

BASIC SCIENCE RESEARCH

ORIGINAL ARTICLES

Reduction of Myocardial Ischemia-Reperfusion Injury by Mechanical Tissue Resuscitation Using Sub-Atmospheric Pressure

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ABSTRACT ***Background:*** **Reperfusion-induced injury after myocardial infarction is associated with a well-defined sequence of early and late cardiomyocyte death. Most present attempts to ameliorate this sequence focus on a single facet of the complex process in an attempt to salvage cardiomyocytes. We examined, as proof of concept, the effects of mechanical tissue resuscitation (MTR) with controlled negative pressure on myocardial injury following acute myocardial infarction.** ***Methods:*** **Anesthetized swine were subjected to 75 minutes of left coronary artery occlusion and three hours of reperfusion. Animals were assigned to one of three groups: (A) untreated control; treatment of involved myocardium for 180 minutes of MTR with (B) −50 mmHg, or (C) −125 mmHg.** ***Results:*** **All three groups were subjected to equivalent ischemic stress. Treatment of the ischemic area with MTR for 180 minutes significantly ($p < 0.001$) reduced infarct size (area of necrosis/area at risk) in both treatment groups compared to control: 9.3 ± 1.8% (−50 mmHg) and 11.9 ± 1.2% (−125 mmHg) versus 26.4 ± 2.1% (control). Total area of cell death was reduced by 65% with −50 mmHg treatment and 55% in the −125 mmHg group.** ***Conclusions:*** **Treatment of ischemic myocardium with MTR, for a controlled period of time during reperfusion, successfully reduced the extent of myocardial death after acute myocardial infarction. These data provide evidence that MTR using subatmospheric pressure may be a simple, efficacious, nonpharmacological, mechanical strategy for decreasing cardiomyocyte death following myocardial infarction, which can be delivered in the operating room.** doi: 10.1111/j.1540-8191.2009.00972.x *(J Card Surg 2010;25:247-252)*

Cardiomyocyte death due to ischemia-reperfusion follows a tri-phasic pattern. Acutely, cell death is related to the initial degree of ischemia, the timing and subsequent inflammatory response to reperfusion. This is followed in a few days by a wave of apoptotic death (second phase) in the periinfarct tissue, and even later by a wave of generalized autophagy (third phase).

Many mediators acting through multiple mechanisms have been proposed to be involved in causing reperfusion injury. These include accumulation of neutrophils,[1] the production of oxygen radicals,[2] endothelial dysfunction,[3] and the activation of complement.[4] Research to develop interventional therapy to decrease the ultimate size of myocardial death has focused primarily on chemotherapeutic inhibitors to specific portions of the reperfusion injury cascade. While significant promise has been generated with animal studies, clinical trials have demonstrated few significant benefits.[5] We speculate that this lack of success is due to the fact that these therapeutics act selectively on a single point within a cascade of events, or on a single aspect of a very complex, multifaceted process.

Application of a controlled vacuum (negative pressure wound therapy) has been shown to remove soluble inflammatory mediators such as TNF-alpha, decrease tissue edema, increase local tissue blood flow, and increase cell viability.[6,7] Application of a brief, precise mechanical intervention to control local factors (such as inflammatory cytokines and mediators), edema, and decreased blood flow within the region that surrounds the area of "irreversible" death would allow for resuscitation of these compromised tissues and decrease the ultimate magnitude of tissue death. This therapy could be delivered as an adjunct to urgent coronary bypass surgery (CABG) and, perhaps, even as a part of other forms of reperfusion therapy. Such a mechanical tissue resuscitation (MTR) was postulated in this "proof-of-concept" study to be of benefit in reducing the extent of myocardial death after acute myocardial infarction.

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MATERIALS AND METHODS

Surgical preparation

Studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals (1996) published by the National Institutes of Health under a protocol approved by the Wake Forest University Health Sciences Animal Care and Use Committee.

Twenty-two female swine (25 kg) were procured and allowed to acclimate to the housing conditions. The animals were divided into three groups including: (A) an untreated control group, (B) a group that was treated with continuous subatmospheric pressure of 50 mmHg (MTR 50) applied directly to the area at risk, and (C) a group treated with continuous subatmospheric pressure of 125 mmHg (MTR 125).

The animals were pretreated with atropine (0.05 mg/kg) and sedated with ketamine (22 mg/kg), xylazine (1 mg/kg), and acepromazine (1.1 mg/kg) by intramuscular injection. Animals were intubated, and surgical anesthesia was established and maintained with isoflurane. Ventilatory parameters were adjusted to maintain physiologic oxygenation and carbon dioxide levels. Femoral arterial and venous access was obtained for hemodynamic monitoring and fluid administration. A median sternotomy was performed, the pericardium opened and the atrium cannulated for blood flow analysis. Prolene suture ligatures were placed around 2 to 3 diagonal branches of the left anterior descending (LAD) coronary artery so as to create an anterior, free-wall infarct. Amiodarone (5 mg/kg) and lidocaine (2.2 mg/kg) were given by intravenous injection to control arrhythmias. Heparin (400 U/kg/90 min) was also administered to prevent clot formation on the catheters.

Ischemia-reperfusion protocol

Ischemia was induced by tightening snares around the ligatures, occluding the branches of the previously described portion of LAD distribution for 75 minutes. This created an anterior, free-wall area-at-risk (AAR) with a reproducible infarct size while limiting mortality. Following the ischemic period, the snares were released and ischemic tissues were allowed a reperfusion period of three hours. Hemodynamic and blood gas analysis were performed at baseline, end of ischemia and at 30, 60, 120, and 180 minutes of reperfusion (R30, R60, R120, and R180). Myocardial blood flow using neutron activated microspheres (BioPAL, Worcester, MA, USA) was determined at baseline, end of ischemia, 30 minutes of reperfusion, and at the end of the experiment (180 minutes of reperfusion). For animals in groups B and C, MTR was accomplished by placing an operational open-cell polyvinyl alcohol (PVA) matrix (V.A.C.® WhiteFoam, KCI) cut to conform to the size of the area at risk, directly on the area at risk. The distal end of an evacuation tube was attached to the PVA matrix, with the proximal end attached to a collection vessel, then to a microprocessor controlled vacuum pump (V.A.C., KCI). The matrix was covered with an oversized, pliable, liquid-impermeable sheet (Allo-derm, LifeCell Corp., Branchburg, NJ, USA). The edges of the cover extended beyond the matrix and onto the epicardium. The edges of the covering were further sealed to the epicardium using fibrin glue (Evicel, Johnson & Johnson, Somerville, NJ, USA). Controlled subatmospheric pressure at either 50 mmHg or 125 mmHg was continuously applied to the myocardium through the open cell matrix via the microprocessor controlled vacuum pump. This pump was able to establish and maintain controlled continuous subatmospheric pressure for the duration of reperfusion.

Determination of infarct size

Infarct size was determined as has been previously described.[3] At the end of the reperfusion period, the snares were retightened and the AAR was determined by negative staining using patent blue, in which the perfused areas were stained blue and the ischemic area remained unstained. The heart was excised and rinsed in saline. The atria, great vessels, valvular structures, and right ventricular free wall were dissected from the left ventricle (LV). The LV was sectioned into 3 mm to 4 mm thick sections perpendicular to the long axis of the heart. The stained tissue (blue, non-ischemic tissue, NI) was dissected free from the nonstained (ischemic) tissue and both tissues were weighed. The AAR was determined by gravimetric analysis and expressed as a percentage of the entire left ventricle. The nonstained AAR was then sliced into small segments (to increase surface area for staining) and stained in a 1% solution of triphenyltetrazolium chloride (TTC, Sigma Chemical) for five minutes to differentiate the viable tissue (TTC positive, red; ISC) from the necrotic tissue (pale, NEC). The TTC positive tissue (ischemic, nonnecrotic tissue: ISC) was dissected apart from the TTC-negative tissue (necrotic, pale; NEC). Similar to the AAR, the area of necrosis (AN) was determined gravimetrically and expressed as a percentage of the area at risk. Samples used for the analysis of regional myocardial blood flow were taken following the infarct sizing procedure.

Determination of regional myocardial blood flow

In order to determine regional myocardial blood flow and confirm ischemia, 15 micron neutron-activated microspheres (BioPAL, Inc, Worcester, MA, USA) were injected into the left atrium at baseline, end of ischemia, 30 minutes into reperfusion, and at 180 minutes of reperfusion (end of the experiment). A reference sample of arterial blood was simultaneously drawn from the femoral artery at a rate of 7 mL/min for ninety seconds. Following infarct sizing procedures, tissue samples (mixed epicardial and endocardial, nonseptal) from the nonischemic, ischemic nonnecrotic, and necrotic areas were collected and sent to the manufacturer for blood flow analysis (BioPAL, Inc., Worchester, MA). Blood flow was calculated as $(F_R \times CPM_T)/CPM_R)$/ tissue weight in grams, where F_R = reference sample flow rate (7 mL/min), CPM_T = counts per minute in tissue samples, and CPM_R = counts per

TABLE 1
Hemodynamic Parameters Measured Throughout Experiment

				Reperfusion			
	Group	**Baseline**	**Ischemia**	**30**	**60**	**120**	**180**
MAP (mmHg)	Control	70 ± 7	73 ± 7	65 ± 7	73 ± 6	68 ± 6	66 ± 5
	MTR 50	58 ± 3*	53 ± 1*	54 ± 1	55 ± 2*	52 ± 2*	50 ± 3*
	MTR 125	56 ± 2*	55 ± 2*	55 ± 2	52 ± 2*	53 ± 2*	51 ± 3*
Heart Rate (beats/min)	Control	90 ± 4	76 ± 3	72 ± 2	71 ± 3	75 ± 4	73 ± 4
	MTR 50	62 ± 5	67 ± 8	68 ± 9	66 ± 10	66 ± 11	71 ± 11
	MTR 125	74 ± 5	72 ± 6	70 ± 6	70 ± 7	74 ± 7	84 ± 9

Hemodynamic parameters were recorded from a femoral artery catheter at the designated time points. Data are presented as Mean ± SEM. *$p < 0.05$ versus Control within a time period.

minute in the reference blood sample. Blood flow is reported as mL/min/gram tissue.

Exclusion criteria and statistics

Animals were excluded from analysis if: (1) the AAR/LV was <8%; (2) blood flow during the ischemic period was greater than 0.15 mL/min/g tissue; or (3) an incident of ventricular fibrillation occurred that was nonresponsive to cardioversion. Group differences in infarct size and AAR were compared using one-way analysis of variance followed by Tukey's method for multiple comparisons (SigmaStat). For blood pressure and hemodynamic variables, a two-way repeated measures analysis of variance was performed with *post hoc* Holm-Sidak test for comparing group differences at individual time points (SigmaStat). All data are presented as mean ± standard error of the mean (SEM).

RESULTS

Exclusions

Fifteen of the 22 animals entered into the study were used for analysis. Of the seven animals excluded, four were excluded for intractable or prolonged ventricular fibrillation (three in control group, one in MTR 125). One animal was excluded for each of the following reasons: (a) unexplained protracted loss of blood pressure (control); (b) technical TTC staining malfunction (control); and (c) AARwas too small (MTR 50 mmHg). None of the animals were excluded as a result of complications occurring because of the application of the treatment modality.

Hemodynamics and blood flow

Mean arterial pressure and heart rate were monitored throughout the experiment. There were no significant differences in the heart rates between any of the groups at any time during the experiment (Table 1). The mean blood pressures in the two treated groups were lower than the control group, including at baseline (prior to ischemia and reperfusion). However, there were no time related changes in mean pressure indicating that baseline pressures were maintained throughout the time course, and that the lower pressures were not related to the treatment modalities.

Regional myocardial blood flow was determined in three regions of the myocardium for each group: the normally perfused, nonischemic zone (NI); the ischemic, nonnecrotic zone (ISC); and the ischemic, necrotic tissue (NEC). Analysis of blood flow reveals that both treated groups had similar baseline blood flows in all three regions. In the normally perfused NI zone, blood flow remained relatively constant throughout the experiment with no significant group or time related differences (Table 2). In the ischemic, nonnecrotic and ischemic, necrotic zones, ischemia was characterized by an equivalent and nearly completes loss of blood flow among all three groups. These zones also exhibited the normal reactive hyperemia (30 minutes after reperfusion), and blood flow that returned approximated baseline flow levels by the end of the three hour reperfusion time. There were no statistically significant group differences in either area (ISC and NEC) at any of the time points with blood flow equivalent

TABLE 2
Regional Myocardial Blood Flow (mL/min/g tissue)

	Control			MTR 50			MTR 125		
	NI	**Isc**	**Nec**	**NI**	**Isc**	**Nec**	**NI**	**Isc**	**Nec**
Baseline	0.60 ± 0.16	0.57 ± 0.09	0.56 ± 0.07	0.52 ± 0.04	0.61 ± 0.13	0.61 ± 0.06	0.43 ± 0.06	0.63 ± 0.18	0.49 ± 0.09
Ischemia	0.47 ± 0.21	0.06 ± 0.03	0.02 ± 0.00	0.48 ± 0.06	0.03 ± 0.01	0.05 ± 0.01	0.57 ± 0.16	0.07 ± 0.01	0.04 ± 0.01
R30	0.41 ± 0.04	1.39 ± 0.35	0.95 ± 0.16	0.57 ± 0.07	1.33 ± 0.22	1.46 ± 0.27	0.62 ± 0.27	0.92 ± 0.16	0.90 ± 0.16
R180	0.60 ± 0.17	1.10 ± 0.21	1.2 ± 0.33	0.65 ± 0.09	0.50 ± 0.11	0.73 ± 0.08	0.42 ± 0.04	0.39 ± 0.03	0.70 ± 0.06

Regional myocardial blood flow was determined in three regions of the heart: NI = non-ischemic left ventricle; ISC = ischemic, non-necrotic left ventricle; NEC = necrotic left ventricle throughout the experiment. R30 = 30 minutes of reperfusion; R180 = 180 minutes of reperfusion. Data are presented as mean ± SEM.

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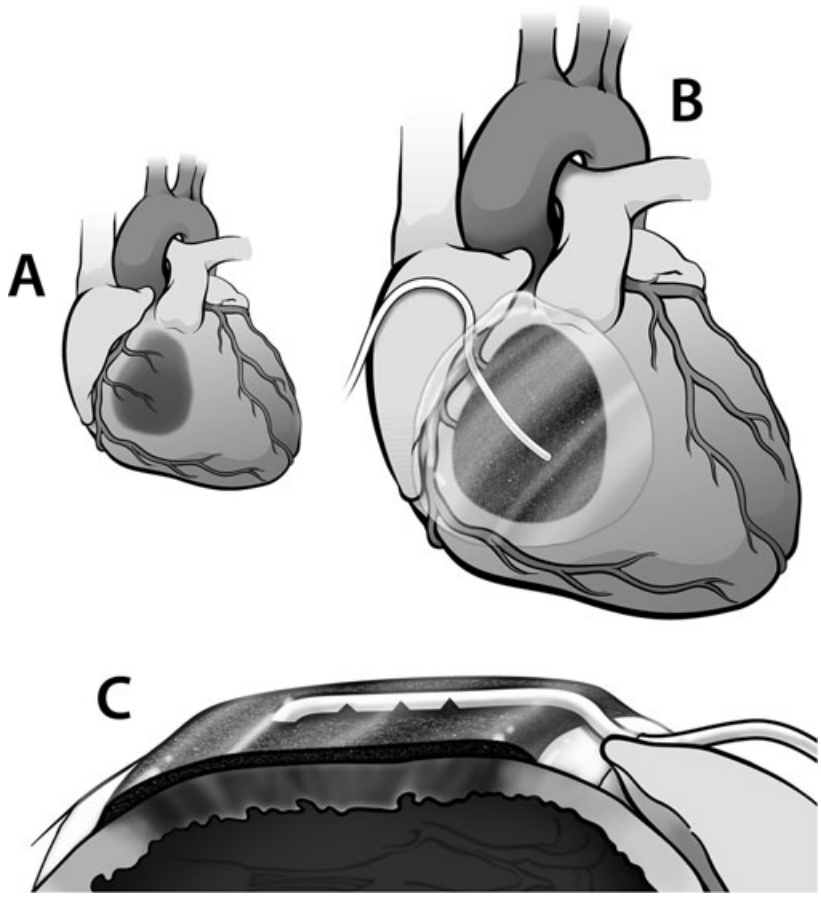

Figure 1. *Application of device Panel A. The ischemic area was made by ligation of the branches off the left anterior descending coronary artery to create a free wall ischemic defect. Panel B. Mechanical tissue resuscitation (MTR) was accomplished by placing the PVA sponge directly over the area at risk. The distal end of the evacuation tubing was connected to the sponge before the patch was covered with a pliable, gas-impermeable sheet material. The edges of this cover sheet extend beyond the sponge to aid in sealing the patch to the epicardial surface. Panel C shows a cross sectional view of the device with the foam sponge completely covering the ischemic area (shaded region of the myocardium) and the film extending beyond the border zones. The tubing has multiple holes to facilitate transfer of the negative pressure from the pump to the myocardium via the foam sponge.*

to or greater than baseline in all groups at the end of reperfusion.

Infarct size

As a measure of ischemia-reperfusion injury, the amount of NEC following 75 minutes of ischemia and three hours of reperfusion was assessed. All three groups of animals were subjected to equivalent ischemic stresses (Fig. 2A). The areas of ischemia (AAR/LV $\times$ 100%; AARas a percentage of the left ventricle) were not statistically different in the treatment groups (11.9 $\pm$ 1.4% and 11.8 $\pm$ 2.0% for treatment with 125 mmHg and 50 mmHg, respectively) compared to the control group (12.9 $\pm$ 1.2%). Infarct size, however, was significantly reduced in both treatment groups (Fig. 2B). Application of 50 mmHg subatmospheric pressure reduced infarct size by 65% [i.e., to 35% of control levels (9.3 $\pm$ 1.8% vs. 26.4 $\pm$ 2.1%; $p<0.001$)], while application of 125 mmHg subatmospheric pressure reduced infarct size by 55% (i.e., to 45% of control levels [11.9 $\pm$ 1.2 vs. 26.4 $\pm$ 2.1%; $p < 0.001$]).

CONCLUSIONS

Extensive laboratory and clinical studies have addressed the chronologic progression of events and the pathophysiology of myocardial infarction and reperfusion injury. In the first 24 hours following myocardial infarction, "irreversible" ischemic cellular necrosis occurs rapidly within the central area of infarction. This period is the clinically useful treatment window for reperfusion therapies whose aim is to rapidly restore blood flow in an attempt to minimize the extent of inflammation and the necrosis that it engenders. Approximately 24 hours after the initial ischemic injury, cardiomyocyte death begins to extend into the periinfarction zone. This results in waves of myocardial cell death through the periinfarct and border zones. It is thought that this phenomenon of progressive cell death, which persists for up to three weeks, is the result of an apoptotic state induced into myocardiocytes within the periinfarct tissues.[8,9] It has been suggested that reperfusion following revascularization procedures results in the generation of oxygen free radicals, which may trigger or contribute to this injury.[8,9] While there have been no clinically successful treatment

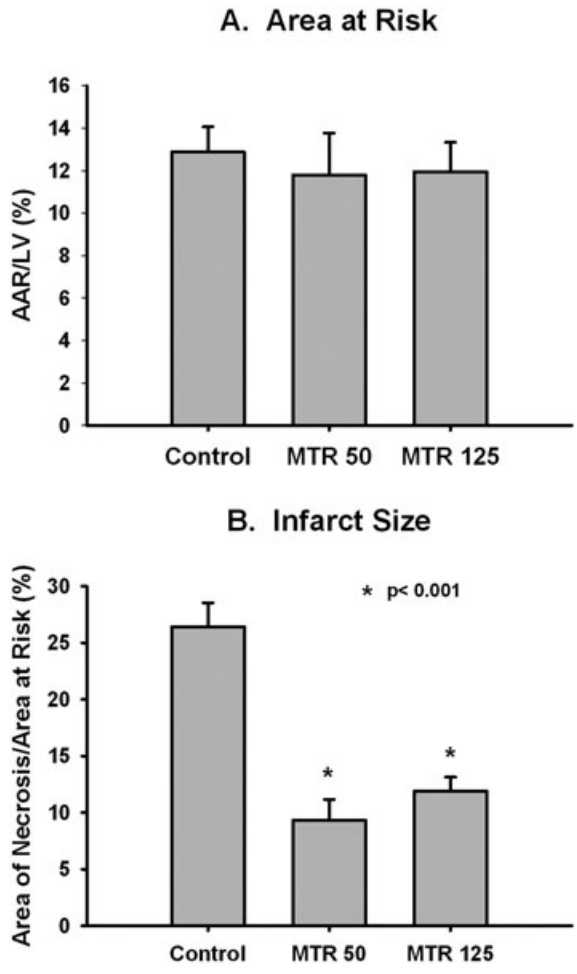

Figure 2. *Area at risk and infarct size. Panel A. Area at risk (AAR) expressed as a percentage of the entire left ventricle. There are no differences between the groups in ischemic zone. Panel B. Infarct size is expressed as area of necrosis as a percentage of AAR. Application of MTR at both 50 and 125 mmHg significantly reduce infarct size. Data are presented as Mean ± SEM. * $p < 0.001$ versus control.*

modalities for this stage of injury, interruption of the cascade which results in production of the chemical modulators that produce apoptosis, or removal of the modulators after they are produced, could potentially obviate this series of events. A third phase of injury follows over a period of months after an infarction with a further general loss of adjacent cardiomyocytes. This "autophagy" stage is thought to be secondary to tissue damage initiated during the initial infarction or in the period immediately postinfarction. It has been postulated that if myocardial cells could be prevented from dying secondary to apoptosis and thus interrupt the downward spiral of function, then the reactive autophagic response might also be ameliorated or prevented.[10]

We postulate that resuscitation of such severely compromised myocardial cells in the initial ischemic area provides the potential to preserve and restore myocardial function even within the area we presently characterize as the "irreversible zone of death" and also may decrease the degree of apoptotic death and autophagy. This study focused on the ability of MTR to decrease the magnitude of acute cell death in the ischemic area. Determination of the ability of MTR to decrease apoptotic death and autophagy are planned future studies.

Mechanical interventions have previously been shown to modulate cell and tissue response to ischemia-reperfusion injury. Both ischemic preconditioning and postconditioning have been shown to limit reperfusion injury in the experimental setting.[11-13] Preconditioning may occur naturally in humans secondary to brief ischemic episodes and the gradual occlusion prior to infarctions, but it is not currently possible to use preconditioning as a treatment modality. Postconditioning in humans has been studied in a small study with promising results showing an improvement of myocardial perfusion and a limitation of enzyme release.[14]

In this study, MTR was utilized, in which controlled subatmospheric pressure was applied directly to the involved areas of ischemic myocardium during reperfusion as a means to reduce the size of the ultimate injury. This proof of concept study used a relatively small, discrete area of ischemia that allowed for nearly complete treatment of the ischemic zone with the treatment device. The device was easily applied directly to the epicardial surface of the heart. Application of this MTR device had no discernable effects on myocardial function. Hemodynamically, the three groups maintained similar basal pressures at a relatively constant level throughout the experiment. Since neither direct measurements of myocardial function (i.e., pressure-volume, echocardiogram) or wall stress were employed during this study, it is not possible to determine if substantial differences in energy utilization or myocardial work existed between groups. The normal blood gas profiles suggest that blood pressure differences between the groups of animals did not cause important decreases in cardiac output with resultant acidosis. The observed blood pressures in all groups were within normal physiologic range for swine under isoflurane anesthesia.[15] A more thorough examination of these parameters will require further refined trials on larger infarctions, which are underway.

One potential mechanism for the observed protection is an increase of local blood flow induced on the ischemic-reperfused myocardium by the mechanical resuscitation device treatment. Lindstedt et al. demonstrated that epicardial blood flow is enhanced with low (−50 mmHg), but not high (125 mmHg) magnitudes of negative pressure.[16] In their model an open cell dressing (Granufoam, KCI, San Antonio, TX) was fixed in place between the cut edges of the sternum and, in contrast to our study, specifically did not contact the myocardium. Lindsted, et al. strongly advocate placing multiple nonadherent layers between the foam dressing and the myocardial tissues. Increased flow occurred in normal, ischemic, and ischemic-reperfused epicardium in their model.[17,18] This blood flow increase secondary to negative pressure was seen during hypothermia as well.[19] While this is an attractive explanation for the protective effects observed in the present study, there is as yet no direct evidence that the change

in microvascular blood flow occurs beyond the epicardial surface. Indeed, it is the subendocardium that is most at risk from ischemia-reperfusion. However, localized blood flow analysis was not performed in this study. For this preliminary study design, there was no attempt to quantify changes in localized blood flow between the endocardium and epicardium as a mechanism for the observed protection. Blood flow analysis used random transmural pieces of myocardium from the NI and ischemic zones as a means to verify lack of blood flow during the ischemic interval.

The conclusions reached from this proof of concept study are limited because of the relative simplicity of the study design. Measures of myocardial function such as pressure-volume analysis or echocardiography would provide important information regarding myocardial contractility and compliance. Additionally, histologic analysis of apoptosis and autophagy and measures of inflammatory mediators may provide insight into the mechanisms involved in the observed protective effects of negative pressure therapy.

In summary, this study demonstrates that intervention by application a MTR device in direct contact with the injured zone can protect the heart from ischemia-reperfusion injury and decrease the ultimate size of the infarct. This therapy was applied during reperfusion only, suggesting that the observed protection could be applied to a number of clinically important patient populations. For example, this technique would be a straight forward adjunct during surgical revascularization of acute myocardial infarctions and should not interfere with the placement or function of bypass grafts. In addition, this technique could potentially be adapted to be used in patients reperfused using percutaneous interventions by use of thoracoscopic application of the negative pressure patch. This nonpharmacological intervention does not target a single specific point in the cascades of deleterious events. Rather, the topical application of the MTR device is likely to act through multiple mechanisms and therefore could be potentially more effective than many of the pharmacologic interventions attempted previously. This therapeutic modality also has the potential to be used in concert with pharmacologic and nonpharmacologic therapies that could function in a potentially synergistic manner with both open- and closed-chest reperfusion strategies.

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REFERENCES

1. Jordan JE, Zhao ZQ, Vinten-Johansen J: The role of neutrophils in myocardial ischemia-reperfusion injury. Cardiovasc Res 1999;43:860-878.
2. Jordan JE, Thourani VH, Auchampach JA, et al: A_3 adenosine receptor activation attenuates neutrophil function and neutrophil-mediated reperfusion injury. Am J Physiol 1999;277:H1895-H1905.
3. Jordan JE, Zhao Z-Q, Sato H, et al: Adenosine A_2 receptor activation attenuates reperfusion injury by inhibiting neutrophil accumulation, superoxide generation and coronary endothelial adherence. J Pharmacol Exp Ther 1997;280:301-309.
4. Yellon DM, Hausenloy DJ: Myocardial reperfusion injury. N Engl J Med 2007;357:1121-1135.
5. Dirksen MY, Laarman GJ, Simoons ML, et al: Reperfusion injury in humans: A review of clinical trials on reperfusion injury inhibitory strategies. Cardiovasc Res 2007;74:343-355.
6. Morykwas MJ, Argenta L, Shelton-Brown EI, et al: Vacuum-assisted closure: A new method for wound control and treatment: Animal studies and basic foundation. Ann Plast Surg 1997;45:553-562.
7. Stechmiller JK, Kilpadi DV, Childress B, et al: Effect of vacuum-assisted closure therapy on the expression of cytokines and proteases in wound fluid of adults with pressure ulcers. Wound Repair Regen 2006;14:371-374.
8. Kajstura J, Cheng W, Reiss K, et al: Apoptotic and necrotic myocyte cell deaths are independent contribution variables of infarct size in rats. Lab Invest 1996;74:86-107.
9. Gottlieb RA, Burleson KO, Kloner RA, et al: Reperfusion injury induces apoptosis in rabbit cardiomyocytes. J Clin Invest 94:1621-1628.
10. Dorn G, Diwan A: The rational for cardiomyocyte resuscitation in myocardial salvage. J. Mol Med 2008;86:1085-1095.
11. Kloner RA, Jennings RB: Consequences of brief ischemia: Stunning, preconditioning, and their clinical implications Part 1. Circ 2001;104:2981-2989.
12. Kloner RA, Jennings RB: Consequences of brief ischemia: Stunning, preconditioning, and their clinical implications Part 2. Circ 2001;104:3158-3167.
13. Zhao ZQ, Corvera JS, Halkos ME, et al: Inhibition of myocaridal injury by ischemic postconditioning during reperfusion: Comparison with ischemic preconditioning. Am J Physiol Heart Circ Physiol 2003;285:H579-H588.
14. Staat P, Rioufol G, Piot C, et al: Postconditioning the human heart. Circ 2005;112:2143-2148.
15. Heerdt PM, Gandhi CD, Dickstein ML: Disparity of isoflurane effects on left and right ventricular afterload and hydraulic power generation in swine. Anesth Analg 1998;87:511-521.
16. Lindstedt S, Malmsjo M, Ingemansson R: No hypoperfusion is produced in the epicardium during application of myocardial topical negative pressure in a porcine model. J Cardiothorac Surg 2007;2:53.
17. Lindstedt S, Malmsjo M, Gesslein B, et al: Evaluation of continuous and intermittent myocardial topical negative pressure. J Cardiovasc Med (Hagerstown) 2008;9:813-819.
18. Lindstedt S, Malmsjo M, Ingemansson R. Blood flow changes in normal and ischemic myocardium during topically applied negative pressure. Ann Thorac Surg 2007;84:568-573.
19. Lindstedt S, Johansson M, Hlebowicz J, et al: Myocardial topical negative pressure increases blood flow in hypothermic, ischemic myocardium. Scand Cardiovasc J 2008;1-9.