

BASIC SCIENCE RESEARCH

ORIGINAL ARTICLE

Mechanical Tissue Resuscitation (MTR): A Nonpharmacological Approach to Treatment of Acute Myocardial Infarction

James E. Jordan, Ph.D.,* Beatriz D. Pereira, M.D.,* Magan R. Lane, B.S.,* Michael J. Morykwas, Ph.D.,† Maria McGee, Ph.D.,† and Louis C. Argenta, M.D.†

**Department of Cardiothoracic Surgery, Wake Forest School of Medicine, Winston-Salem, North Carolina; and †Department of Plastic & Reconstructive Surgery, Wake Forest School of Medicine, Winston-Salem, North Carolina*

ABSTRACT ***Background and Aim:*** **Myocardial ischemia-reperfusion injury is known to trigger an inflammatory response involving edema, apoptosis, and neutrophil activation/accumulation. Recently, mechanical tissue resuscitation (MTR) was described as a potent cardioprotective strategy for reduction of myocardial ischemia-reperfusion injury. Here, we further describe the protective actions of MTR and begin to define its therapeutic window.** ***Methods:*** **A left ventricular, free-wall ischemic area was created in anesthetized swine for 85 minutes and then reperfused for three hours. Animals were randomized to two groups: (1) untreated controls (Control) and (2) application of MTR that was delayed 90 minutes after the initiation of reperfusion (D90). Hemodynamics and regional myocardial blood flow were assessed at multiple time points. Infarct size and neutrophil accumulation were assessed following the reperfusion period. In separate cohorts, the effect of MTR on myocardial interstitial water (MRI imaging) and blood flow was examined.** ***Results:*** **Both groups had similar areas at risk (AAR), hemodynamics, and arterial blood gas values. MTR, even when delayed 90 minutes into reperfusion (D90, 29.2 ± 5.0% of AAR), reduced infarct size significantly compared to Controls (51.9 ± 2.7%, $p = 0.006$). This protection was associated with a 33% decrease in neutrophil accumulation ($p = 0.047$). Improvements in blood flow and interstitial water were also observed. Moreover, we demonstrated that the therapeutic window for MTR lasts for at least 90 minutes following reperfusion.** ***Conclusions:*** **This study confirms our previous observations that MTR is an effective therapeutic approach to reducing reperfusion injury with a clinically useful treatment window.** doi: 10.1111/jocs.12580 *(J Card Surg 2015;30:659–667)*

Pro-inflammatory cytokines,[1,2] neutrophils,[1,3] oxygen radicals,[4] and complement[5,6] are among the many mediators of injury released or activated with the restoration of blood flow to the ischemic myocardium. Myocardium exposed to these factors may succumb to early necrotic cell death or a delayed death due to apoptosis.[7,8] Mechanobiology is the science of effecting biological change in living tissues by the application of mechanical forces. While standard pharmacological therapies attempt to block or modify chemical processes to achieve a therapy goal, mechanical tissue resuscitation (MTR) uses the application of physical forces to effect change in tissue whose survival has been compromised or impaired. MTR uses a controlled negative pressure over a period of time on at-risk tissues and their surroundings to produce a physiological state that is more compatible with survival of compromised cells. This form of treatment is especially valuable in terminally differentiated tissue such as brain and heart where regrowth of functional tissue is limited. Previously, MTR was shown to reduce tissue injury in models of myocardial infarction,[9,10] traumatic brain

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Address for correspondence: Louis C. Argenta, M.D., Department of Plastic and Reconstructive Surgery, Wake Forest School of Medicine, Winston-Salem, NC 27157. Fax: +1-336-713-6524; e-mail: largenta@wakehealth.edu

injury,[11] and burns.[12] This protection appears to be the result of increased blood flow,[9,13,14] decreased edema, cytokine modulation, and inhibition of apoptosis.[9,15]

Present treatment of myocardial ischemia-reperfusion may require pretreatment or the loading of pharmacologic agents to therapeutic levels at or immediately before reperfusion which may limit the clinical application of these treatments. Based on previous studies, we hypothesize that MTR could decrease infarct size and limit reperfusion injury without the need for pretreatment. MTR is achieved with a porous matrix to homogenously distribute a controlled negative pressure directly to the ischemic-reperfused myocardium. The ability to afford protection without pretreatment requirements expands the clinical potential of this therapeutic modality.

MATERIALS AND METHODS

All studies complied with the Guide for the Care and Use of Laboratory Animals using protocols approved by the Wake Forest Baptist Health Institutional Animal Care and Use Committee.

Surgical preparation

Female swine were anesthetized and, following intubation, mechanically ventilated with anesthesia maintained using isofluorane. Drug and fluid administration was given through the femoral vein. Blood pressure was measured from the femoral artery. A median sternotomy provided access to the heart which was instrumented with a Millar catheter into the left ventricle. A second Millar catheter was advanced to the aortic arch via a femoral artery. The left atrium was cannulated for central administration of microspheres. Finally, monofilament sutures were passed around diagonal branches of the left anterior descending coronary arteries.

Ischemia-reperfusion protocol

Reversible tourniquets were tightened around several coronary artery branches of the left anterior descending artery (LAD) for 85 minutes creating a reproducible, anterior ischemic area. Release of the tourniquets initiated the three hour reperfusion period (Fig. 1). Hemodynamics and arterial blood gases were recorded at baseline, middle, and end of ischemia (mid- and end-isc), as well as at 30 (R30), 60 (R60), 120 (R120), and 180 (R180) minutes of reperfusion. MTR-treatment was applied 90 minutes after reperfusion and was accomplished using a polyvinyl–alcohol sponge, nitrile sheet, and a vacuum tube attached to a computer-controlled vacuum pump as previously described.[9,10] Evicel glue (Ethicon, Somerville, NJ, USA) was used to seal the edges of the nitrile to the myocardium creating an airtight patch. Negative pressure (−125 mmHg) was continuously applied to the ischemic-reperfused myocardium for the final 90 minutes of reperfusion.

Infarct size

Infarct size was determined by gravimetric analysis as previously described.[9,10] Briefly, the coronary ligatures were permanently tightened and a blue dye was used to negatively stain the area at risk (AAR). The heart was removed from the body, the right ventricle removed, and the left ventricle was cut into sections perpendicular to the long axis. Each tissue segment was then divided into epicardial and endocardial sections, the blue tissue separated from the unstained tissue which was then subjected to TTC staining. Tissue samples for myeloperoxidase and blood flow were taken after infarct size measurements were complete.

Myeloperoxidase

Myeloperoxidase (MPO) was measured in transmural tissue samples taken from the AAR as previously described.[5,16] Weighed tissues were homogenized first in phosphate buffer to remove hemoglobin and other extracellular peroxidases. The pellets were further homogenized and sonicated in hexadecyltrimethylammonium bromide buffer. Supernatants were added to a reaction buffer containing hydrogen peroxide and o-dianisidine and measured spectrophotometrically.

Regional myocardial blood flow

Regional myocardial blood flow was assessed at baseline, end of ischemia, R30, and R180 using

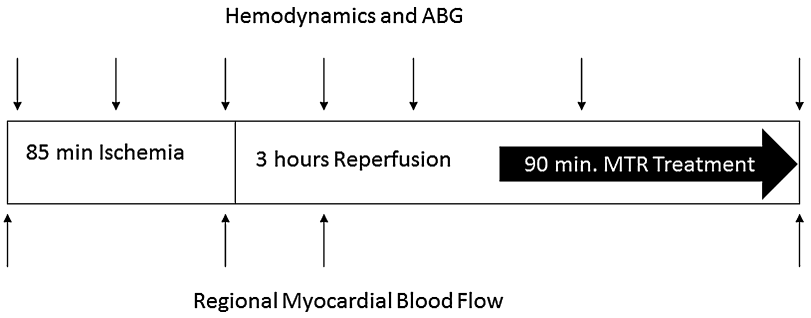

Figure 1. *Experimental time course. Animals for infarction studies were subjected to 85 minutes of ischemia and three hours of reperfusion. MTR treatment was given to one group of animals beginning 90 minutes after the initiation of reperfusion. Hemodynamics, arterial blood gas analysis, and regional myocardial blood flow data were recorded as indicated in the figure.*

neutron-activated microspheres as previously described.[9,10] Microspheres were injected centrally into the left atrium and reference blood samples were collected from the femoral artery.

Magnetic resonance imaging

To understand the effect of MTR on myocardial structure and water handling, MRI imaging and analyses, including T_2-weighted, diffusion tensor, and fiber-tracking were performed on hearts from an independent cohort of animals using a protocol where MTR is known to be protective.[9] Following reperfusion, full-thickness samples ($\sim 4 \times 3$cm) from both the AAR and nonischemic (NI) regions were taken immediately after euthanasia. Both tissue samples from each heart were sealed in a 3 cm-diameter cylinder placed in a 7 T animal scanner (Bruker Biospin, Ettlinger, Germany) fitted with a shim insert, producing a gradient of 400 mT/m.

The T_2-weighted images were generated using a multislice, multi-echo, spin-echo sequence achieving a plane resolution of 234 μm^2/pixel in a field of vision covering 3 cm^2 with 128×128 pixels. The scans included eight echoes from 20 slices, each 1 mm thick. Repetition time was 300 ms and echo times of 10–80 ms. T_2 values were calculated for each of the four central slices by fitting a single exponential equation to the eight pairs of intensity-versus-time values. Using Image J software (NIH, USA), images were processed by manually choosing regions of interest (ROI) covering approximately 90% of voxels in each slice and excluding hyperintense edges and pockets between the samples where condensed water vapor might collect.

Diffusion tensor images were generated at a resolution of $469 \times 469\ \mu m^2$ in four central slices, each 2 mm thick, with 2 mm separation. The B factor was 342 s/mm^2, and scans included six different directions and 5 B_0 images. Repetition time was 2000 ms; echo time 18.5 ms; diffusion gradient duration 5 ms; and diffusion gradient separation 9 ms. Scalar diffusion parameters, including fractional anisotropy (FA), apparent diffusion coefficient (ADC), and the three eigenvalues (λ_1, λ_2, λ_3) of the diffusion tensor, were calculated for each slice in manually selected ROIs, covering most of the image. For fiber tracking, the FA threshold was set at either 281 or 342 with a minimum fiber length of 10 mm. Images were processed and analyzed using the MEDINRIA software package (ASCLEPIOS Sophia Antipolis Cedex, France).

Direct effect of MTR on myocardial blood flow

To differentiate direct actions on blood flow changes from indirect changes to blood flow due to decreased intravascular leukocyte adherence, an independent cohort of animals was utilized. Swine were anesthetized as above and instrumented with pressure transducers and a flow probe (Transonic Systems, Inc., Ithaca, NY, USA) on the proximal left anterior coronary artery. Baseline recordings were made following a stabilization period. Following ~30 minutes of MTR-treatment (−125 mmHg, relative to atmospheric) on the myocardium perfused by the LAD, a second set of measurements was recorded. The negative pressure was removed and blood flow was allowed to normalize for a further 30 minutes before a final recording was made. Changes in blood flow, blood pressure, and heart rate were normalized to baseline values.

Exclusion criteria and statistics

All data from a given animal were excluded from analyses if any of the following exclusion criteria were met: (1) AAR <8% of the left ventricle (one Control); (2) transmural blood flow was >0.15 mL/min/g tissue (two Control, three D90); (3) ventricular fibrillation required more than five attempts at cardioversion (one Control); and (4) staining problems (two Controls). All data are presented as mean ± SEM, except for the MRI data which are presented as mean ± SD so that the number of imaging slices analyzed does not unfairly reduce variability and impact group differences. Treatment group data of nonrepeated measures data were compared using t-tests. Where appropriate, non-parametric rank-sum tests were performed. Two-way repeated measures analyses were used to assess treatment differences in hemodynamic and blood gas parameters. Blood flow, blood pressure, and heart rate from isolated flow studies were analyzed by one-way repeated measures ANOVA. Post hoc Holm–Sidac multiple comparisons were used to determine any significant group differences.

RESULTS

A total of 26 animals entered and completed the experimental protocol (100% survival through the timed ischemia-reperfusion). After excluding animals using the above criteria, data from 17 animals were analyzed (eight Controls, nine MTR). Physiologically, the two groups were similar with no group differences at any time for any arterial blood gas (Table 1) or hemodynamic variable (Table 2). Rectal temperatures varied slightly but not significantly from normal physiologic ranges.

Infarct size

Both groups had equivalent areas of the left ventricle made ischemic (Fig. 2A). The AARs were 15.5 ± 1.0% in Control animals and 13.7 ± 1.1% in MTR-treated animals ($p = 0.256$). Infarct size (Fig. 2B) was significantly decreased with MTR (29.2 ± 5.0%) compared to Controls (51.9 ± 2.7%, $p = 0.006$).

Neutrophil accumulation

Neutrophil accumulation was determined in both the AAR and the NI regions by assessing MPO activity (Fig. 2C). MPO activity in NI regions was low in both groups, representing a background number of trapped neutrophils. There were no group differences in MPO activity in this region ($p = 0.352$). In the ischemic-

TABLE 1
Physiological Parameters Throughout the Experiment

	RR (min^{-1})	Temp (°C)	pH	pCO_2 (mmHg)	pO_2 (mmHg)	HCT (%)	Calcium (mM)
Baseline							
Control	14 ± 0	37.3 ± 0.2	7.47 ± 0.02	43 ± 2	530 ± 61	27.1 ± 0.8	1.8 ± 0.1
D90	14 ± 1	36.8 ± 0.1*	7.45 ± 0.02	46 ± 2	622 ± 27	28.4 ± 0.9	1.9 ± 0.1
Mid-Isc							
Control	15 ± 1	37.5 ± 0.2	7.44 ± 0.02	43 ± 2	569 ± 46	27.4 ± 1.7	1.9 ± 0.1
D90	14 ± 1	37.0 ± 0.1*	7.42 ± 0.02	48 ± 2	624 ± 35	29.1 ± 1.1	1.9 ± 0.1
End-Isc							
Control	15 ± 0	37.6 ± 0.1	7.42 ± 0.02	47 ± 2	566 ± 62	29.4 ± 1.4	1.8 ± 0.1
D90	15 ± 1	37.1 ± 0.1*	7.40 ± 0.02	48 ± 2	613 ± 32	28.7 ± 1.1	2.0 ± 0.1
R30							
Control	15 ± 0	37.6 ± 0.1	7.42 ± 0.02	46 ± 2	567 ± 70	30.3 ± 1.1	2.0 ± 0.1
D90	15 ± 1	37.3 ± 0.1	7.40 ± 0.02	48 ± 2	622 ± 39	30.0 ± 0.8	2.0 ± 0.1
R60							
Control	15 ± 0	37.5 ± 0.1	7.41 ± 0.02	45 ± 1	587 ± 48	30.3 ± 1.2	2.0 ± 0.1
D90	16 ± 1	37.3 ± 0.1	7.40 ± 0.02	47 ± 2	594 ± 32	29.5 ± 0.8	2.0 ± 0.0
R120							
Control	15 ± 0	37.6 ± 0.1	7.46 ± 0.07	45 ± 2	562 ± 53	29.6 ± 1.3	2.1 ± 0.1
D90	16 ± 1	37.2 ± 0.1	7.41 ± 0.02	43 ± 2	606 ± 33	29.7 ± 1.7	2.0 ± 0.0
R180							
Control	16 ± 1	37.9 ±0.4	7.40 ± 0.03	44 ± 2	552 ± 52	30.5 ± 1.7	2.1 ± 0.1
D90	16 ± 1	37.1 ± 0.1	7.42 ± 0.02	44 ± 1	579 ± 34	28.1 ± 1.9	2.0 ± 0.0

RR = respiratory rate; Temp = temperature; HCT = hematocrit; Mid-Isc = middle of ischemic period; End-Isc = end of ischemic period; R30, R60, R120, R180 = 30, 60, 120 and 180 minutes into reperfusion; D90 = MTR application delayed 90 minutes into a 3 hour reperfusion period.
*$p < 0.05$ vs. Control within same time point.

reperfused myocardium, MTR reduced MPO activity by 33% from 30.2 ± 6.7 to 20.2 ± 2.9. When the AAR was normalized to the NI region, MTR significantly reduced MPO activity (2.27 ± 0.39) compared to Controls (7.16 ± 2.55, $p = 0.047$).

Regional myocardial blood flow

Regional myocardial blood flow was determined in both epicardial and endocardial samples from the NI and ischemic, non-necrotic areas of the myocardium

TABLE 2
Hemodynamic Parameters Throughout the Experiment

	HR (min^{-1})	MAP (mmHg)	AoP_p (mmHg)	LVEDP (mmHg)	LVDP (mmHg)	LVCI (sec^{-1})	+dP/dt (mmHg * sec^{-1})	−dP/dt (mmHg * sec^{-1})
Baseline								
Control	86 ± 11	81 ± 5	96 ± 6	12 ± 2	80 ± 3	37 ± 5	1.12 ± .15	−1.36 ± .05
D90	73 ± 4	75 ± 3	89 ± 2	12 ± 1	79 ± 2	34 ± 3	1.00 ± 0.07	−1.33 ± 0.05
Mid-Isc								
Control	82 ± 9	76 ± 5	89 ± 6	12 ± 2	74 ± 3	36 ± 4	1.03 ± 0.10	−1.23 ± .05
D90	70 ± 3	70 ± 3	84 ± 3	13 ± 2	74 ± 3	38 ± 4	0.99 ± 0.09	−1.23 ± 0.07
End-Isc								
Control	83 ± 8	70 ± 5	82 ± 6	9 ± 2	68 ± 2	38 ± 3	1.00 ± 0.12	−1.16 ± 0.09
D90	73 ± 4	63 ± 3	77 ± 4	12 ± 1	69 ± 3	38 ± 3	1.02 ± 0.09	−1.11 ± 0.07
R30								
Control	105 ± 6	71 ± 3	81 ± 5	7 ± 2	68 ± 4	42 ± 2	1.19 ± 0.14	−1.13 ± 0.10
D90	92 ± 6	65 ± 2	77 ± 3	9 ± 1	69 ± 3	42 ± 3	1.15 ± 0.14	−1.10 ± 0.06
R60								
Control	102 ± 9	75 ± 3	86 ± 5	8 ± 2	73 ± 2	40 ± 3	1.25 ± 0.13	−1.29 ± 0.07
D90	94 ± 5	65 ± 1	77 ± 2	7 ± 1	68 ± 1	40 ± 2	1.06 ± 0.05	−1.14 ± 0.03
R120								
Control	105 ± 14	66 ± 2	78 ± 4	7 ± 1	69 ± 2	40 ± 3	1.07 ± 0.12	−1.21 ± 0.07
D90	95 ± 6	62 ± 1	74 ± 1	8 ± 1	67 ± 2	38 ± 1	1.04 ± 0.05	−1.12 ± 0.04
R180								
Control	113 ± 14	60 ± 1	70 ± 3	6 ± 1	66 ± 2	45 ± 2	1.16 ± 0.08	−1.16 ± 0.05
D90	93 ± 5	61 ± 2	75 ± 2	7 ± 0	70 ± 2	39 ± 1	1.13 ± 0.07	−1.14 ± 0.05

Mid-Isc = middle of ischemic period; End-Isc = end of ischemic period; R30, R60, R120, R180 = 30, 60, 120 and 180 minutes into reperfusion; D90 = MTR application delayed 90 minutes into a 3 hour reperfusion period; HR = heart rate; MAP = mean peripheral artery pressure; AoP_p = peak aortic pressure; LVEDP = left ventricular end-diastolic pressure; LVDP = left ventricular developed pressure; LVCI = left ventricular contractility index.

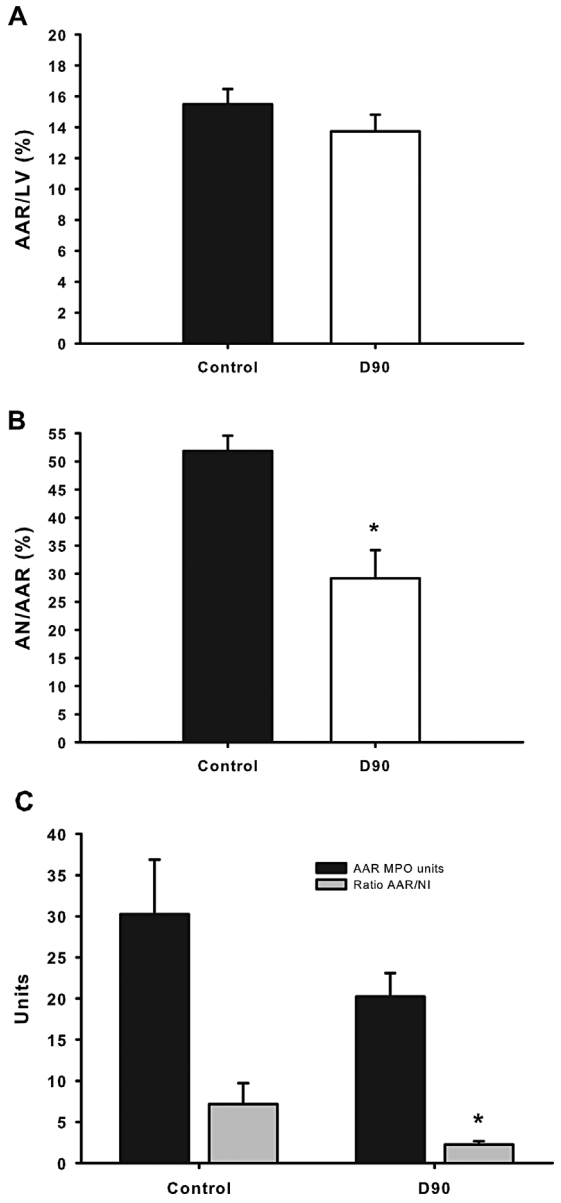

Figure 2. *Area at risk, infarct size, and neutrophil accumulation following myocardial ischemia reperfusion. (A) Both Control and D90 groups had equivalent areas of the left ventricle subjected to 85 minutes of ischemia and 3 hours of reperfusion. (B) Even when delayed 90 minutes, MTR (D90) significantly reduces infarct size compared to untreated Controls. Infarct size is presented as the amount of necrotic tissue normalized to the area at risk. (C) Myeloperoxidase activity was used to assess neutrophil accumulation within the ischemic-reperfused myocardium. Both raw activity (black bars) and the activity in the AAR normalized to the non-ischemic (NI) regions (gray bars) are presented. AAR, area at risk; LV, left ventricle; AN, area of necrosis; MPO, myeloperoxidase; $^*p < 0.05$ vs. Control. Data are mean ± SEM.*

(Table 3). There were no group or time-based differences in blood flow within the NI myocardium. For ischemic-reperfused tissues, blood flow was reduced to near zero during ischemia and above baseline values during early reperfusion as expected. Since MTR was only applied after 90 minutes of reperfusion, no differences in blood flow at any time point prior to MTR application were expected or observed. To test the hypothesis that MTR would increase blood flow, a comparison of flow at R180 was performed. R180 was the only time during the experiment where blood flow was measured and MTR was active (Fig. 3). Here, a significant preservation of blood flow was observed in MTR-treated epicardium, but not in endocardial samples. Furthermore, the decay rate in blood flow (slope of the line from R30 to R180) was about half as fast in both the epicardium ($-0.004 \pm .001$ vs. -0.009 ± 0.002) and the endocardium (-0.005 ± 0.001 vs -0.009 ± 0.002) in MTR-treated animals compared to Controls.

Magnetic resonance imaging

Myocardial edema was shown to increase in the AAR compared to NI tissue (Fig. 4A, 44.4 ± 4.8 vs. 35.1 ± 1.9 ms, $p < 0.001$), as a result of ischemia-reperfusion injury. MTR treatment reduced T_2 values (38.9 ± 4.0 vs. 44.4 ± 4.8 ms $p < 0.0001$, Fig. 4A) in the AAR, a finding consistent with a decrease in interstitial edema. MTR also significantly reduced the apparent diffusion coefficient in the AAR (Fig. 4B, $1.109 \pm .048$ vs. $1.189 \pm .068$, $p < 0.001$). In addition, MTR increased FA ($p < 0.03$, Fig. 4C) suggesting that MTR-treatment influences myocardial tissue structure and order. This change in anisotropy was also reflected in a concomitant reduction in all three eigenvalues from the diffusion tensor imaging analysis, with the most pronounced effect on motion perpendicular to the muscle fibers in the heart; λ_1 (parallel to muscle fibers, 180% difference, $p = 0.0006$), λ_2 (perpendicular to muscle fibers, 130% difference, $p = 0.0125$), and λ_3 (perpendicular to muscle fibers, 282% difference, $p < 0.0001$). The observed increased anisotropy with MTR is consistent with a partial reversal of structural damage caused by interlaminar and interfiber accumulation of water.

Direct effect of MTR on myocardial blood flow

In a naïve (no ischemia-reperfusion) cohort of animals, blood flow changes in response to MTR were measured with an ultrasonic flow probe (Fig. 5). Blood flow during MTR treatment at −125 mmHg was $28.7 \pm 6.3\%$ greater than at baseline. During this same time, neither heart rate nor blood pressure changed from baseline values. Within 30 minutes of removing the negative pressure from the myocardium, all measured variables returned to baseline levels.

DISCUSSION

In this study, MTR was applied to the ischemic-reperfused myocardium after 90 minutes of reperfusion had elapsed whereas prior studies had treatment beginning immediately upon reperfusion. While early reperfusion remains the most important variable in preserving myocardium following an acute myocardial infarction, there is still substantial injury that occurs because of reperfusion. Having a 90 minute window following revascularization to administer therapy against this reperfusion injury would eliminate the need of establishing therapeutic pharmacological levels

TABLE 3
Regional Myocardial Blood Flow Assessed by Microspheres

	NI-EPI	NI-ENDO	ISC-EPI	ISC-ENDO	NEC-EPI	NEC-ENDO
Baseline						
Control	0.816 ± 0.134	0.809± 0.086	0.790 ± 0.106	0.892 ± 0.094	0.768 ± 0.088	0.796±0.096
D90	0.869± 0.097	0.767 ± 0.102	0.769 ± 0.105	0.774 ± 0.098	0.778 ± 0.123	0.890±0.171
End-Isc						
Control	0.804 ± 0.122	.0759 ± 0.091	0.096 ± 0.012	0.093± 0.018	0.045 ± 0.024	0.031±.0.015
D90	0.748 ± 0.111	0.615 ± 0.087	0.086 ± 0.014	0.079 ± 0.010	0.026 ± 0.010	0.039±0.031
R30						
Control	0.879 ± 0.148	0.777 ± 0.118	1.930 ± 0.378	2.138 ± 0.330	2.094 ± 0.436	1.515±0.408
D90	0.842 ± 0.101	0.677 ± 0.080	1.763 ± 0.188	1.719 ± 0.219	1.922 ± 0.289	1.684±0.376
R180						
Control	0.702 ± 0.112	0.612 ± 0.089	0.589 ± 0.067	0.788 ± 0.109	0.949 ± 0.129	0.703 ± 0.182
D90	1.128 ± 0.154	0.872 ± 0.184	1.159 ± 0.186*	1.189 ± 0.310	2.064 ± 0.458*	1.549 ± 0.443

NI = non-ischemic region; ISC = ischemic, non-necrotic region; NEC = necrotic region; EPI = epicardial samples; ENDO = endocardial samples; End-Isc = end of ischemia timepoint; R30/R180 = 30 or 180 minutes following reperfusion.
*<0.05 vs. Control group.

of therapy prior to the start of reperfusion. This potential widens the window of opportunity for treatment of patients who undergo surgical, percutaneous, or pharmacological revascularization.

MTR administered immediately upon reperfusion has previously been shown to reduce myocardial edema, infarct size, and apoptosis.[9,10] Here, MTR reduced infarct size by nearly 50% even though treatment was delayed for 90 minutes (half of the total reperfusion period). This magnitude of protection is comparable to that observed previously (39% to 55% reduction) in similar models with immediate therapy.[9,10] The protection afforded to the at-risk myocardium came with no untoward effects, altered hemodynamics, oxygenation, or other physiological measures. Together, these findings suggest that the observed preservation of myocardial tissue is the result of MTR treatment.

Neutrophils are major contributors to the inflammatory response of reperfusion injury.[1,3] They induce myocardial injury via a variety of mechanisms including oxygen radical production, release of inflammatory mediators and cytokines, and mechanically obstructing arteriolar flow. There is usually a strong correlation between myocardial preservation and reduction of neutrophil accumulation.[17] In this study, myeloperoxidase activity was reduced 33% in transmural samples of MTR-treated animals. As shown in our previous studies, there are regional differences in how MTR impacts the tissue. The effect of MTR on apoptosis and edema[9] is more robust in the epicardium than in the endocardium, suggesting a proximity effect. The relationship between MTR and neutrophil accumulation at various depths within the myocardium will require more sophisticated examination.

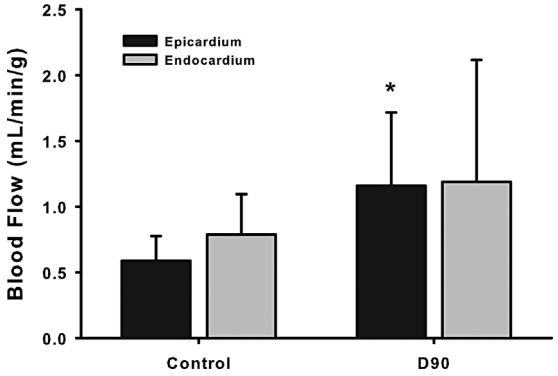

Figure 3. *Regional myocardial blood flow evaluated by microspheres. Blood flow was measured at four time points throughout the experiment by the microsphere technique. This figure shows blood flow changes that occurred in the epicardium and endocardium of ischemic, non-necrotic myocardial tissue samples at R180, the only timepoint during which MTR treatment was active. *$p < 0.05$ vs. Control within a region. Data are mean ± SEM.*

Tissue edema is a consequence of reperfusion injury and is known to interfere with electrical conduction,[18] oxygenation,[19] and both systolic and diastolic myocardial function.[20–22] We used MRI to assess both the magnitude of extracellular water and its effect on tissue structure and order. The increase in T_2 signal may reflect both an absolute increase in water and/or a change in the extent of hydration status of primarily structural proteins such as collagen. In previous studies, we uncoupled this uncertainty with the use of histology (increase interstitial spacing due to ischemia reperfusion) suggesting that the observed increase in T_2 signal here is reflective of an increase in extracellular water.[9] MTR reduced the T_2 signal in the AAR suggesting an accelerated resolution of myocardial edema in agreement with our previous work.[9] In fact, MTR reduced edema to levels comparable to those observed in the nonischemic myocardium. In the process of reducing edema, structural order (anisotropy) was partially resolved in MTR-treated myocardium by reducing interlaminar and interfiber spacing. The restoration of normal structure is reflected in the eigenvalues that represent water movement parallel (λ_1) and perpendicular (λ_2 and λ_3) to the orientation of muscle fibers within the region of interest of MRI images.

Another mechanism thought to play an important role in the myocardial protection of MTR is the direct effect on local myocardial blood flow. Multiple studies have

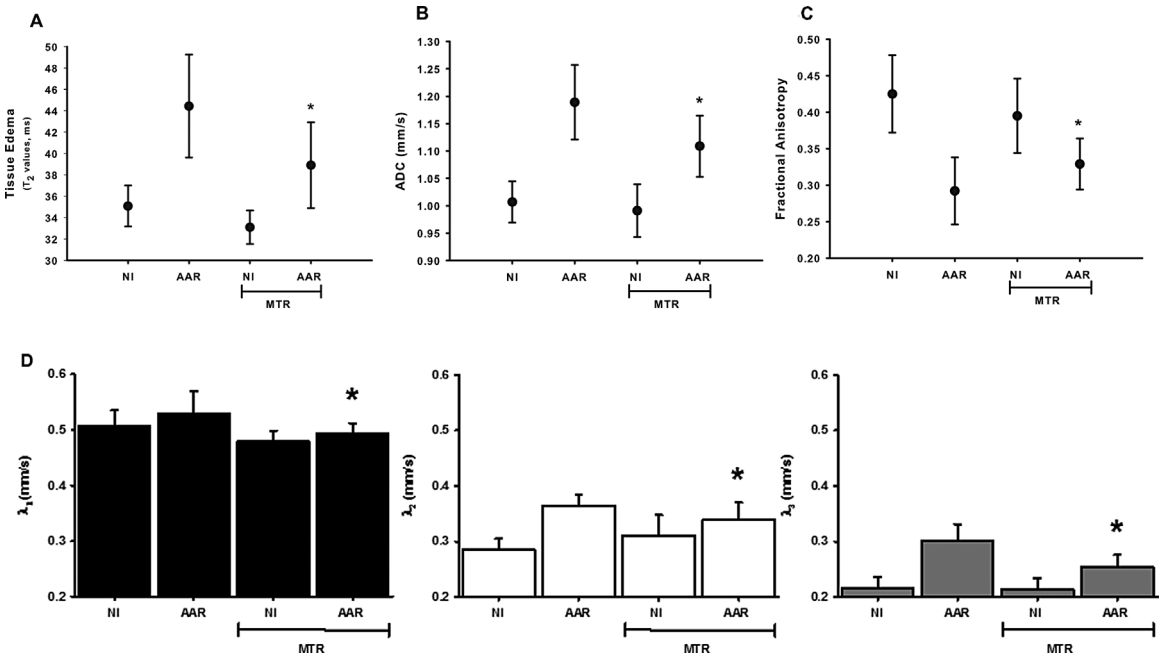

Figure 4. *Magnetic resonance imaging analyses. Effect of MTR on both edema and structure. Both ischemic-reperfused (AAR) and nonischemic (NI) regions of hearts were imaged using MRI. Ischemic regions were compared with and without MTR treatment. Each data point is mean and standard deviation of 16 different central myocardial slices from four different hearts (four slices from each heart). (A) T_2 values. The T_2 values in the AAR are higher than in NI tissues, consistent with edema in injured myocardial tissue. MTR treatment reduces the T_2 value within the AAR compared to the untreated ischemic tissue, consistent with a decrease in edema. (B) Apparent diffusion coefficient. Ischemic tissues develop larger diffusion coefficients (faster water transfer) than do NI tissues. MTR reduces this diffusion coefficient compared to the untreated hearts. (C) Fractional anisotropy. Structural order is maintained in NI tissues compared to the AAR, reflected by larger anisotropy values. MTR treatment partially restores order to the myocardium compared to untreated tissue within the AAR. (D) Eigenvalues of the diffusion tensor, λ_1, λ_2, λ_3. Diffusion tensor imaging suggests a disruption in structure between AAR and NI tissues. Again, MTR treatment of the AAR restores some structure (reduced water movement parallel and perpendicular to muscle fibers) to the ischemic-reperfused tissue. These changes are most prominent in the first and third eigenvalues. AAR, area at risk; MTR, mechanical tissue resuscitation; $^*p < 0.05$ vs. untreated AAR. Data are mean $\pm$ SD.*

shown a positive relationship between MTR and blood flow in other tissues.[13,14,23] Here, blood flow was similar in each group prior to MTR treatment, including during ischemia and the reactive hyperemia of early reperfusion. The effect of MTR was captured in the final measurement where blood flow was significantly higher in MTR-treated epicardial samples. While MTR-treated blood flow was also greater in the endocardium than in Controls, these differences did not reach statistical significance. This difference may be attributable to a proximity effect of MTR with a more robust effect on tissues closer to the source of negative pressure.

To provide further proof of the direct action of MTR on blood flow, a separate cohort of animals was instrumented to measure only blood flow, pressure, and heart rate in the absence of ischemia. Blood flow increased nearly 30% above baseline levels during MTR treatment, without changes in blood pressure or heart rate. Support for the direct link between MTR and blood flow comes from the fact that blood flow returns to basal levels following removal of the MTR stimulus from these hearts (Fig. 5). These results also agree with previous observations of increased blood flow without concomitant changes in blood pressure or heart rate with MTR treatment.[9]

While we observed that lower levels of negative pressure (−50 mmHg) had little or no effect on blood flow,[9] others have demonstrated that the larger magnitudes of negative pressure (in the range of −125 mmHg) were less effective at changing blood flow.[24] The differences in observations between levels of negative pressure may be related to the methods used to measure blood flow and require further investigation.[25]

In summary, MTR has a wide cardioprotective window, lasting at least 90 minutes following revascularization. MTR reduces neutrophil accumulation and interstitial edema within the ischemic-reperfused myocardium. Finally, we confirm a direct link between MTR and regional blood flow. Together with our previous work which demonstrated MTR reducing myocardial edema and apoptosis, a more complete understanding of how MTR protects the heart is emerging.

The use of MTR in patients having open revascularization via CABG is intuitive: placing the matrix directly over newly revascularized myocardium prior to sternal closure. Although a removable device may be used, our previous studies demonstrate that a biodegradable device that would eliminate the need for removal is possible. MTR use in patients having percutaneous

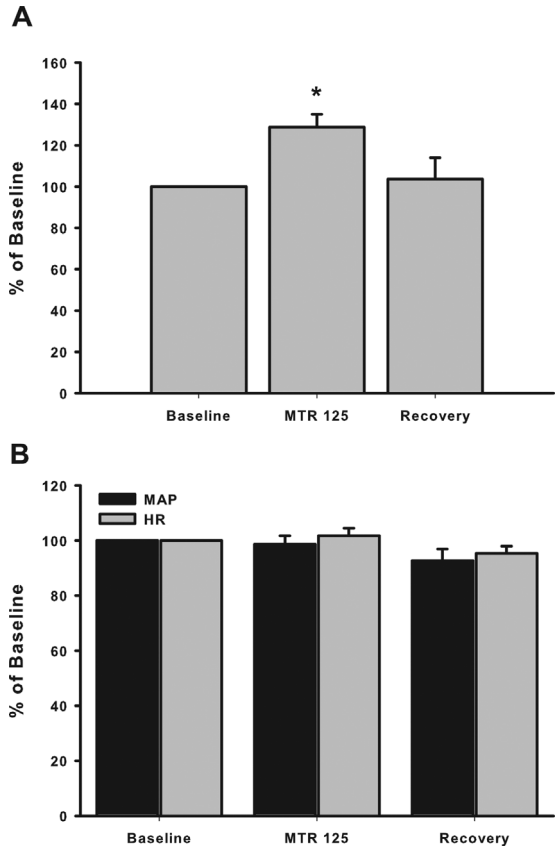

Figure 5. *Direct effect of MTR on myocardial blood flow. Changes in hemodynamics and myocardial blood flow (ultrasonic flow meter) in the absence of ischemia were determined in a separate cohort of animals. (A) MTR (−125 mmHg) significantly increased blood flow by approximately 30% compared to baseline. Blood flow returns to basal levels after removing the negative pressure of MTR. (B) Neither heart rate nor blood pressure was changed with the application of MTR directly to the myocardium. MTR, mechanical tissue resuscitation; $^{*}p < 0.05$ vs. baseline. Data are mean ± SEM.*

revascularization, however, is less obvious. With the understanding that MTR remains an effective therapeutic approach at least 90 minutes after initial revascularization, it is plausible to utilize the tissue sparing actions of MTR delivered in a minimally invasive manner with percutaneous interventions, especially in centers utilizing the ever-expanding hybrid catheterization lab/operating room. For delivery in this manner, the foam matrix, covering film and vacuum tubing would be assembled into a single, layered unit which would be rolled into a cigar-shaped form with the vacuum tube extending out from the main matrix. Using thoracoscopic instruments, a pericardial window would be created over or adjacent to the area at risk and the device inserted and expanded (unrolled). The covering film would then be distributed to seal against the myocardial surface with the pericardium providing additional sealing pressure. Fibrin glue may be used to assist in sealing leaks should that be required. In these cases, a biodegradable device that could be deployed percutaneously would be optimal allowing the vacuum tube to be removed as if it were a chest tube. The possibility of using such a device to preserve myocardium in cases of small vessel myocardial infarction that is not reconstructable by stent or open procedure is also attractive.

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