Left ventricular unloading before reperfusion reduces endothelin-1 release and calcium overload in porcine myocardial infarction

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Objectives: The aim of this study was to test the hypothesis that after an acute myocardial infarction, endothelin-1 release with subsequent calcium overload is a mediator of myocardial reperfusion injury, which can be inhibited, in part, by left ventricular unloading immediately before reperfusion. We recently have reported that left ventricular unloading before reperfusion reduces infarct size after acute myocardial infarction. However, the biologic mechanisms of infarct salvage in unloaded hearts subjected to ischemia/reperfusion remain undefined.

Methods: Twelve pigs were subjected to 1 hour of left anterior descending coronary artery occlusion followed by 4 hours of reperfusion. A left ventricular assist device was initiated 15 minutes before reperfusion and maintained during reperfusion (assist device group, n = 6). A control group (n = 6) was subjected to reperfusion alone. Infarct size, endothelin-1 plasma levels, intracellular calcium levels, and apoptosis were analyzed in both groups.

Results: At reperfusion, left ventricular unloading significantly decreased left ventricular end-diastolic and end-systolic pressures. Infarct size, expressed as a percentage of zone at risk, was also significantly reduced by 54% in the group with the left ventricular assist device compared with controls. Support with a left ventricular assist device reduced endothelin-1 release from the heart at 15 minutes, 30 minutes, and 1 hour of reperfusion. Myocardial release of endothelin-1 was significantly correlated with infarct size at 15 minutes of reperfusion ($r = 0.79$; $P = .008$). Left ventricular unloading caused a significant reduction of calcium overload and of the percentage of apoptotic cells in the ischemic region.

Conclusion: Our findings suggest that endothelin-1 release and calcium overload are important mediators of reperfusion injury and that they can be significantly reduced by left ventricular unloading before coronary artery reperfusion during myocardial infarction.

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Prompt institution of reperfusion after coronary artery occlusion limits myocardial infarct size.[1-3] However, reperfusion after more than 1 hour of ischemia, a common clinical scenario, may result in significant "reperfusion injury," which diminishes the extent of myocardial salvage.[1] Pharmacologic treatment alone, such as the early use of beta-blockers, nitroglycerin, and angiotensin-converting enzyme inhibitors, has not been effective in the prevention of reperfusion injury.[4-6] Increased circulating plasma levels and myocardial tissue content of endothelin-1

Abbreviations and Acronyms

Ca^{2+} = calcium
CS = coronary sinus
ET-1 = endothelin-1
INF/LV = infarct weight/left ventricular weight ratio
IV = intravenous
LAD = left anterior descending coronary artery
LV = left ventricle/ventricular
LVAD = left ventricular assist device
MI = myocardial infarction
TUNEL = terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling
ZR/LV = zone at risk/left ventricular weight ratio

(ET-1), a 21-amino acid peptide with a powerful vasoconstrictor activity, have been detected during ischemia/reperfusion, and a possible role for this peptide in the pathophysiology of ischemia/reperfusion injury has been postulated.[7,8]

Mechanical unloading of the myocardium reduces left ventricular (LV) pressure work and myocardial oxygen consumption.[9-12] We[13] have previously shown that LV unloading during myocardial infarction (MI) limits reperfusion injury as well as infarct size independent of its effects on myocardial blood flow. We[13] have also demonstrated that LV unloading initiated just before reperfusion produced a dramatic reduction in calcium (Ca^{2+}) overload and contraction band necrosis by electron microscopy in the ischemic region compared with reperfusion alone. Others have shown in isolated myocytes subjected to simulated ischemia that ET-1 promotes Ca^{2+} flux into the cells, which is associated with cell death.[14] We therefore tested the hypothesis that LV unloading just before reperfusion reduces ET-1 release on reperfusion, and we proposed that the reduced ET-1 levels would then reduce Ca^{2+} flux into ischemic myocytes, reducing fatal Ca^{2+} overload and consequently reducing infarct size.

Materials and Methods

Animal Model

We used 12 Yorkshire pigs of either sex weighing 50 ± 5 kg. The study protocol was approved by the Animal Welfare Committee of the University of Texas at Houston Medical School, and all experiments were performed according to the Committee's guidelines.

Surgical Procedure

Pigs were sedated with tiletamine HCl and zolazepam HCl (Telazol IM; Wyeth, Madison, NJ, 2.0 mg/kg, to effect) and intubated. General anesthesia was maintained with isoflurane (0.5–2.5 vol%). An 8F sheath was placed in the right carotid artery and a catheter was advanced to the ascending aorta for monitoring aortic pressure and for blood sampling for regional myocardial blood flow and ET-1 sampling. A 6F right multipurpose catheter was placed into the coronary sinus (CS) under fluoroscopic guidance. The A-Med left ventricular assist device (LVAD) (A-Med Systems, Inc, West Sacramento, Calif), a miniaturized circulatory assist device, was inserted into the LV via femoral access as previously described.[13] In control animals, the introducer sheath was inserted in the femoral artery but the pumping was not initiated. The heart was exposed via a sternotomy. A ligature was placed around the left anterior descending coronary artery (LAD) at a position from which the distal third of the artery would be occluded by tightening the ligature. A Doppler flow probe was placed around the artery just proximal to the snare for monitoring coronary blood flow. A catheter was placed in the LV via an apical stab wound for measurement of LV pressure. Catheters were placed in the left atrium for measurement of atrial pressure and for radioactive microsphere injection. All animals received acetylsalicylic acid (325 mg intravenously [IV]) 30 minutes before ischemia, and sodium heparin 5000 IU IV bolus followed by an IV drip to maintain an activated clotting time of greater than 200 seconds until the end of the experiment. An IV lidocaine drip of 1 mg/min was started and maintained throughout the occlusion period for 2 hours into reperfusion or until the animal's condition had stabilized. In addition, bretylium tosylate was infused before the occlusion with an IV drip rate of 5 $mg \cdot kg^{-1} \cdot h^{-1}$ maintained throughout the procedure. Hemodynamic and functional measurements were recorded on a Gould chart recorder (ThermoSpectra Corp, San Diego, Calif) and analyzed as previously described.[13] After baseline measurements, the LAD was occluded and the occlusion was maintained for 1 hour followed by reperfusion for 4 hours. Flow cessation in the LAD was documented by the Doppler flow signal. At the experiment conclusion, the animals were heparinized (4000 IU IV), fully anesthetized with intravenous pentobarbital, and humanely killed with supersaturated potassium chloride. The heart was quickly excised from the chest and washed with tap water.

Experimental Protocol

Baseline measurements included hemodynamic values (arterial blood pressure, left atrial pressure, and LV pressure), coronary blood flow, and arterial and CS ET-1 sampling. Hemodynamic values and flow data were continuously recorded. Blood samples for measuring plasma ET-1 were collected simultaneously from the CS and the descending thoracic aorta at baseline, 45 minutes of ischemia, reperfusion, and 15 and 30 minutes and 1, 2, and 4 hours of reperfusion. Tissue biopsy specimens for Ca^{2+} measurements were taken at baseline, 45 minutes of ischemia, and at 10 minutes, 30 minutes, and 4 hours of reperfusion. Group 1 (n = 6) served as a control group and was not subjected to LV assistance. In group 2 (n = 6), LV unloading was started 15 minutes before reperfusion at maximal rotational speed and maintained for the remainder of the experiment.

Plasma ET-1 Content

ET-1 concentration was determined by the radioimmunoassay technique with the isoform-specific rabbit antibodies against synthetic ET-1 (Peninsula Laboratories, Inc, San Carlos, Calif). Blood samples were collected simultaneously from the CS and the aorta and processed according to the manufacturer's instructions.

Assessment of Intracellular Ca^{2+} in Cardiac Tissue

Myocardial tissue biopsy specimens, 13-mm long, were obtained from the ischemic and control regions with an 18-gauge needle (BioPince; Amedic AB, Sollentuna, Sweden) for Ca^{2+} measurements.

Intracellular Ca^{2+} content was determined with the Ca^{2+}-sensitive fluorescent probe, fluo-3 AM (Molecular Probes, Eugene, Ore), using a fluorescence deconvolution microscope (Applied Precision Delta Vision, Issaquah, Wash), incorporating an Olympus IX70 microscope (Olympus America, Melville, NY), as previously described.[15] A combination of probes was used to determine cell types (myocytes, vascular endothelial cells, and fibroblasts). 4,6-Diamino-2-phenylindole (DAPI) was used to identify nuclei. Smooth muscle actin, cardiac muscle actin, and cardiac myosin were probed with secondary antibody tagged with DIPYrromethene BOron Difluoride (BODIPY) or Texas Red dye. Smooth muscle actin identified vascular elements, whereas actin and myosin patterns and absence of intercalated disks distinguished myocytes from fibroblasts.[15]

Infarct Size Determination

Infarct size quantification by triphenyl tetrazolium chloride staining was measured according to the method of Fishbein and associates.[16] After excision of the heart, the aortic root was perfused with Evans blue dye (1%) while the LAD distal to the occlusion was perfused with triphenyl tetrazolium chloride at equal pressures. The heart was then sliced. The blue-stained control region, the red-stained risk region, and the tan infarct were measured by planimetry and quantified in all slices as described previously.[9]

Regional Myocardial Blood Flow

Regional myocardial blood flow was analyzed with the microsphere technique at baseline, 25 minutes of ischemia, and 5 minutes of reperfusion and was expressed as milliliters per gram per minute.[17] Flows were measured in the endocardial and epicardial areas as well as transmurally.

TUNEL Assay

Terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL)–positive cardiomyocytes were detected in paraffin-embedded LV sections by the protocol described in the DeadEnd Colorimetric TUNEL System (Promega Corporation, Madison, Wis). Positive and negative control sections were included. Microscopic evaluations were performed by a BX40 inverted microscope (Olympus America).

Statistical Analysis

All results are expressed as mean ± SEM. For multiple comparison procedures, including hemodynamic and coronary flow data, infarct size, ET-1 levels, and Ca^{2+} measurements, analysis of variance and the Newman–Keuls multiple-range tests were performed (Statistica; StatSoft, Inc, Tulsa, Okla). Analysis of covariance was performed to compare infarct sizes, with ET-1 levels or collateral blood flow used as a covariate.

Results

Hemodynamic Changes

Hemodynamic changes during ischemia and reperfusion are shown in Table 1. At baseline and during LAD occlusion and reperfusion, there was no significant difference in heart rate, mean arterial pressure, or rate–pressure product between control and unloaded animals. Initiation of the pump resulted in LV unloading, as indicated by significant decreases in end-diastolic and systolic pressures.

Myocardial Blood Flow

Myocardial blood flow, expressed as millimeters per gram per minute, was measured in the area of coronary occlusion as well as in the contralateral area (Table 2). There was a significant decrease in myocardial blood flow with ischemia. However, there was no significant difference in absolute transmural blood flow between controls and treated animals

TABLE 1. Hemodynamic parameters in control and unloaded animals

	LAD occlusion				Reperfusion						
	Baseline	10 min pump off	35 min pump off	45 min pump on	10 min pump on	30 min pump on	1 h pump on	2 h pump on	3 h pump on	4 h pump on	4 h pump off
HR (beats/min)											
Control	113 ± 4	103 ± 3	101 ± 6	94 ± 3	95 ± 2	94 ± 3	92 ± 3	94 ± 5	95 ± 7	99 ± 8	93 ± 8
LVAD	100 ± 8	89 ± 9	94 ± 7	95 ± 9	87 ± 6	85 ± 6	81 ± 7	90 ± 8	98 ± 8	102 ± 12	86 ± 13
MAP (mm Hg)											
Control	93 ± 8	90 ± 8	84 ± 7	88 ± 9	89 ± 10	82 ± 9	80 ± 8	64 ± 7	57 ± 5	45 ± 5	40 ± 4
LVAD	89 ± 11	92 ± 13	91 ± 12	84 ± 11	73 ± 10	66 ± 12	67 ± 12	68 ± 15	66 ± 10	67 ± 13	54 ± 15
LVSP (mm Hg)											
Control	109 ± 9	107 ± 9	101 ± 8	106 ± 10	109 ± 11	101 ± 10	99 ± 9	84 ± 7	79 ± 5	66 ± 6	62 ± 4
LVAD	102 ± 10	105 ± 14	105 ± 12	71 ± 16	42 ± 9*	49 ± 12*	40 ± 12*	55 ± 12	55 ± 9*	41 ± 5*	71 ± 16
LVEDP (mm Hg)											
Control	7 ± 1	8 ± 1	9 ± 1	10 ± 2	9 ± 1	9 ± 1	9 ± 1	7 ± 1	8 ± 1	7 ± 1	6 ± 1
LVAD	6 ± 0	8 ± 1	8 ± 1	5 ± 2	4 ± 1*	3 ± 1*	4 ± 1*	3 ± 1*	5 ± 1	3 ± 1*	8 ± 2
RPP											
Control	121 ± 8	109 ± 9	103 ± 9	100 ± 10	104 ± 12	95 ± 11	92 ± 9	79 ± 9	76 ± 8	65 ± 8	58 ± 8
LVAD	104 ± 16	97 ± 18	99 ± 15	82 ± 12	66 ± 10*	63 ± 13	59 ± 14	67 ± 14	73 ± 8	67 ± 6	66 ± 16

Values are expressed as means ± SEM of 6 experiments. *HR,* Heart rate; *LVAD,* left ventricular assist device; *MAP,* mean arterial pressure; *LVSP,* left ventricular systolic pressure; *LVEDP,* left ventricular end-diastolic pressure; *RPP,* heart rate–pressure product. *$P < .05$ vs control.

TABLE 2. Regional myocardial blood flow (transmural) in control and unloaded animals

	Absolute flows (mL · g^{-1} · min^{-1})			
		LVAD		
	Control	Off	On	*P*
Baseline				
Control region	0.85 ± 0.09	0.87 ± 0.09	—	.86
Ischemic region	0.62 ± 0.06	0.70 ± 0.06	—	.36
LAD occlusion				
Control region	0.70 ± 0.10	1.12 ± 0.28	—	.12
Ischemic region	0.02 ± 0.01	0.05 ± 0.02	—	.16
Reperfusion (5 min)				
Control region	0.74 ± 0.13	—	0.57 ± 0.10	.34
Ischemic region	2.50 ± 0.36	—	2.75 ± 0.45	.67

Values are means ± SEM of 6 experiments. *LAD,* Left anterior descending coronary artery.

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in the ischemic or control regions at baseline, at 25 minutes of occlusion, and at 5 minutes of reperfusion. Endocardial and epicardial flows were also similar between the groups (data not shown).

Infarct Size

No differences were noted in the zone at risk/LV weight ratio (ZR/LV) ($P = .12$), or infarct weight/total LV weight ratio (INF/LV) ($P = .09$) between the groups (Figure 1). There was a significant (54%) reduction of infarct size when expressed as a percent of zone at risk (INF/ZR) in the supported group compared with controls (24.10% ± 6.45% vs 51.59% ± 6.64%; $P = .014$). When the transmural collateral blood flow to the ischemic region was used as a covariate, infarct size (as a percentage of area at risk) was significantly smaller in the LVAD group than in the control group ($P = .025$). A significant reduction in infarct size occurred independent of collateral blood flow (Figure 2).

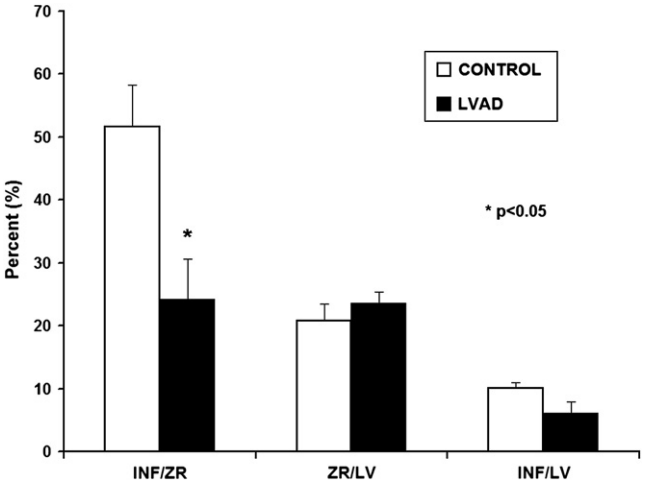

Figure 1. Bar graph showing infarct size *(INF),* expressed as a percent of zone at risk *(ZR),* and zone at risk and infarct zone as a percentage of the LV weight for the LVAD and control groups. Data are expressed as mean ± SEM of 6 independent experiments in each group.

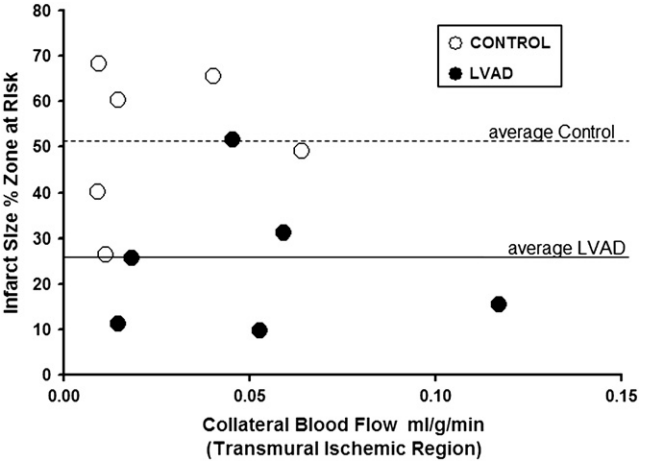

Figure 2. Relationship between infarct size, expressed as a percentage of zone at risk, and collateral blood flow during coronary occlusion in the transmural ischemic zone. By analysis of covariance, a significant reduction in infarct size occurred with unloading, independent of collateral blood flow ($P = .025$).

Plasma ET-1 Levels

The transcardiac release of ET-1 was significantly lower in the unloaded animals than in the controls at 15 minutes, 30 minutes, and 1 hour of myocardial reperfusion (Figure 3). To assess the relationship between myocardial ET-1 release and infarct size, independent of any other variables, we analyzed this relationship for the total group of tested animals using an analysis of covariance. Figure 4 shows the correlation between myocardial release of ET-1 at 15 minutes of

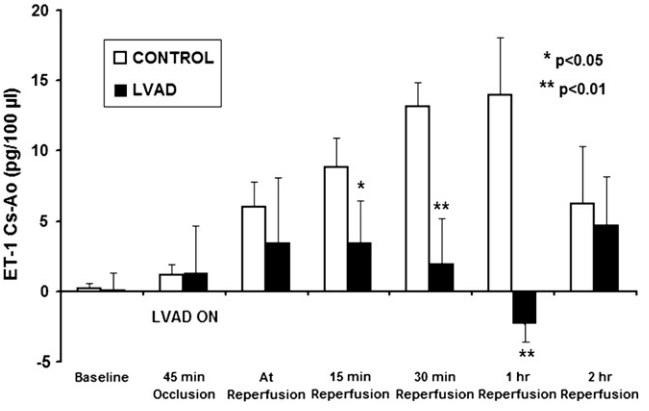

Figure 3. Myocardial ET-1 plasma levels (coronary sinus minus aorta *[Cs − Ao]*) expressed in picograms per 100 µL in the control and unloaded animals (n = 6 pigs in each group).

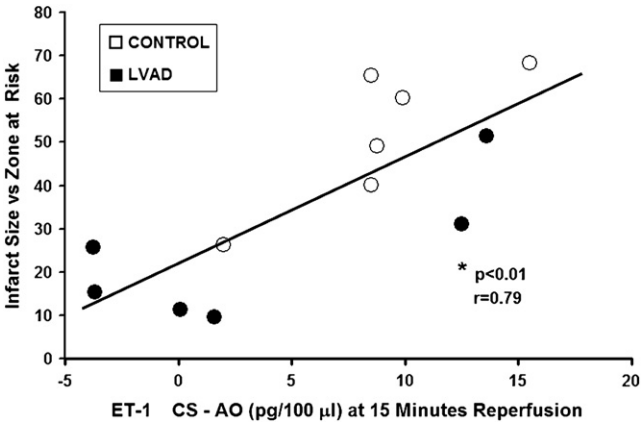

Figure 4. Correlation between myocardial release of ET-1 at 15 minutes of reperfusion and final infarct size. There was a significantly positive correlation between myocardial release of ET-1 and infarct size with an $r = 0.79$, and a $P = .008$. *CS − AO,* Coronary sinus minus aorta.

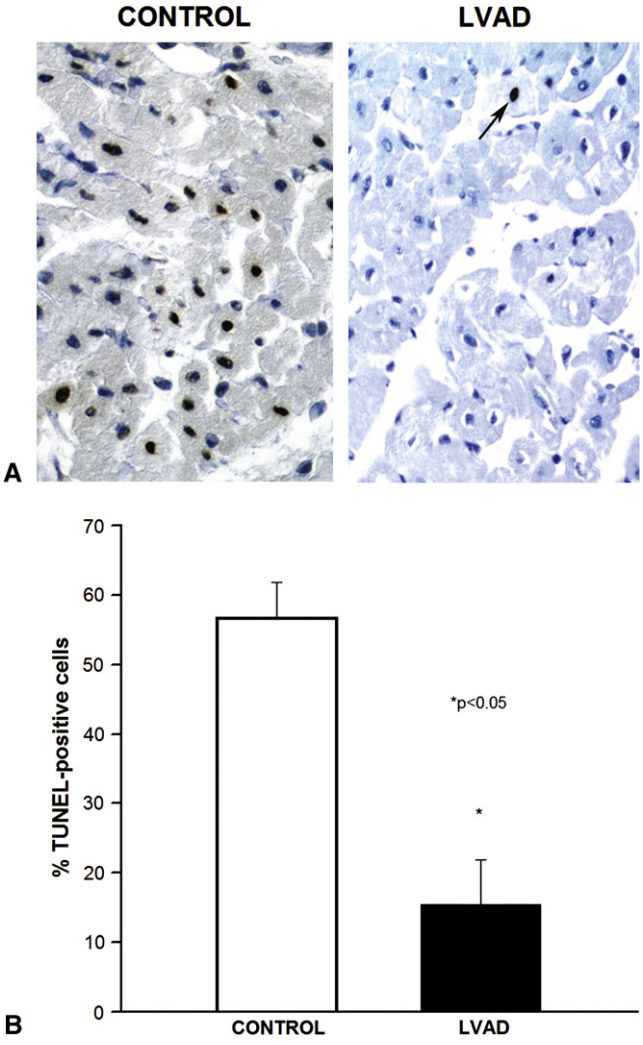

Figure 5. Effect of LV unloading on ischemia/reperfusion–induced apoptosis in the heart. Apoptotic cardiomyocyte nuclei appear brown-stained, whereas TUNEL-negative nuclei appear blue with hematoxylin and eosin. Heavy staining of numerous TUNEL-positive cardiomyocytes was observed in the central ischemic region of the LV of the sham-operated pig after 1 hour of LAD ischemia and 4 hours of reperfusion. In contrast, few TUNEL-positive cells were detected in the LV ischemic region of the unloaded animal *(arrow)* (n = 3 to 4 counts for 6 hearts in each group.

reperfusion and final infarct size. There was a significantly positive correlation between myocardial ET-1 release and infarct size, regardless of animals' treatment, indicating that myocardial ET-1 release was an independent predictor of infarct size at 15 minutes of reperfusion ($r = 0.79$; $P = .008$) (Figure. 4).

Ischemic Zone Apoptosis

LV unloading reduced the number of apoptotic cells in ischemic zones. Of 400 nuclei counted in the central ischemic region of the unloaded animals, 15.15% ± 6.4% displayed TUNEL-positive staining. In contrast, for the same number of nuclei, 56.69% ± 5.17% displayed TUNEL-positive staining in the ischemic region of the control animals ($P = .01$) (Figure 5).

Myocardial Ca^{2+} Content

When we compared ischemic and nonischemic ratios of fluo-3 fluorescence (Figure 6, *top* and *bottom*), there was no significant change in intracellular Ca^{2+} content during reperfusion in the unloaded group. However, in the control group, a significant rise in the ischemic/nonischemic intracellular Ca^{2+} ratio was seen in the reperfusion period compared with the occlusion period (4.86 ± 1.20 vs 1.18 ± 0.28; $P = .016$).

Discussion

We found that the transcardiac release of ET-1 was significantly lower in the unloaded animals as opposed to the untreated animals during reperfusion This study, for the first time, demonstrated normalization of plasma ET-1 levels in the ischemic myocardium by hemodynamic unloading of the LV.

In the management of acute MI, the primary focus has been to achieve early reperfusion. This intervention can reduce infarct size, but it is successful only when reperfusion is achieved early after occlusion.[18] When reperfusion is delayed, infarct size increases and reperfusion injury ensues. Several groups have shown beneficial effects of mechanical unloading during acute MI.[11,19,20] Complete mechanical unloading of the LV with cardiopulmonary bypass, LV venting, and cardioplegia before reperfusion has led to a 77%

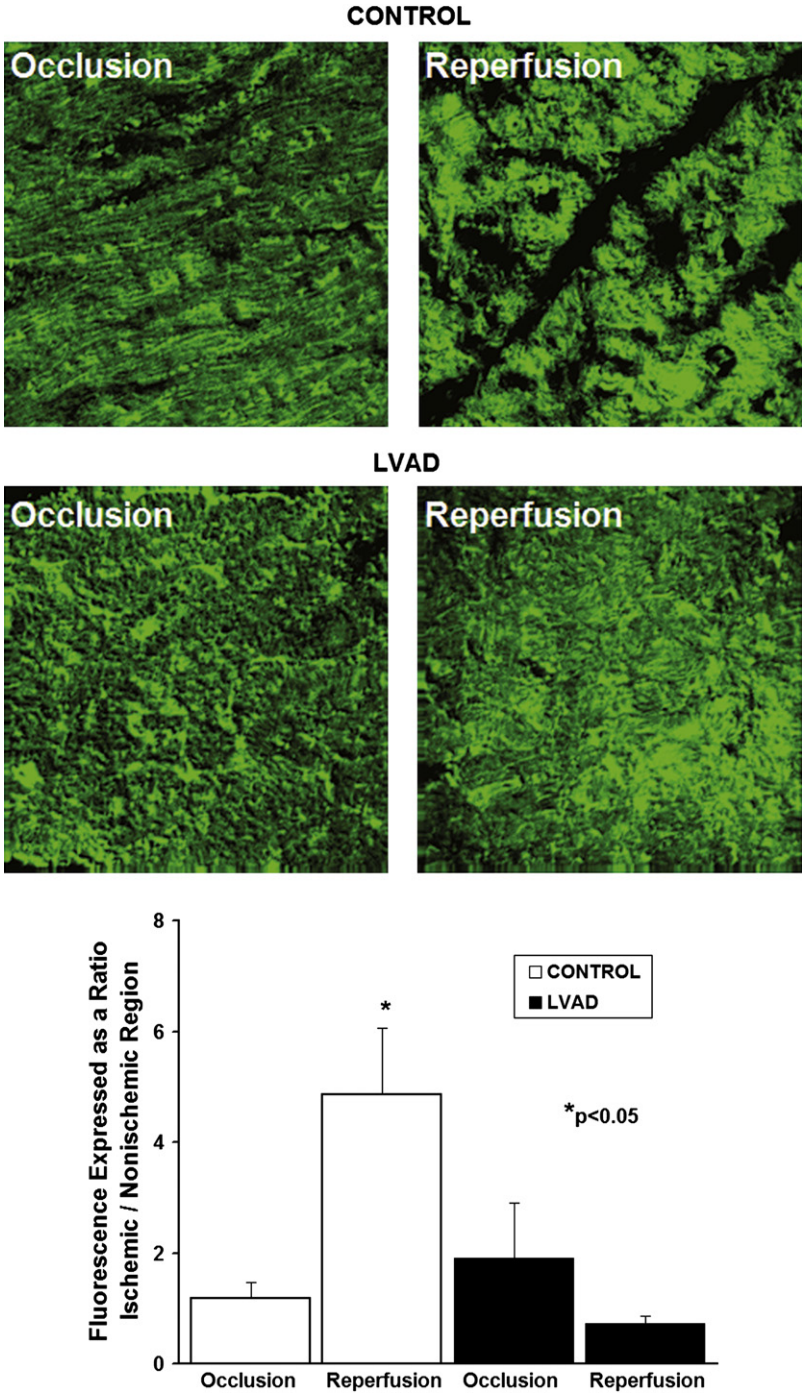

Figure 6. Effect of LV unloading on myocardial Ca^{2+} content. ***Top,*** **Fluorescence deconvolution images of LV sections (ischemic region) probed with fluo-3 fluorescence.** ***Bottom,*** **Ischemic/nonischemic ratios of fluo-3 fluorescence during occlusion and reperfusion period in control and unloaded animals. Data are expressed as mean ± SEM of 12 to 15 slices of 4 hearts in each group.**

reduction of infarct size.[11] This technique, however, is not applicable as a primary approach for MI owing to the significant time delays associated with initiating total cardiopulmonary bypass in patients having an MI. A more practical approach would be percutaneous unloading of the LV. Although it has been reported that chronic LVAD unloading results in regression of hypertrophy and improvement of myocyte function and LV geometry, the cellular and molecular mechanisms responsible for these beneficial effects, particularly in acute ischemia, remain undefined. Preliminary experience in our laboratory in canine ischemia/reperfusion injury has demonstrated that LV unloading, intravenous administration of liposomal prostaglandin E_1, intracoronary heparin distal to the coronary occlusion, and, to a certain

extent, intracoronary adenosine just before reperfusion provide significant infarct salvage, improvement in ischemic collateral blood flow, and reduction in contraction band necrosis paralleling a reduction in the ischemic region end-diastolic wall thickness during early reperfusion.[9,13] Some investigators suggested that the exaggerated flow of Ca^{2+} into the myocytes results in what is called "contraction band necrosis,"[21] but the causes of enhanced Ca^{2+} loading have not been defined.

ET-1 was first described as a vasoactive peptide[22] released from vascular endothelium. ET-1 is released from ischemic myocardium during reperfusion and may contribute to ischemia/reperfusion injury in the intact heart.[23] ET-1 has been shown to be involved in the overload-induced release of Ca^{2+}.[24] ET-1 can influence myocyte intracellular Ca^{2+} concentration by several mechanisms: (1) It stimulates Ca^{2+} release from internal stores[25]; (2) it enhances L-type Ca^{2+} current through the activation of protein kinase C[26]; (3) it activates the Na^+–H^+ exchanger[27]; and (4) it activates the outward I_{NaCa} (ie, reverse mode Na^+–Ca^{2+} exchanger, in which Ca^{2+} influx is induced).[28] Ca^{2+} entry via reverse mode Na^+–Ca^{2+} exchanger is a major cause of ischemia/reperfusion injury.[29] It has been shown that activation of ET receptors on myocytes may trigger phospholipase C activity, resulting in hydrolysis of inositol phosphates and the subsequent release of intracellular Ca^{2+}. This leads to an increased susceptibility of myocardium to ischemia and reperfusion injury. ET-1–induced rise in intracellular Ca^{2+} concentration then causes cell hypercontracture and cell death.[30] In this study, we found that LV unloading, just before reperfusion, resulted in a significant inhibition in the rise of Ca^{2+} induced by reperfusion.

Similar to our findings, Morawietz and associates[31] showed up-regulation of the ET-A receptors in patients with heart failure that was normalized by LV unloading. It is likely that ET-1 is synthesized de novo and produced locally in the ischemic heart.[32] Endothelial cells, vascular smooth muscle cells, and cardiomyocytes all are potential sources of ET-1 production. A study using in situ hybridization and immunohistochemical analyses showed that cardiomyocytes are the main site of ET-1 synthesis and production in porcine ischemic hearts.[33] An increased myocardial expression of ET-1 messenger RNA has been observed in porcine cardiomyocytes subjected to ischemia, suggesting stimulated cardiac production during MI.[33] Galiuto and coworkers[34] showed, in a similar canine model, that LU 135252, an ET_A receptor blocker, when given intravenously at the time of reperfusion, significantly limited the increase in end-diastolic wall thickness, which was also associated with a reduction in myocardial necrosis. We have now demonstrated a significant positive correlation of the transcardiac ET-1 gradient in the ischemic region with myocardial infarct size at 15 minutes of reperfusion, indicating that ET-1 is released across the ischemic heart and promotes LV damage via ET-1 receptors in these animals. Support with the LVAD, just before reperfusion, resulted in a significant reduction in final infarct size independent of regional myocardial blood flow. Moreover, these findings indicate that the transcardiac release of ET-1 is a significant predictor of myocardial infarct size independent of regional myocardial blood flow and the degree of LV unloading. These results are consistent with the findings of Meyns and colleagues,[35] who demonstrated that in a sheep ischemia/reperfusion model known to be devoid of preformed coronary collaterals, "partial LV support" with a modest decrease in preload resulted in a significant reduction in infarct size without any measured increase in myocardial perfusion in the ischemic area. In isolated cultured cardiomyocytes, ET-1 expression is induced by stretch.[36] During acute MI, sarcomere stretching by diastolic Laplace overloading may lead to an increase in synthesis and release of ET-1 locally in the myocardium. A significant correlation between diastolic wall stress and ET-1 expression in the remote area of infarcted rat heart has been documented,[37] suggesting that stretching of the myocardium during diastole contributes significantly to the induction of ET-1 in acute MI. Also, the mechanical support provided by LVAD unloading has been shown to induce a significant reduction in LV wall stress/stretch.[38]

Another key finding of the present study was that LV unloading just before reperfusion resulted in a reduction in apoptotic cells in the ischemic and infarcted areas when compared with untreated animals. Programmed cell death plays a central role in the context of diverse heart diseases of ischemic and nonischemic origin. Recent evidence indicates that simulated ischemia followed by simulated reperfusion results in cardiomyocyte death with typical apoptotic features.[39] It is now widely established that after ischemia/reperfusion, both necrosis and apoptosis may contribute independently to infarct size.[40] After MI, the occurrence of apoptosis has been demonstrated in the infarct area and in the border zone of the infarction, whereas necrotic cells are mainly found in the central zone of the infarct.[41] Myocyte apoptosis appears to be the prevailing form of cell death after MI. Conflicting results exist concerning the role of ET-1 in cardiomyocyte apoptosis. Oie and associates[42] showed that bosentan, a mixed ET_A/ET_B receptor antagonist, attenuated cardiomyocyte cell loss through apoptosis in the viable peri-infarcted area (area at risk) after MI in rats. On the other hand, ET-1 has been reported to be an antiapoptotic factor in vascular smooth muscle cells and endothelial cells.[43] In addition, administration of ET-1 before ischemia has been shown to result in a preconditioning-like cardioprotective effect.[44] ET-1 appears to promote apoptosis only under extreme conditions and instead exerts antiapoptotic influence over a range of physiologic concentrations.

Evidence now indicates that Ca^{2+} overload may promote cell death.[45] In vitro experiments have shown that addition of intracellular Ca^{2+} buffering agents or extracellular Ca^{2+}

chelators significantly inhibit caspase activation, DNA fragmentation, and apoptotic cell death.[45] In contrast, agents that directly mobilize Ca^{2+}, such as Ca^{2+} ionophores, have been shown to trigger apoptosis in diverse cell types.[45] Cell death caused by Ca^{2+} overload has been implicated in reperfusion injury of myocardium after ischemia.[46] Moreover, it has been demonstrated that addition of Ca^{2+} blockers abrogates apoptosis in cardiac myocytes subjected to chemical hypoxia, further supporting the claim that Ca^{2+} influx plays a significant role in apoptotic cell death.[47] Our finding indicates some potential of LV unloading as an intervention to the vicious cycle of distention-induced proapoptotic phenotype shifts.

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Study Limitations

We chose to study the effect of unloading using a porcine model of myocardial ischemia/reperfusion to avoid a collateral flow artifact. In a species like the dog, naturally occurring collateral flow can significantly contribute to cell viability in both the permanently occluded and reperfused heart. However, the degree of collateral circulation in patients varies enormously and most often is not predictable. The pig, on the other hand, has virtually no epicardial collateral connections and only sparse endocardial connections.[48] Inasmuch as it has no pre-existing coronary collateral vessels, its infarctions have sharply defined borders, and coronary anatomy varies little between pigs.[49]

The question that remains to be resolved in this study is whether ET release accounts for the injury or whether the release of ET-1 adds to the injury, resulting from the altered hemodynamics that accompanies ischemia/reperfusion injury. The use of ET antagonism would help to address this question and could establish the cause–effect relationship between ET release and infarction and determine whether reducing ET release per se is a protective maneuver or whether it requires the physical unloading of the LVAD. A straightforward approach would be the use of an ET receptor antagonist, which would possibly abolish any difference between control and unloaded animals.

Conclusion

Mechanical unloading of the LV, just before reperfusion, reduces infarct size in a porcine ischemia/reperfusion model. A possible mechanism for this effect is a reduction of myocardial release of ET-1 by LV unloading before reperfusion, which, in turn, reduces Ca^{2+} overload into ischemic myocytes as well as apoptosis in the ischemic region. Taken together, our findings suggest that ET-1 release and Ca^{2+} overload are important mediators of reperfusion injury and that they can be significantly reduced by LV unloading before coronary artery reperfusion during MI. The potential for developing new therapeutic strategies aimed at preventing reperfusion injury in patients subjected to myocardial revascularization in the setting of acute MI is significant.

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