerable to overstretching. If that were the mechanism, then it is unknown how long the unloading would have to be continued to achieve a sustained protection.

Infarct size was estimated in the present study by incubation of the tissue with TTC. Although most studies find that a TTC estimate of infarct size after 2 or more hours of reperfusion does represent the ultimate infarct size as determined by histology, recent studies from this laboratory suggest that the ability of TTC to discriminate between living and dead tissue might be altered by some therapeutic interventions. In that study, superoxide dismutase caused TTC to stain, as viable, tissue that was clearly necrotic by histological criteria. The mechanism for this artifact is currently unknown. We, therefore, have to consider the possibility that the unloading conditions in group 4 simply might have altered the TTC staining characteristics of the heart and that the actual infarct size might not have been reduced in that group. This would have to be verified by recovery studies in which a histological measurement of infarct size is done at least 48 hours after reperfusion, an impossibility with the current model. Because there is no clinical counterpart to group 4, however, it would be difficult to justify the necessary follow-up studies to validate that finding. On the other hand, it is still the prevailing opinion that TTC negative tissue is unambiguously dead and unrecoverable. Thus, it seems unlikely that any actual protection could have been obscured by artifact in the critical hearts in group 2, which were vented only during reperfusion.

The isolated blood-perfused rabbit heart preparation used in the present report has the following advantages over the previous dog models used to address the effect of unloading: The degree of ischemia (i.e., amount and duration of flow reduction) is under rigid control because rabbits possess very few native coronary collaterals, and thus, variability in infarct size resulting from variations in collateral flow to ischemic tissue is reduced. Heart rate, a major determinant of myocardial oxygen consumption, is held constant, thus obviating any changes in infarct size potentially caused by changes in this variable. It has been suggested that previous studies showing no effect of left ventricular or left atrial bypass on infarct size were hampered by incomplete decompression of the left ventricle. In the isolated blood-perfused rabbit heart, complete decompression was assured by venting the left ventricular cavity directly to atmosphere.

The present results differ from the results obtained from in vivo left heart bypass preparations, Axelrod and coworkers reported that the volume of infarct (expressed as percentage of area at risk) was reduced from 32.5% in control dogs (n=6) to 17.5% in dogs with pulsatile left ventricular bypass (n=9). One possible explanation is that measurements of collateral blood flow were not made in those studies. Dogs display a wide variation in collateral flow, which in turn has a profound effect on resulting infarct size. The difference in infarct size between groups might simply reflect an unfortunate sampling artifact caused by failure to consider this variable. This can be a serious problem when the group sizes are small, as in the Axelrod et al. study. In the follow-up study of Axelrod and colleagues, pulsatile left ventricular bypass was performed during reperfusion in fibrillating dog hearts and was reported to confer significant cardioprotection. Although neither fibrillating nor empty beating hearts perform external work, fibrillating hearts have a higher myocardial oxygen consumption, concomitant with ventricular distension. Thus, it is possible that decompression of the left ventricle on reperfusion does limit infarct size in the setting of ventricular fibrillation but has little effect when a normal cardiac rhythm is maintained.
Left ventricular decompression during reperfusion, an intervention currently feasible in the clinic, was not associated with a reduction in infarct size. Venting of the left ventricle only during occlusion also did not proffer cardioprotection. Our results indicated that the ventricle must be decompressed during both occlusion and reperfusion before limitation of infarct size can be realized.

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