Mechanical Pre-Conditioning With Acute Circulatory Support Before Reperfusion Limits Infarct Size in Acute Myocardial Infarction

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ABSTRACT

OBJECTIVES This study tested the hypothesis that first reducing myocardial work by unloading the left ventricle (LV) with a novel intracorporeal axial flow catheter while delaying coronary reperfusion activates a myocardial protection program and reduces infarct size.

BACKGROUND Ischemic heart disease is a major cause of morbidity and mortality worldwide. Primary myocardial reperfusion remains the gold standard for the treatment of an acute myocardial infarction (AMI); however, ischemia–reperfusion injury contributes to residual myocardial damage and subsequent heart failure. Stromal cell-derived factor (SDF)-1α is a chemokine that activates cardioprotective signaling via Akt, extracellular regulated kinase, and glycogen synthase kinase-3β.

METHODS AMI was induced by occlusion of the left anterior descending artery (LAD) via angioplasty for 90 min in 50-kg male Yorkshire swine (n = 5/group). In the primary reperfusion (1/C14 Reperfusion) group, the LAD was reperfused for 120 min. In the primary unloading (1/C14 Unloading) group, after 90 min of ischemia the axial flow pump was activated and the LAD left occluded for an additional 60 min, followed by 120 min of reperfusion. Myocardial infarct size and kinase activity were quantified.

RESULTS Compared with 1/C14 Reperfusion, 1/C14 Unloading reduced LV wall stress and increased myocardial levels of SDF-1α, CXCR4, and phosphorylated Akt, extracellular regulated kinase, and glycogen synthase kinase-3β in the infarct zone. 1/C14 Unloading increased antiapoptotic signaling and reduced myocardial infarct size by 43% compared with 1/C14 Reperfusion (73 ± 13% vs. 42 ± 8%; p = 0.005). Myocardial levels of SDF-1 correlated inversely with infarct size (R = 0.89; p < 0.01).

CONCLUSIONS Compared with the contemporary strategy of primary reperfusion, mechanically conditioning the myocardium using a novel axial flow catheter while delaying coronary reperfusion decreases LV wall stress and activates a myocardial protection program that up-regulates SDF-1α/CXCR4 expression, increases cardioprotective signaling, reduces apoptosis, and limits myocardial damage in AMI. (J Am Coll Cardiol HF 2015;3:873–82) © 2015 by the American College of Cardiology Foundation.

Despite timely reperfusion therapy, nearly 10% of patients presenting with acute myocardial infarction (AMI) die during their index hospitalization and 25% of survivors progress to develop chronic heart failure, a major public health problem (1). Recent data confirm that more rapid primary reperfusion does not improve clinical outcomes after AMI (2). One explanation for
ABBREVIATIONS AND ACRONYMS

1 Reperfusion = primary reperfusion group
1 Unloading = primary unloading group
AMI = acute myocardial infarction
BCL = B-cell lymphoma
CK-Mb = creatine kinase myocardial band
Erk-1/2 = extracellular regulated kinase
GSK = glycogen synthase kinase
LAD = left anterior descending artery
LV = left ventricle
pGSK = phosphorylated glycogen synthase kinase
SDF = stromal cell-derived factor
TUNEL = terminal uridine nick-end labeling

these poor outcomes is that reperfusion of ischemic myocardium causes cardiomyocyte death and microvascular damage through a process referred to as myocardial ischemia-reperfusion injury (3). These data suggest that a major scientific problem is the need for novel approaches to limit myocardial damage and promote myocardial salvage due to reperfusion injury.

We previously reported that activating an extracorporeal centrifugal pump and delaying reperfusion limits infarct size in a swine model of AMI (4). Yet, the mechanisms governing myocardial salvage in the setting of acute mechanical left ventricle (LV) unloading remain poorly understood. Prior studies examining the effect of intentional exposure to brief periods of ischemia and reperfusion before the onset of coronary occlusion, known as ischemic pre-conditioning, have reported activation of myocardial salvage pathways including extracellular regulated kinase (Erk)-1/2 and the serine/threonine kinase, Akt (5). More recently, the chemokine, stromal cell-derived factor (SDF)-1α and its cognate receptor, CXCR4, have been implicated in ischemic pre-conditioning-mediated myocardial salvage by activating Erk-1/2 and Akt and inhibiting glycogen synthase kinase (GSK)-3β (6,7). No studies have examined whether mechanically “conditioning” the myocardium by first reducing LV wall stress using a circulatory support device and delaying coronary reperfusion impacts these protective signaling pathways in AMI.

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In this study, we explored the central hypothesis that first mechanically reducing LV wall stress using a novel intracorporeal axial flow catheter while extending the delay to coronary reperfusion limits infarct size and activates a myocardial protection program within the infarct zone in the setting of AMI.

METHODS

EXPERIMENTAL PROTOCOL OF MYOCARDIAL INFARCTION AND MECHANICAL CIRCULATORY SUPPORT. Studies were conducted in 14 adult, male Yorkshire swine weighing 63 ± 8 kg. The study protocol was approved by the Institutional Animal Care and Use Committee at Tufts Medical Center. All experiments were performed according to the committee’s guidelines. Animals were pre-medicated with Telazol (Zoetis, Florham Park, New Jersey) (0.8 ml/kg, intramuscular). General anesthesia was induced and maintained with isoflurane (1% to 2%). All animals were intubated and mechanically ventilated (Harvard Apparatus Inc., Holliston, Massachusetts) with room air and supplemented oxygen to maintain physiologic pH and oxygen saturation. Surface electrocardiography leads, an oesogastric tube, peripheral 18-G venous catheters, and a rectal thermistor were placed in all animals. Heating pads were used as needed to maintain a core body temperature ≥99°F. Vascular access sheaths were then deployed into the right internal jugular vein (10-F), left carotid artery (7-F), and both femoral arteries (7-F) and veins (10-F). A pulmonary artery catheter was deployed via the right internal jugular vein. Unfractionated heparin boluses with a goal activated clotting time of 300 to 400 s, continuous lidocaine infusion (1 mg/kg), and noradrenaline (0.16 µg/min) were initiated in all animals. A 6-F Judkins right coronary catheter (Boston Scientific, Marlborough, Massachusetts) engaged the left coronary artery via the right femoral artery, and baseline angiograms were recorded. A 180-cm guidewire was delivered into the distal LAD and a 3.0 × 8-mm metal stent (Boston Scientific) deployed in the mid-LAD after the first diagonal branch with angiographic confirmation of LAD occlusion. Coronary angiography performed immediately after reperfusion and again after the end of the study protocol confirmed patency of the LAD. Animals were humanely killed with pentobarbital and phenytoin after 120 min of reperfusion.

To explore the impact of ischemia-reperfusion injury, 14 adult male swine were subjected to 90 min of mid-LAD occlusion. Of the 14 animals in which myocardial infarction (MI) was induced, 4 animals died within 60 min of LAD occlusion due to refractory ventricular fibrillation. The surviving 10 animals were randomly assigned to either primary reperfusion (1° Reperfusion; n = 5), or the primary unloading (1° Unloading; n = 5) groups (Figure 1A). In the 1° Reperfusion group, 90 min of LAD occlusion was followed directly by 120 min of reperfusion without mechanical support, at which time the animals were humanely killed and LV samples obtained. In the 1° Unloading group, 90 min of LAD occlusion was followed by deployment and activation of a novel intracorporeal axial flow catheter (Impella CP, Abiomed Inc., Danvers, Massachusetts) as described (8). In the 1° Unloading group, the axial flow catheter was deployed into the LV via a 14-F sheath in the left femoral artery and maintained at maximal support (44,000 rotations/ min). Coronary reperfusion was then delayed for an additional 60 min in this group (150 min of LAD
occlusion total). After reperfusion, the mechanical pump remained active throughout the 120 min of reperfusion, followed by humane killing and tissue harvesting as in the 1/C14 Reperfusion group. Finally, 3 sham-operated animals were intubated, anesthetized, and mechanically ventilated without MI or mechanical unloading. LV tissue samples obtained from sham controls were used for tissue analysis.

PHYSIOLOGICAL CHARACTERIZATION IN VIVO. Changes in LV pressure and volume were assessed using a 5-F conductance catheter system (Sigma-M, CD Leycom, Zoetermeer, the Netherlands) deployed via the left carotid into the LV (Figure 1C). Ventricular pressure and volume were measured at baseline, after 90 min of LAD occlusion, and after 120 min of reperfusion using a solid-state pressure transducer and dual-field excitation mode respectively as previously described (4,9,10). Stroke volume is calculated as the difference in conductance volumes at +dP/dt\text{max} and -dP/dt\text{min}. An estimated end-systolic pressure volume relationship was calculated as peak LV end-systolic pressure divided by stroke volume for a single cardiac cycle. LV stroke work was calculated as the product of peak LV peak systolic pressure and stroke volume. To evaluate LV wall stress, 3-dimensional echocardiography was performed using the Artida System (Toshiba Medical Systems, Tustin, California). LV peak wall stress, LV mean wall stress, and LV systolic wall tension were also calculated as previously described (11-13).
QUANTIFICATION OF SDF-1α AND CXCR4 LEVELS.

Total protein was extracted from tissue homogenates isolated as previously described (14). SDF-1α protein levels were quantified in LV tissue isolated from sham-operated animals and infarct zones using Western blot and an enzyme-linked immunosorbent assay. Circulating serum levels of SDF-1α were quantified by enzyme-linked immunosorbent assay (R&D Systems, Inc., Minneapolis, Minnesota). CXCR4 levels in LV tissue isolated from sham-operated animals and infarct zones were quantified by Western blot (Abcam, Cambridge, United Kingdom). Immunoblot analysis was then performed as previously described (4,14).

QUANTIFICATION OF KINASES ASSOCIATED WITH MYOCARDIAL SALVAGE.

Immunoblot analysis was performed using antibodies against porcine total ERK (Cell Signaling, Danvers, Massachusetts), phosphorylated ERK-1/2 (Cell Signaling), total Akt (Cell Signaling), phosphorylated Akt (Cell Signaling), total GSK-3β (Cell Signaling), p-GSK-3β (Cell Signaling), total Stat-3 (Cell Signaling), pStat-3 (Cell Signaling), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Millipore, Darmstadt, Germany). Expression of phosphorylated protein levels was normalized to both total protein levels and GAPDH. Hydrogen peroxide levels were measured within the infarct zone as a marker of oxidative stress using an Amplex Red assay kit (Life Technologies, Carlsbad, California).

QUANTIFICATION OF APOPTOTIC SIGNALING PATHWAYS.

Immunoblot analysis was performed using antibodies against porcine B-cell lymphoma (BCL)-2 (Cell Signaling), BAX (Cell Signaling), BCL-XL (Cell Signaling), and GAPDH. Expression of apoptosis regulatory protein levels were normalized to both total protein levels and GAPDH. Terminal uridine nick-end labeling (TUNEL) staining was performed using 10-micron-thick sections fixed in 4% paraformaldehyde/phosphate-buffered saline for 20 min. Slides were permeabilized on ice with 0.1% Triton X-100 in 0.1% sodium citrate, and sections were labeled in the dark at 37°C for 60 min. Slides were rinsed with phosphate-buffered saline and nuclei were labeled with ProLong Gold anti-fade with DAPI (Life Technologies). Images were acquired using a Nikon Eclipse E800 fluorescent microscope and Openlab version 5 software (Perkin Elmer, Waltham, Massachusetts). TUNEL-positive cells were counted at 10× magnification by an investigator blinded to experimental group and expressed as a percentage of all nuclei.

DETERMINATION OF MI SIZE.

Upon completion of the study protocol, methylene blue was used to delineate the area at risk followed by sectioning of the LV. Biopsy specimens were obtained from the anteroapical LV distal to the site of stent deployment (infarct zone) and from the posterobasal wall (noninfarct zone) for molecular analysis and LV slices then incubated in 1% triphenyltetrazolium chloride as previously described. Blood samples obtained during the study protocol were centrifuged at 5,000 rotations/min for 15 min for isolation of serum and plasma, which were then stored at −80°C. Creatine kinase myocardial band (CK-Mb) levels were measured by Ani Lytics (Gaithersburg, Maryland).

STATISTICAL ANALYSIS.

Results are presented as mean ± SD. D’Agostino and Pearson normality testing confirmed a normal distribution for all comparisons. All data were analyzed by 2-way repeated measures analysis of variance on ranks followed by a Holm-Sidak comparison if warranted. Simple linear regression analysis was used to evaluate for a correlation between 2 parameters. All statistical analyses were performed with SigmaStat version 3.1 (Systat Software, Inc., San Jose, California). An α-level of p < 0.05 was considered to indicate a significant effect or between-group difference.

RESULTS

MECHANICAL UNLOADING IN AMI PROMOTES HEMODYNAMIC STABILITY AND REDUCES LV WALL STRESS.

The hemodynamic effects of ischemia-reperfusion injury with and without mechanical unloading are shown in Online Table 1. Compared with baseline values, 90 min of LAD occlusion increased pulmonary artery occlusion pressure, but did not change mean arterial pressure, dP/dt maximum, LV stroke volume, LV systolic pressure, or LV end-diastolic pressure in either the 1st Reperfusion or the 1st Unloading groups (Online Table 1). After 120 min of 1st Reperfusion, LV end-systolic pressure was decreased and pulmonary artery occlusion pressure remained increased compared with baseline values. In the 1st Unloading group, activation of the axial flow pump generated 2.9 ± 0.3 l/min of flow. After 120 min of reperfusion with the pump active, 1st Unloading decreased LV end-diastolic pressure, pulmonary artery occlusion pressure, and LV systolic pressure compared with baseline values. Compared with 1st Reperfusion, 1st Unloading reduced LV stroke volume, stroke work, systolic pressure, end-diastolic pressure, and end-diastolic volume, whereas mean arterial pressure and estimated end-systolic pressure volume relationship were not changed after 120 min of reperfusion. In addition, 1st Unloading significantly reduced LV mean wall stress.
Figure 2: Primary Left Ventricular Unloading Increases Stromal Derived Factor-1α and CXCR4 Levels

(A) Left ventricular (LV) tissue protein levels by quantitative enzyme-linked immunosorbent assay of stromal derived factor-1α (SDF-1α) from sham-operated controls and infarct zones after primary reperfusion (1st Reperfusion) or primary unloading (1st Unloading). (B) LV tissue protein expression of SDF-1α from sham-operated controls and infarct zones after 1st Reperfusion or 1st Unloading. (C) Circulating levels of SDF-1α before and after 1st Reperfusion or 1st Unloading. (D) LV tissue protein expression of CXCR4 from sham-operated controls and infarct zones after 1st Reperfusion or 1st Unloading. Quantification of western blots for protein levels normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). *p < 0.05 versus Sham; †p < 0.05 versus 1st Reperfusion; ‡p < 0.05 versus Baseline.

(45 ± 10 dynes/cm² vs. 21 ± 5 dynes/cm², 1st Reperfusion vs 1st Unloading; p = 0.006) and left ventricular peak wall stress (66 ± 20 vs. 30 ± 6 × 10² dynes/cm², 1st Reperfusion vs 1st Unloading; p = 0.02) with a trend toward decreased LV systolic wall tension (1,131 ± 190 dynes/cm vs. 863 ± 199 dynes/cm, 1st Reperfusion vs 1st Unloading; p = 0.05) after 120 min of reperfusion (Figures 1B and 1C). These findings support the finding that, despite identical degrees of hemodynamic compromise due to LAD occlusion, activation of an axial flow catheter in the 1st Unloading group decreased LV wall stress and stroke work, while supporting mean arterial pressure.

MECHANICAL UNLOADING INCREASES SDF-1α AND CXCR4 EXPRESSION IN THE INFARCT ZONE. To begin exploring whether mechanical unloading promotes cardioprotective signaling in AMI, we first quantified LV tissue levels of SDF-1α and its cognate receptor, CXCR4. Compared with sham controls, 1st Reperfusion decreased protein levels of SDF-1α and did not change CXCR4 levels in LV tissue from the infarct zone (Figure 2). In contrast, 1st Unloading increased levels of SDF-1α and CXCR4 in the infarct zone. In addition, 1st Unloading increased circulating serum levels of SDF-1α compared with the 1st Reperfusion group. Levels of SDF-1α directly correlated with reduced left ventricular peak wall stress (R = 0.8; p < 0.01, for both circulating and myocardial SDF-1α levels). Compared with sham controls, levels of SDF-1α were not changed in the noninfarct and peri-infarct zones from both groups (data not shown). These findings suggest that...
Unloading may mediate cardioprotection by up-regulating SDF-1α and CXCR4 expression within the infarct zone.

MECHANICAL UNLOADING PROMOTES CARDIO-PROTECTIVE SIGNALING. We next explored whether 1st Unloading increased downstream signaling associated with SDF-1α/CXCR4 activity in AMI. Compared with sham controls, 1st Reperfusion decreased levels of both phos-ERK-1/2 and phos-Akt and increased levels of phos-Stat3. Compared with sham controls or 1st Reperfusion, 1st Unloading increased phos-ERK-1/2, phos-Akt, and phos-GSK-3β levels without affecting phos-Stat3 levels in LV tissue from the infarct zone (Figures 3A to 3E). Next, we observed increased accumulation of hydrogen peroxide as a marker of oxidative stress within the infarct zone after 1st Reperfusion, not 1st Unloading (Online Figure 1). Compared with sham controls, levels of phos–ERK-1/2, phos-Akt, phos–GSK-3β, and phos-Stat3 were not significantly changed in the noninfarct zones from both groups (data not shown). These findings suggest that 1st Unloading activates downstream signaling pathways that protect against reperfusion injury during AMI.

MECHANICAL UNLOADING ATTENUATES MYOCARDIAL APOPTOSIS. To further explore whether 1st Unloading limits cell death, we quantified levels of BCL-2-associated proteins known to regulate mitochondrial permeability and cardiomyocyte apoptosis. Compared with sham controls, 1st Reperfusion increased
levels of BCL-2, BAX, and BCL-XL, but decreased the BCL-2/BAX ratio. Compared with sham controls, 1° Unloading also increased levels of BCL-2, BAX, and BCL-XL, but preserved the BCL-2/BAX ratio. Compared with 1° Reperfusion, 1° Unloading increased levels of BCL-2, BCL-XL and the BCL-2/BAX ratio (Figures 3F to 3J). In addition, 1° Unloading reduced TUNEL-positive staining for deoxyribonucleic acid fragmentation in LV tissue from the infarct zone compared with 1° Reperfusion (Online Figure 1). These findings suggest that 1° Unloading before reperfusion promotes antiapoptotic signaling and limits apoptosis within the infarct zone during AMI.

**MECHANICAL UNLOADING AND DELAYING CORONARY REPERFUSION REDUCES MYOCARDIAL INFARCT SIZE.**

We next explored whether 1° Unloading affects myocardial infarct size. Compared with 1° Reperfusion alone, 1° Unloading reduced the percent of infarcted LV myocardium normalized to the area at risk by 43% (74 ± 11% vs. 42 ± 8%, 1° Reperfusion vs. 1° Unloading; p = 0.005) (Figure 4). No difference in the area at risk quantified as a percent of total myocardium was observed between the 1° Reperfusion and the 1° Unloading groups (91 ± 13% vs. 46 ± 15%; p = 0.57). In both groups, LAD occlusion led to a similar increase in CK-Mb values (7 ± 2% vs. 7 ± 3%, 1° Reperfusion vs. 1° Unloading; p = 0.91) after 90 min of ischemia. In contrast, after 120 min of reperfusion, 1° Unloading reduced CK-Mb values (8.7 ± 0.7% vs. 5.1 ± 1.2%, 1° Reperfusion vs. 1° Unloading; p = 0.01) (Figure 4). Among all animals, a direct correlation was observed between peak LV wall stress and percent MI (R = 0.80; p = 0.01). An inverse correlation was observed between myocardial levels of SDF-1α within the infarct zone and percent MI (R = 0.89; p < 0.01).

**DISCUSSION**

The number of patients with heart failure is projected to exceed 8 million individuals by 2030 with associated health care costs of approximately $70 billion annually (15). A history of AMI is associated with a 5-fold increase in the incidence of heart failure over a 5-year period (16). Independent risk factors for the development of systolic heart failure 90 days after an index AMI include infarct size, the extent of microvascular obstruction, and wall tension (17). Although major system-wide improvements have led to more rapid primary reperfusion therapy to replenish myocardial oxygen supply during an AMI, the number of patients surviving to hospital discharge and subsequently developing heart failure continues to grow. There exists a need for improved methods to prevent the onset of heart failure by reducing myocardial damage sustained during an AMI (Figure 5).

The findings of this study demonstrate for the first time that, compared with the well-established strategy of rapid primary reperfusion, initially reducing LV wall stress and myocardial oxygen demand with an axial flow catheter (primary LV

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**FIGURE 4** Primary LV Unloading Reduces Infarct Size in Acute Myocardial Infarction

(A) Quantification of myocardial infarct size as percent of left ventricular (LV) area at risk (AAR) after primary reperfusion (1° Reperfusion) or primary unloading (1° Unloading). Regression plots of infarct size as a percent of the LV AAR (A) versus (B) LV mean wall stress ($r^2 = 0.68$; $R = 0.80$; $p = 0.01$) and (C) myocardial levels of stromal derived factor (SDF)-1α ($r^2 = 0.75$; $R = -0.89$; $p < 0.01$) after 1° Reperfusion or 1° Unloading. *p < 0.05 versus 1° Reperfusion; n = 5/group.
unloading), and then delaying reperfusion for 1 h reduces myocardial infarct size. This study also introduces that the beneficial effects of acute circulatory support may not simply stem from a passive decrease in myocardial oxygen demand, but rather may involve activation of a “myocardial protection program” that up-regulates SDF-1α/CXCR4 expression, increases cardioprotective signaling, reduces apoptosis, and hence limits myocardial damage in AMI. This concept of “mechanically conditioning” the myocardium before reperfusion may be a viable approach to decrease the number of patients who develop heart failure due to myocardial damage sustained during an AMI treated with primary reperfusion. Future clinical studies are needed to examine the usefulness of primary unloading versus primary reperfusion in AMI.

By first initiating mechanical support to reduce native LV work while sustaining systemic perfusion, we postulated that the process of myocardial injury would be slowed and that delaying coronary reperfusion would further limit the potentially deleterious effects of reperfusion injury. To explore this hypothesis, we used a novel axial flow catheter that pumps blood directly from the LV to ascending aorta and observed a significant reduction in LV wall stress and stroke work comparable to prior reports demonstrating that axial flow catheters reduce LV wall stress and myocardial oxygen demand (18,19). With confirmation that this pump reduced LV wall stress, we explored whether a prolonged period of mechanical unloading and a delay to reperfusion improves myocardial protection or exacerbates cardiomyocyte death by prolonging myocardial ischemia. We studied this question by extending the delay to reperfusion for 60 min in the primary unloading group. Despite a total of 150 min of LAD occlusion in the 1st Unloading group compared with 90 min in the 1st Reperfusion group, we observed a 43% reduction in infarct size normalized to the area at risk and a 51% reduction in infarct size normalized to the total LV cross-sectional area by employing a strategy of first unloading the LV, then waiting to reperfuse the coronary artery. This observation of reduced infarct size with primary LV unloading is supported by a recent analysis of the USPella registry, which reported improved survival to hospital discharge with implantation of an Impella 2.5LP device immediately before coronary revascularization in patients presenting with an AMI and cardiogenic shock (20).

In this analysis, mean door-to-balloon time was delayed by 60 min in patients receiving the Impella device before versus after primary reperfusion. Collectively, these data suggest that first mechanically reducing LV wall stress may represent a new approach to improve clinical outcomes in patients presenting with an AMI.

The mechanisms governing myocardial salvage due to primary unloading in AMI remain poorly understood. We postulated that primary LV unloading activates cardioprotective signaling pathways that have been implicated in ischemic pre-conditioning, which include activation of phosphor-inositol-3 kinase, Akt, and Erk, leading to reduced formation of the mitochondrial permeability transition pore (5,21,22). More recent evidence supports that increased expression of SDF-1α promotes Akt-mediated phosphorylation and inactivation of GSK-3β, which also limits mitochondrial permeability transition pore formation and myocardial infarct size (6,7,23). Whether “mechanically conditioning” the myocardium before reperfusion activates these multiple signaling pathways has not been reported previously. Several prior studies have established an important role for GSK-3β as a downstream target of ERK and Akt.
signaling and further shown that GSK-3β regulates antiapoptotic signaling effectors such as BCL-2 and BCL-XL (23). We now identify for the first time that activation of an axial flow catheter regulates SDF1/CXCR4 levels, GSK-3β phosphorylation, and apoptotic signaling within the infarct zone in AMI. These observations advance the science of circulatory support because they establish a potential biological effect of acute LV unloading on signaling within the infarct zone, not previously reported by others.

**STUDY LIMITATIONS.** Limitations of the present study include the small number of animals studied due in part to a 30% mortality rate among animals subjected to LAD occlusion and the prohibitive costs associated with high numbers in large animal studies. Additionally, the long-term effect of primary unloading on late ventricular remodeling in AMI is not known. We have shown that short-term unloading activates cardioprotective signaling and acutely reduces infarct size, but future studies are needed to address the short- and long-term effects on cardiac structure and function after pump removal using primary unloading as a therapeutic approach to limit myocardial infarct scar, reduce LV volumes, and improve LV ejection fraction weeks after an AMI. Finally, we have now introduced that mechanical unloading promotes expression of SDF-1α and CXCR4 along with phosphorylation of multiple downstream kinases. Whether SDF-1α/CXCR4 is associated causally with reduced infarct size and whether this signaling pathway is the dominant mechanism of infarct salvage in primary unloading as opposed to decreased myocardial demand alone requires further study. Furthermore, alternate mechanisms should be explored, including gain-of-function and loss-of-function studies to further clarify the role of cardioprotective signaling in the setting of primary LV unloading with acute circulatory support pumps.

**REFERENCES**


KEY WORDS myocardial infarction, reperfusion injury, ventricular assist devices

APPENDIX For a supplemental figure and tables, please see the online version of this article.