EXPERIMENTAL STUDIES

A Comparison of Mechanical and Laser Transmyocardial Revascularization for Induction of Angiogenesis and Arteriogenesis in Chronically Ischemic Myocardium

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OBJECTIVES
The purpose of the present study was to compare the use of a mechanical transmyocardial implant (TMI) device with transmyocardial laser revascularization (TMR) for induction of therapeutic angiogenesis and arteriogenesis in the chronically ischemic heart.

BACKGROUND
Prior experimental studies have demonstrated evidence for neovascularization after both mechanical and laser transmyocardial revascularization, although a long-term comparison of the two techniques has not been performed.

METHODS
Using an established model of chronic hibernating myocardium, mini-swine underwent 90% proximal left circumflex (LCx) coronary artery stenosis. One month later, baseline positron emission tomography (PET) and dobutamine stress echocardiography (DSE) were performed to quantify regional myocardial blood flow (MBF) and function. Animals then underwent TMR with a holmium:yttrium-aluminum-garnet (holmium:YAG) laser (n = 5), TMI (n = 5), or sham redo-thoracotomy (n = 5). In the TMR group, the entire LCx region was treated with transmural laser channels at a density of 1/cm². Transmyocardial implants were placed transmurally at a similar density in the LCx region of the TMI group. Six months later, the PET and DSE studies were repeated, and the animals were euthanized.

RESULTS
Six months after TMR, there was a significant increase over baseline in resting MBF to the lased LCx region (68.9 ± 4.6% vs. 89.3 ± 3.0% reference non-ischemic septal segments; p < 0.001). This increased MBF was accompanied by a significant improvement in LCx regional wall motion during peak dobutamine stress (p = 0.04). Compared with baseline, there was no change in LCx region MBF six months after either TMI (72.9 ± 4.8% vs. 85.7 ± 3.4%; p = 0.10) or sham redo-thoracotomy (75.6 ± 4.6% vs. 80.1 ± 5.0%; p > 0.2). Likewise, there was no significant change in rest or stress wall motion by DSE six months postoperatively in either group. Overall vascular density was increased only in the TMR-treated regions six months postoperatively. The difference between groups was most notable for a twofold increase in the number of small arterioles seen in the lased (4.4 ± 0.3 arterioles per high power field; p < 0.001 vs. both TMI and sham) compared with TMI (2.2 ± 0.2) and sham (1.9 ± 0.2)-treated regions.

CONCLUSIONS
Mechanical transmyocardial revascularization with a TMI device does not appear to promote physiologically significant angiogenesis or arteriogenesis in the chronically ischemic porcine heart and cannot be recommended for clinical trials at this time. Infrared laser-mediated injury mechanisms may be important for inducing therapeutic neovascularization with direct myocardial revascularization techniques. (J Am Coll Cardiol 2002;39:1220–8) © 2002 by the American College of Cardiology Foundation
torias in patients not amenable to bypass surgery or angioplasty (11).

Numerous studies (12–14) have demonstrated experimental evidence for therapeutic angiogenesis after TMR, and although the long-term benefits of the procedure are as yet unproven (15), TMR may prove beneficial in treating angina pectoris in the thousands of patients with end-stage CAD. However, there are several disadvantages involved in the use of TMR, including the need for expensive equipment, the potential for peri-procedural laser malfunction, the need for regular maintenance and highly specialized technical support and the risk of laser-induced injury of patients or medical personnel, among others. Consequently, if a “non-laser” alternative were available that provided equivalent clinical benefit, this might be preferred to TMR. Recent experimental studies using mechanical drilling (16) and needle punctures (17,18) have suggested that non-laser mechanical therapies might be capable of promoting angiogenesis. The purpose of the present study was to examine long-term (six months) changes in regional myocardial perfusion using quantitative positron emission tomography (PET), function with dobutamine stress echocardiography (DSE), and overall vascular and arteriolar density after mechanical and laser transmyocardial revascularization in an established model of chronic hibernating myocardium (19). The mechanical means of performing transmyocardial revascularization in this study was a novel transmyocardial implant (TMI) device (20). A prior pilot study, in which six to eight implants were placed into the lateral wall of the left ventricle (LV) of non–ischemic swine, demonstrated no adverse events related to implant placement up to three months postoperatively. Postmortem histology revealed evidence for blood vessel growth in the region of the implants (20).

**METHODS**

**Animals.** Adult male mini-swine (40 kg) were obtained from Harlan-Sinclair (Indianapolis, Indiana) and housed under standard conditions and fed a regular diet. The Animal Care and Use Committee of Duke University approved all procedures and protocols. Animals received humane treatment in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication 85-23, revised 1996).

**Experimental model.** Using a previously described porcine model of chronic hibernating myocardium (12,13,19), animals underwent placement of a hydraulic occluder and ultrasonic flow probe (Transonic Systems, Ithaca, New York) around the proximal left circumflex (LCx) coronary artery (Fig. 1). Three days postoperatively, the occluder was inflated to reduce resting blood flow immediately distal to the occluder to 10% of baseline. Animals were then kept in this low-flow state for the duration of the experiment, with blood flow recordings performed three times weekly to assure continued occlusion.

**Positron emission tomography and DSE.** After one month in the low-flow state, animals underwent PET and DSE to document the presence of hibernating myocardium in the LCx distribution. After an overnight fast, dynamic PET imaging of the heart using $^{13}$N-ammonia and $^{18}$F-fluorodeoxyglucose (FDG) was performed as previously described (12,13,19) to obtain regional estimates of myocardial blood flow (MBF) (ml/g per min) and glucose utilization (nmol/g per min). The PET scans were interpreted as showing hibernating myocardium if reduced absolute values of MBF were noted in the lateral and posteroinferior walls of the LV supplied by the LCx, accompanied by normal or increased FDG uptake in these same regions (both as compared with the non-ischemic septum) (21).

The DSE test was performed in 3-min stages with incremental doses of dobutamine beginning with 5 μg/kg/min and increasing to 40 μg/kg per min as previously described (12,13,19). Based on a standard 16-segment model (22), wall motion was graded as 1 = normal, 2 = hypokinetic, 3 = akinetic, or 4 = dyskinetic. Regional wall motion score index (WMSI) was calculated at rest, low dose and peak stress. Echocardiograms were interpreted in a blinded manner by a cardiologist with expertise in stress echocardiography. Using DSE, viability in the LCx region was defined as an improvement in systolic wall thickening with low-dose dobutamine in myocardial regions with severe hypocontractility at rest. Viable segments were considered ischemic if systolic wall motion deteriorated with stress (biphasic response) (23).

**TMI/sham redo-thoracotomy/TMR.** Once hibernating myocardium in the LCx distribution was demonstrated by PET and DSE, animals were randomly assigned to either TMI (n = 5), sham redo-thoracotomy (n = 5), or TMR with a holmium:yttrium-aluminum-garnet (holmium:YAG) laser (Cardiogenesis, Sunnyvale, California) (n = 5). All procedures in all groups were performed within three days of completion of the baseline PET and DSE studies by a
single surgeon using previously described techniques (12,13). For animals undergoing TMR, 20 channels were created at 1-cm intervals in the hibernating LCx region. This number of channels consistently treats the entire LCx region in this experimental model (12,13). Holmium:YAG channels were created using multiple 2-J pulses, with a total energy level of approximately 20 J per channel. Transmural penetration of laser channels was confirmed by visible spurting of blood from the channels during systole as well as a change in the pitch of the sound emitted by the laser as it passed through the wall and into the blood-filled ventricle. Laser settings were in accordance with manufacturer recommendations.

For animals randomized to TMI, an average of 16 (range 14 to 16) transmyocardial implants (Fig. 2) were placed at approximately 1-cm intervals in the LCx region similar to TMR. Because of the somewhat larger size of the implants, slightly fewer numbers, compared with laser channels, were needed to treat the entire LCx region (Fig. 3). The occluder and flow probe were left intact. The pericardium was left widely open. Those animals randomized to the sham group underwent an identically repetent thoracotomy; the pericardium was opened, but TMI/TMR was not performed. In all cases, continuous LCx occlusion was confirmed postoperatively by weekly flow monitoring with the flow probe.

**Follow-up PET and DSE.** Six months after TMI, sham redo-thoracotomy, or TMR animals underwent repeat PET and DSE. This follow-up time point was chosen because it corresponds to the period of maximal anginal relief seen in clinical studies of TMR (2). To allow comparisons between studies performed at baseline and six months and to correct for the known inter-study variability of absolute values of MBF by PET (24), normalization of the data was performed using previously described techniques (13,24). For
each PET study, sectors representing the anterior septum were used as the normal reference segments (19). The $^{13}$N-ammonia activity in sectors representing the LCx distribution was then expressed as a percentage of the activity measured in the reference segments.

**Analysis of angiogenesis and arteriogenesis.** Animals were euthanized six months after TMI, sham thoracotomy, or TMR for histologic and histochemical staining to assess overall vascular and arteriolar density in the LCx region (12). At the time of euthanasia, the location of the TMIs was readily apparent owing to their visible anchor coils on the epicardial surface. Likewise, the TMR channels were identified as punctate regions of scar tissue easily visible at the endocardial surface (12). Of the original 16 to 20 mechanical or laser channels per animal, 6 were randomly chosen for histologic analysis using previously described techniques (12). Routine histologic staining was performed with hematoxylin–eosin and Masson trichrome. Angiogenesis was assessed using endogenous endothelial alkaline phosphatase as previously described (12,25,26). Arteriogenesis was assessed using immunohistochemical staining for HHF-35 (Dako, Carpinteria, California), a murine monoclonal antibody directed against human smooth muscle actin. The HHF-35 stains primarily medium and large arteries (12). Overall vascular density was quantitated in a blinded fashion by two independent observers using previously described techniques (26). Endogenous endothelial alkaline phosphatase staining intensity was measured using an image analysis system (Olympus IX70 inverted microscope, Optronics DEI-750 image-capturing hardware; PowerTower Pro 180 CPU). Images were captured using Adobe Premiere and quantified using NIH image software. Arteriogenesis was likewise quantitated in a blinded fashion by two independent observers using a modification of previously described techniques (12). Four randomly selected samples, each containing at least one channel remnant, were analyzed per animal for a total of 20 samples per group. Three random high-power ($\times 200$) fields were examined per sample. Both vascular and arteriolar density levels were analyzed for the TMI and TMR channel remnants and myocardium within 0.5 cm of the channel remnants. For the sham animals, vascular density was analyzed on 20 randomly selected samples (4 per animal) from the ischemic LCx distribution.

**Statistical analysis.** Results are presented as the mean ± SE. Both MBF and glucose utilization by PET, as well as WMSI by DSE, were compared within groups using a paired Student $t$ test with a Bonferroni correction for multiple comparisons. One-way between-groups analysis of variance (ANOVA) was used to compare MBF, WMSI, and vascular density among groups. A $p$ value $< 0.05$ was considered statistically significant.
RESULTS

All animals survived to their predetermined euthanasia dates. There were no major perioperative complications related to laser or myocardial implant therapy. There was no migration or breakage of any implant as documented radiographically in the immediate postoperative period, two weeks postoperatively and again at the time of euthanasia (Fig. 4). All implants were confined to the wall of the LV at the time of euthanasia.

Positron emission tomography. Myocardial blood flow by PET for all groups at baseline and six months’ post treatment are shown in Table 1. In all animals at baseline, PET demonstrated a significant decrease in LCx-region absolute MBF compared with the corresponding non-ischemic septum and a significant increase in glucose utilization in the regions of decreased blood flow consistent with myocardial viability and ischemia (data not shown) (21). There was no difference in normalized baseline LCx-region MBF among any of the three groups by one-way ANOVA. No significant change in MBF was seen after either mechanical TMI or sham redo-thoracotomy. However, six months after holmium:YAG TMR, there was a significant increase in MBF to the lased regions.

Dobutamine stress echocardiography. The DSE data for the LCx distribution in all groups at baseline and six months’ post treatment are shown in Table 2. Baseline DSE in all animals demonstrated severe hypocontractility at rest.

Table 1. Normalized Left Circumflex Region Myocardial Perfusion* by PET

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>6 Months Postoperatively</th>
<th>p Value</th>
</tr>
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<tbody>
<tr>
<td>TMR</td>
<td>68.9 ± 4.6</td>
<td>89.3 ± 3.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TMI</td>
<td>72.9 ± 4.8</td>
<td>85.7 ± 3.4</td>
<td>0.10</td>
</tr>
<tr>
<td>Sham</td>
<td>75.6 ± 4.6</td>
<td>80.1 ± 5.0</td>
<td>&gt; 0.2</td>
</tr>
</tbody>
</table>

*Normalized myocardial perfusion (%) = mean perfusion (ml/g per min) LCx region/ mean perfusion (ml/g per min) nonischemic septum.

PET = positron emission tomography; TMI = transmyocardial implant; TMR = transmyocardial laser revascularization.

Table 2. Regional Wall Motion Score Index (WMSI) for Hibernating Left Circumflex Distribution by Dobutamine Stress Echocardiography

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Low Stress</th>
<th>Peak Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMR</td>
<td>2.1 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>TMI</td>
<td>2.0 ± 0.2</td>
<td>1.7 ± 0.3</td>
<td>1.6 ± 0.2*</td>
</tr>
<tr>
<td>Sham</td>
<td>2.3 ± 0.2</td>
<td>2.0 ± 0.3</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>TMR</td>
<td>2.1 ± 0.1</td>
<td>1.4 ± 0.2</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>TMI</td>
<td>2.1 ± 0.1</td>
<td>1.4 ± 0.2</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Sham</td>
<td>2.3 ± 0.2</td>
<td>1.8 ± 0.4</td>
<td>2.2 ± 0.3</td>
</tr>
</tbody>
</table>

WMSI: 1 = normal, 2 = hypokinetic, 3 = akinetic, 4 = dyskinetic. *p = 0.04 for peak stress regional WMSI six months’ post-TMR vs. baseline.

TMI = transmyocardial implant; TMR = transmyocardial laser revascularization.
in the LCx region. As shown in Table 2, wall motion in these regions demonstrated a biphasic response of initial improvement (reduced WMSI) during low-dose dobutamine infusion followed by deterioration with high-dose dobutamine stimulation consistent with ischemic, viable myocardium in the LCx distribution (23). There was no difference in baseline rest or stress LCx-region WMSI between any of the three groups by one-way ANOVA. There was no significant change in rest WMSI six months postoperatively in any group. Likewise, there was no significant improvement in peak stress regional WMSI six months after sham redo-thoracotomy or mechanical TMI. In fact, there was a trend toward increased inducible ischemia at peak dobutamine stress in the TMI group (p = 0.06). On the contrary, there was a significant improvement (p = 0.04) in regional WMSI for the lased segments at peak stress, consistent with a reduction in ischemia, six months after holmium:YAG laser TMR.

Vascular and arteriolar density. The TMI and TMR channel remnants were easily identified on histologic staining as hypocellular regions filled with connective tissue. Similar-appearing regions (Fig. 5) were observed six months after sham redo-thoracotomy or mechanical TMI. In fact, there was a trend toward increased inducible ischemia at peak dobutamine stress in the TMI group (p = 0.06). On the contrary, there was a significant improvement (p = 0.04) in regional WMSI for the lased segments at peak stress, consistent with a reduction in ischemia, six months after holmium:YAG laser TMR.

DISCUSSION

TMR-induced angiogenesis and arteriogenesis. To date, six prospective randomized controlled trials have demonstrated the efficacy of surgical TMR for decreasing anginal symptoms in patients ineligible for traditional methods of revascularization (1–5,27). One of the proposed mechanisms of action of TMR is improved regional perfusion via angiogenesis (11). This angiogenesis hypothesis holds that laser-induced injury and the subsequent inflammatory response leads to new blood vessel growth with a secondary improvement in anginal symptoms due to an increase in local oxygen delivery. A number of animal studies in various ischemic models lend support to this hypothesis (12–

Figure 5. Masson trichrome staining (×100) showing (A) hypocellular transmyocardial implant channel remnant filled with blue-staining connective tissue. Large region devoid of connective tissue within channel remnant is prior location of coil of implant device, which has been removed post mortem. Similar-appearing region six months post-transmyocardial laser revascularization is shown in (B).

Figure 6. Endogenous endothelial alkaline phosphatase staining (original magnification ×100) of representative sections from hibernating myocardium treated with sham redo-thoracotomy (A), transmyocardial implant (TMI) (B), and holmium yttrium:aluminum:garnet transmyocardial laser revascularization (TMR) (C). Note the significantly greater blue-staining intensity, characteristic of endothelial cells, in (C) compared with (B) and (A). The number of blue-staining blood vessels is greater both within and adjacent to the TMR channel remnant (C) compared with that seen with TMI (B).
However, several studies have suggested that the angiogenic response to TMR is a non-specific response to injury, which may be produced using mechanical means. Malekan et al. (16) found no significant difference in vascular density in normal ovine myocardium four weeks after treatment with CO₂ laser or mechanical drill, both of which significantly increased vascular density over untreated control regions. Likewise, in chronically ischemic porcine hearts, Chu et al. (17) found a similar increase in vascular endothelial growth factor (VEGF) protein levels as well as vascular density one week after TMR using either a CO₂ laser or 18-gauge hypodermic needle. A follow-up study from this same laboratory found an increase in angiogenic growth factor expression and neovascularization up to four weeks after transmyocardial revascularization using an 18-gauge needle (18).

The most distal experimental end point for assessing therapeutic angiogenesis and arteriogenesis is assessment of regional function and perfusion, because these represent the ultimate desired effect of angiogenic interventions (25). However, no study to date has compared functional data, including measurements of regional perfusion and function, after laser and mechanical revascularization. In addition, there are no long-term studies (>4 weeks) comparing mechanical and laser therapies. Consequently, the purpose of the present study was to compare the long-term angiogenic and arteriogenic response in the chronically ischemic heart after treatment with a novel TMI device or holmium:YAG TMR. The device used (Fig. 2) has previously been demonstrated safe in a pilot study in non-ischemic swine (20). The present study demonstrates that the mechanical TMI device employed does not significantly improve regional MBF, function or vascular density six months after treatment in hibernating porcine myocardium. On the contrary, consistent with prior experimental work (12–14,28), there was a significant increase in MBF by PET and contractile reserve by DSE six months after holmium: YAG TMR. These functional improvements were accompanied by significant neovascularization in the lased regions. No changes in MBF, function, or vascular density were seen in control animals undergoing sham redo-thoracotomy.

**Mechanical versus laser tissue effects.** Angiogenesis refers to the sprouting of new capillaries from pre-existing ones and is mainly caused by hypoxia and mediated via activation of hypoxia-inducible factor (HIF-1α), which serves to increase transcription of VEGF and its receptors and stabilize VEGF mRNA (30). Arteriogenesis, on the other hand, is the growth of arteries from pre-existing arterioles, and it is the only relevant type of vascular growth capable of carrying significant blood flow (31). Primary arteriogenic stimuli include shear stress and inflammation where an...
invasion of monocytes and other white blood cells leads to the production of growth factors such as the fibroblast growth factors with subsequent vascular growth (30–32). Mechanical means of performing transmyocardial revascularization produce tissue effects generally confined to their path through the myocardium. Lasers on the other hand, produce a zone of reversible injury distant from the laser channels (33), the degree of which varies with the type of laser used (34). Consequently, one might hypothesize that the use of laser energy, with its increased inflammatory response, might yield greater arteriogenesis compared with mechanical means. This is supported by the finding in the present study of a twofold increase in the number of arterioles in the lased regions compared with those treated with the mechanical implant device or sham thoracotomy. Because nearly equal numbers of TMI and TMR channels were placed within the ischemic regions, the “efficiency” of TMR, described as the amount of arteriogenesis for a given number of channels (18), appears to be superior to that of mechanical transmyocardial revascularization.

Two recent studies support this hypothesis. The first was a comparison of holmium:YAG, CO2, and xenon chloride excimer lasers performed in our laboratory (26). Both holmium:YAG and CO2 are infrared lasers that use thermal ablation to create transmyocardial channels. Excimer lasers, in contrast, are “cold” lasers that operate deep within the ultraviolet spectrum and produce tissue ablation via dissociation of molecular bonds (26). Consequently, excimer lasers are more purely ablative and produce less damage of surrounding myocardium than the infrared lasers, similar to mechanical means of transmyocardial revascularization.

Consistent with the results of the present work, that study (35) demonstrated that the holmium:YAG and CO2 lasers produced a greater neovascularization response in ischemic porcine myocardium than excimer laser TMR. Additionally, Hamawy and colleagues (36) have demonstrated a dose response to the number of channels produced with an excimer laser. Their study (36), which found no increase in perfusion four weeks after treatment of a given area of ischemic porcine myocardium with 10 or 25 excimer-lased channels but a significant increase when the same area was treated with 50 channels, also supports the theory that the efficiency of infrared laser TMR is greater than that of mechanical means, as a greater number of channels were needed to produce a given level of neovascularization.

Finally, one might hypothesize that, because infrared laser TMR appears to require fewer channels than mechanical means to produce a given degree of angiogenesis, the larger number of mechanical punctures might ultimately result in greater scarring and less functional improvement.

Study limitations. Limitations of the present study are several. First, the number of animals per group is somewhat small, and consequently the possibility of a type II error exists. Specifically, the trend toward improved regional myocardial perfusion in the mechanical TMI group potentially might have reached significance had the pattern held up in a larger number of animals. However, the complete absence of improvement in regional function in the TMI group (unlike TMR) would suggest that the lack of statistical significance in MBF is accurate.

Another limitation is that the presence of the LV metal implants in the TMI group made blinding of their follow-up echocardiograms impossible, thus allowing for the potential introduction of observer bias. Finally, the physiologically significant angiogenesis and arteriogenesis seen after laser TMR in the present study, although in agreement with prior experimental work (12–14,28,36), does contrast with the results of human trials that have not consistently demonstrated improvements in regional perfusion or function after TMR (1,3,4). The reasons for this discrepancy are not entirely clear but may relate to differences between the animal model utilized and human subjects. Unlike the human subjects with severe multi-vessel disease enrolled in the clinical trials, the experimental model used in this and most other preclinical studies of pro-angiogenic therapies is one of single-vessel disease where the remaining vessels are normal and thus potentially more able to form collateral vessels capable of improving MBF to the ischemic regions.

Conclusions. This study demonstrates that the mechanical TMI device tested does not significantly improve regional MBF, function, or vascular density six months after treatment in hibernating porcine myocardium. On the contrary, TMR with a holmium:YAG laser did increase regional myocardial perfusion and improve function while being accompanied by a significant arteriogenic response as demonstrated on immunohistochemical staining. Because both therapies were administered in relatively equivalent “doses,” the results suggest that infrared laser may be more efficient than mechanical therapies for the induction of therapeutic angiogenesis and arteriogenesis in the ischemic heart.

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