EXPERIMENTAL STUDIES

Early Postoperative Changes in Regional Systolic and Diastolic Left Ventricular Function After Transmyocardial Laser Revascularization

A Comparison of Holmium:YAG and CO₂ Lasers

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OBJECTIVES

The purpose of this study was to determine the short-term effects of transmyocardial laser revascularization (TMR) on regional left ventricular systolic and diastolic function, myocardial blood flow (MBF) and myocardial water content (MWC).

BACKGROUND

Clinical studies of TMR have noted a significant incidence of cardiac complications in the early postoperative period. However, the early post-treatment effects of laser therapy on the myocardium and their potential contribution to postoperative cardiac morbidity are unknown.

METHODS

Swine underwent holmium:yttrium-aluminum-garnet (holmium:YAG) (n = 12) or carbon dioxide (CO₂) (n = 12) laser TMR. Regional systolic function for the lased and nonlased regions was quantitated using preload recruitable work area (PRWA) and regional diastolic function with the ventricular stiffness constant alpha.

RESULTS

Preload recruitable work area was significantly decreased in the lased regions both 1 (59.8 ± 13.0% of baseline, p = 0.02) and 6 h (64.2 ± 9.4% of baseline, p = 0.02) after holmium:YAG TMR. This decreased PRWA was associated with a significant reduction in MBF to the lased regions (13.2% reduction at 1 h, p = 0.02; 18.4% decrease at 6 h post-TMR, p = 0.01). These changes were not seen after CO₂ laser TMR. A significant increase in MWC (1.4 ± 0.3% increase with holmium:YAG, p = 0.004; 1 ± 0.2% increase with CO₂, p = 0.002) and alpha (217.4 ± 44.2% of baseline 6 h post-holmium:YAG TMR, p = 0.05; 206 ± 36.7% of baseline 6 h post-CO₂ TMR, p = 0.03) was seen after TMR with both lasers.

CONCLUSIONS

In the early postoperative setting, impaired regional systolic function in association with regional ischemia is seen after TMR with a holmium:YAG laser. Both holmium:YAG and CO₂ lasers are associated with increased MWC and impaired diastolic relaxation in the lased regions. These changes may explain the significant incidence of early postoperative cardiac morbidity. The impact of these findings on anginal relief and long-term outcome are not known. (J Am Coll Cardiol 2000;35:1022–30) © 2000 by the American College of Cardiology

Transmyocardial laser revascularization (TMR) is emerging as a potential treatment option in end stage coronary artery disease (1–8). The procedure involves using a high powered laser to create transmural channels from the epicardial to endocardial surface of the left ventricular free wall in regions of chronically ischemic yet viable myocardium. Most commonly, the procedure is performed through a small anterolateral thoracotomy although reports of thoracoscopic TMR are increasing (9,10). In addition, the procedure has been suggested as a potential treatment option in an expanding array of clinical settings including cardiac allograft vasculopathy (11,12) and for use in combined procedures with percutaneous transluminal coronary angioplasty (PTCA) (13), coronary artery bypass grafting (CABG) (6,13) and valve replacement (14). Clinical studies have consistently demonstrated significant reductions in anginal class after the procedure although these benefits are usually not apparent until at least 3 months postoperatively (1–7). The mechanism of action of TMR may potentially be mediated via angiogenesis (15,16), and the time needed for new blood
vessel growth might account for the delay in clinical improvement after the procedure.

Because the benefits of TMR are delayed, patients undergoing the procedure appear to be at high risk for postoperative complications. Clinical studies of TMR have noted the majority of these adverse events to be cardiac in nature, primarily ischemia and congestive heart failure (1–4,8,13–19). However, the degree to which these complications potentially stem from direct myocardial injury after laser application is unknown. Consequently, the purpose of this study was to determine the early postoperative effects of TMR on regional left ventricular systolic and diastolic function, myocardial blood flow (MBF) and myocardial water content (MWC). Because most clinical studies to date have used either carbon dioxide (CO$_2$) (1–4,7,8,13) or holmium:yttrium-aluminum-garnet (holmium:YAG) (5,6) laser energy for channel production and because both are now FDA approved for the treatment of medically refractory angina in patients with end stage coronary disease, these were chosen for this study.

**METHODS**

A total of 24 crossbred swine (50 kg) were used. Animals were obtained from Walnut Hill Farms (Hillsborough, North Carolina), 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 care 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 1985).

Experimental preparation. All animals underwent induction of anesthesia with ketamine (22 mg/kg IM) and diazepam (10 mg IV). Orotracheal intubation was performed and anesthesia maintained with continuous infusions of diazepam (15 mg/h IV) and fentanyl (30 µg/kg/h IV) while the animals were mechanically ventilated (20). Additional doses of IV fentanyl and diazepam were given throughout the experiment as needed. Bretylium tosylate (5 mg/kg IV) was given preoperatively to prevent cardiac arrhythmias (20). An 8 Fr introducer sheath was placed in the left carotid artery with its tip in the aorta. The sheath was used for continuous arterial blood pressure monitoring during the experiment and as the reference standard withdrawal port during injection of radioactive microspheres as described below. A 16-gauge angiocatheter was placed in the left anterior jugular vein for administering drugs and fluids. A median sternotomy was performed and the heart suspended in a pericardial cradle. Pneumatic occluders were placed about the superior and inferior vena cavae. Pairs of ultrasonic dimension transducers were implanted in the subendocardium of the anterolateral (region to be lased) and posterolateral (control region) left ventricular free walls to assess regional myocardial segment length. Transducers were aligned 10 to 15 mm apart along the circumference of the minor axis of the left ventricle. A flexible 19-gauge catheter was placed in the base of the left atrial appendage for microsphere injections (Fig. 1). A micromanometer (Millar Instruments Inc., Model PC-350, Houston, Texas) was balanced, calibrated and passed into the left ventricle via the apex. Continuous electrocardiographic monitoring was used throughout the procedure to ensure a stable cardiac rhythm. Serial blood gas analysis was used to ensure adequate oxygenation and ventilation. Ventilator settings were adjusted to maintain pH 7.35–7.45 and pO$_2$ ≥ 100 mm Hg. Body temperature was monitored and maintained at 37°C. Ringer’s lactate was infused at a rate of 5 ml/kg/h during the course of the experiment (20).

Regional function data collection and analysis. Regional systolic contractile function was assessed using the preload recruitable work area (PRWA), a load insensitive index of regional myocardial performance (21,22). Data were collected at baseline, 1 and 6 h after TMR in anesthetized animals. Data collection was performed under steady-state conditions during rapid venous caval occlusion to obtain data over a range of loading conditions.

The analog data were filtered by a 50 Hz low-pass filter and digitized in real time at an eight-channel sweep rate of 200 Hz by an analog to digital converter (ADAC model 1012, Woburn, Massachusetts). After data collection, analysis was performed on a microprocessor (MicroVAX II/GPX Work Station, Digital Equipment Corp., Maynard, Massachussets) with custom interactive programs (Physiologic Monitoring Inc., Durham, North Carolina). The first derivative of left ventricular transmural pressure (dP/dt) was calculated from the digital pressure waveform as a running five-point polyorthogonal transformation.

The cardiac cycle was defined automatically using dP/dt...
as described previously (21,22). Diastole was defined as beginning 15 ms after the first zero crossing of dP/dt after peak negative dP/dt and ending 15 ms before the beginning of the systolic upstroke of left ventricular pressure. Beginning ejection was placed at peak positive dP/dt and end ejection defined as peak negative dP/dt. Beat point definitions were visually confirmed on all data with a videographics display system.

An analog of segmental stroke work (SW) was then calculated as the integral of left ventricular pressure (P) and myocardial segment length (L) over each cardiac cycle according to the following equation:

$$SW = \int PdL.$$  

For each vena caval occlusion, linear regressions were performed on data from 10 beats before onset of vena caval occlusion to achievement of steady state at end vena caval occlusion (<0.5% beat-to-beat change in segment length at beginning diastole). Only data where heart rate changed by <10% were considered.

Data obtained during transient vena caval occlusion were fitted to the regional preload recruitable stroke work (PRSW) relationship relating SW to end-diastolic segment length (EDL) (21):

$$SW = M_{SW} (EDL - L_W)$$

where $M_{SW}$ and $L_W$ are the slope and relationship x-intercept, respectively. Preload recruitable work area, a normalized index of contractility defined as the area under the SW versus EDL regression line, was calculated from the formula:

$$PRWA = M_{SW}/2 (1.2 L_{Wmax} - L_W)^2$$

where $L_{Wmax}$ is the maximal x-intercept obtained for a given myocardial segment over the entire experiment. The factor 1.2 was chosen so that PRWA was always positive but still diminished significantly as $L_W$ approached $L_{Wmax}$ (21,22).

Steady-state pressure and segment length relations during diastole were used to quantify regional diastolic function. Regional segment length (l) was normalized as Lagrangian strain (epsilon) using the equation (23):

$$\varepsilon = (l - l_0)/l_0$$

where $l_0$ is the unstressed segment length obtained from maximal vena caval occlusion. Static pressure-strain relations during the passive filling phase of diastole were then fitted to a monoexponential equation:

$$P = \beta e^{\alpha t} + P_0$$

where P is left ventricular pressure and both $\alpha$ and $\beta$ are constants that reflect regional material properties. For the purposes of this study, $\alpha$ was chosen to compare changes in regional myocardial stiffness during diastole (24,25).

All steady-state data are reported as mean values over 10 to 20 cardiac cycles. Slopes, intercepts and linear correlation coefficients were derived from least squares regression procedures.

MBF measurements. Myocardial blood flow in the lased and control regions was determined 1 and 6 h after TMR by injecting radionuclide-labeled microspheres by the reference withdrawal method (26). For each blood flow determination, approximately $1 \times 10^6$ microspheres of 15 μm diameter labeled with one of two different isotopes ($^{95}$Nb, $^{103}$Ru) (New England Nuclear, Boston, Massachusetts) were injected via the left atrial line. Each dose of microspheres was injected via the left atrial catheter within approximately 5 s and then flushed with 10 cc of saline. The arterial reference sample was withdrawn via the carotid catheter at a constant rate of 5 ml/min beginning before microsphere injection and continuing for 3 min. Microsphere suspensions were vortex agitated followed by warm water sonication for at least 30 min before injection, according to manufacturer recommendations. Injections of microspheres presented herein resulted in no changes in heart rate, blood pressure, left ventricular pressure or left ventricular wall dimensions. The myocardial and reference blood samples were counted in a Packard Auto-Gamma
eral wall region containing the TMR channels was excised, post-TMR data. Hearts were harvested and the anterolateral wall region was excised under general anesthesia after collection of the 6-h tissue water content measurements. Animals were sacrificed to avoid all epicardial and intramyocardial vessels. Carbon dioxide laser channels were created using a single 40 J pulse (50 ms pulse duration), whereas holmium:YAG channels were created using multiple 2 J pulses, with a total energy level of approximately 20 J per channel. All laser settings were in accordance with manufacturer recommendations. Transmural penetration of laser channels was confirmed by visible spurting of blood from the channels during systole. Hemostasis was obtained by manual compression. One and 6 h post-TMR, measurements of regional myocardial function and MBF were collected as described above.

Tissue water content measurements. Animals were sacrificed under general anesthesia after collection of the 6-h post-TMR data. Hearts were harvested and the anterolateral wall region containing the TMR channels was excised, blotted dry and weighed. The presence of transmural penetration of the laser channels was confirmed grossly at this time. The nonlased posterolateral wall control region was likewise excised and weighed. After measurement of the wet weight of the lased and control regions, they were dried at 65°C for 72 h and reweighed (27). Myocardial water content (MWC) was calculated as follows (28):

\[
\text{MWC} = \frac{\text{wet weight} - \text{dry weight}}{\text{dry weight}} \times 100\%
\]

Statistical analysis. All data were analyzed in a blinded fashion. Statistical comparisons were performed using STATISTICA for windows version 5.1 (StatSoft, Inc., Tulsa, Oklahoma). All data is presented as the mean ± standard error. Serial measurements of hemodynamic data, PRWA and regional diastolic stiffness over time throughout the experiment were compared using a one-way analysis of variance (ANOVA) test. When overall significance was found within the ANOVA, Newman-Keuls test was used to delineate which comparisons were significantly different (29). Values of MBF and water content were compared for lased and nonlased regions using a paired Student t test. Changes in percent tissue water content were compared between holmium:YAG and CO2 lased regions using an unpaired Student t test. Statistical significance was considered a p value <0.05.

RESULTS

Hemodynamics. Mean baseline, 1 h and 6-h post-TMR global hemodynamic and arterial pH data for all animals is presented in Table 1. As analyzed using a one-way ANOVA, there was no significant difference in any of the hemodynamic parameters measured at any time point during the study. This held true when all of the animals were grouped together, as in Table 1, and when the holmium:YAG and CO2 groups were compared individually (data not shown).

Regional left ventricular function. Digitized data for a representative study at baseline, 1 h and 6 h post-TMR is shown in Figure 2. Representative regional SW versus end-diastolic segment length curves used to derive PRWA are shown in Figure 3. Preload recruitable work area, a load-insensitive index of regional myocardial contractile function, was significantly reduced in the lased regions at both 1 and 6 h after TMR with a holmium:YAG laser (Fig.

<table>
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<th>Table 1. Hemodynamic and pH Data*</th>
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<td>Heart Rate</td>
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*There was no statistically significant difference between any of the measured parameters at any time point during the study.

LVEDP = left ventricular end-diastolic pressure; MAP = mean arterial pressure; TMR = transmyocardial laser revascularization.
There was no change in the PRWA of control nonlased regions in the holmium:YAG group. Likewise, there was no significant change in the PRWA of the lased or control regions after TMR with a CO₂ laser (Fig. 4B).

Representative regional steady-state pressure-strain relations during diastole for the lased region in a single animal at baseline, 1 h and 6 h post-TMR are shown in Figure 5. By 6 h postoperatively, regional diastolic relaxation was significantly impaired in regions treated with both holmium:YAG and CO₂ lasers (Fig. 6). In both groups, alpha 6 h post-TMR was more than double its baseline value, indicating a two-fold increase in regional myocardial stiffness. No change in diastolic relaxation was seen in the nonlased regions of hearts treated with either laser.

**Regional MBF.** Absolute values of MBF per g dry tissue weight for the lased and nonlased regions are presented in Table 2. There was a statistically significant decrease in transmural MBF in the regions lased with a holmium:YAG laser both 1 and 6 h post-TMR. This reduction in transmural blood flow (13.2% decrease at 1 h and 18.4% decrease at 6 h post-TMR) potentially explains the observed impairment in systolic function, as evidenced by a significant decrease in PRWA, in the holmium:YAG lased regions. No significant change in MBF was seen in the CO₂ treated regions, which correlates with the functional data as well since no change in PRWA was seen in the CO₂ lased hearts.

**Tissue water content.** Changes in MWC after TMR are presented in Table 3. Values of MWC in the nonlased control regions are consistent with normal values found in pigs by prior investigators (25,28). Overall, there was a 1.4 ± 0.3% increase in MWC in regions lased with a holmium:YAG laser over the corresponding nonlased regions. Likewise, there was a 1 ± 0.2% increase in tissue water in CO₂ lased regions. The increased myocardial edema in regions treated with holmium:YAG versus CO₂ laser trended towards, but did not reach, statistical significance (p = 0.13). These findings likely explain the impaired diastolic relaxation observed in the lased regions after TMR with both lasers (25,28).

**DISCUSSION**

Transmyocardial laser revascularization is emerging as a potential treatment strategy for thousands of patients with ischemic heart disease not amenable to CABG or PTCA. However, despite the apparently increasing use of the procedure, the direct effects of the more commonly used lasers on myocardial function have been incompletely characterized. Prior studies of post-TMR function have used either load sensitive indexes of contractile performance without specifically controlling loading conditions (8,30,31), channel densities much greater than utilized clinically today (30–32) or have created nontransmural channels (30,32). In addition, no study to date has examined MWC and diastolic function after TMR. This study is the first to fully describe the early postoperative effects of both holmium:YAG and CO₂ lasers on regional left ventricular systolic and diastolic function, MBF and tissue water content. The major findings of the study are that, in the early postoperative setting, TMR using both holmium:
YAG and CO₂ lasers results in myocardial edema and impaired diastolic relaxation in the lased regions. Secondly, impaired regional systolic function, in association with regional ischemia, is seen in the treated regions after TMR with the holmium:YAG laser, but not the CO₂ laser.

**Laser-specific tissue effects.** The word “laser” is an acronym for light amplification by stimulated emission of radiation, and laser systems are defined by their physical parameters including wavelength, energy and pulse duration (33,34). Carbon dioxide lasers operate at a wavelength of 10.6 μm, which is highly absorbed by water, whereas the wavelength of holmium:YAG is 2.1 μm. This shorter wavelength is much less absorbed by water. The CO₂ laser, because of its high coefficient of absorption, produces relatively confined photothermal ablative effects in myocardium with only a small zone of lateral thermal necrosis.
Holmium:YAG lasers, on the other hand, work via both photothermal and photoacoustic effects, are less purely ablative and produce greater lateral thermal damage than CO₂ lasers (33,35). Based on the known tissue effects of these lasers, one hypothesis to explain the findings of this study is that the holmium:YAG laser, with its greater lateral thermal damage, produced regional ischemia via coagulation of myocardial arterioles and capillaries in the lased region after absorption of peripheral laser energy (30). The CO₂ laser, because of its more confined ablative effects, may have produced less regional small vessel damage and, consequently, no measurable change in regional MBF or systolic function. As with holmium:YAG, the CO₂ laser did produce enough tissue damage to lead to a significant increase in tissue water content in the lased regions along with impaired diastolic relaxation.

Perioperative cardiac morbidity post-TMR. Clinical studies of TMR have noted a relatively high incidence of cardiac complications in the early postoperative period (1–8,13,17–19), with the majority of this morbidity occurring in the first 24 h after surgery. Several authors (5,8,13,19) have hypothesized that these adverse cardiac events may stem from myocardial edema after the procedure although this concept was previously unproven. In addition, early postoperative myocardial ischemia has been noted to occur relatively frequently post-TMR (18), and yet the possibility of laser treatment as a potential cause of this ischemia has not been described. Patients with preoperative unstable angina and congestive heart failure (1,8,19) have been noted to experience significantly greater post-TMR morbidity and mortality than stable patients. Based on the findings of this study, it seems logical that when laser induced injury is superimposed on significant preexisting left ventricular dysfunction, worsening heart failure with its associated morbidity results. In addition, these findings suggest that TMR with a holmium:YAG laser might be expected to produce greater early postoperative cardiac dysfunction than CO₂ laser TMR due to the associated changes in both systolic and diastolic function versus diastolic dysfunction only with CO₂. The published experience with holmium:YAG TMR to date is relatively small (5,6) compared with CO₂ laser (1–4,7,8,13), and, consequently,
it is too early to tell whether the findings of this study will be reflected in the clinical setting. Finally, although the holmium:YAG laser appears to affect systolic as well as diastolic function in the early postoperative period, it is unknown whether these changes will have any adverse effects on the long-term functional outcome of the procedure. The experience with the CO₂ laser suggests that short-term changes in diastolic function do not adversely affect the clinical outcome and benefits of the procedure. Randomized trials comparing the CO₂ and holmium:YAG lasers have not been performed, and further research in this area is needed.

Implications for postoperative management. If the findings of this study can be extrapolated to the human clinical arena, one might anticipate that the use of perioperative diuretic therapy may potentially minimize myocardial edema and diastolic dysfunction. This is supported by a recent study from our institution demonstrating improved outcomes for TMR patients receiving a furosemide infusion beginning in the immediate postoperative period (19). In addition, a recent study found a mortality benefit for patients with unstable angina and reduced ejection fraction undergoing TMR who had an intra-aortic balloon pump placed preoperatively (8), suggesting the balloon pump may help compensate for transient laser-induced myocardial ischemia and dysfunction. Whether the laser-induced dysfunction becomes clinically relevant likely depends on the extent of myocardium lased and the functional status of the nonlased areas and their potential to compensate for impaired function in the lased regions.

Study limitations. A limitation of this study is that TMR was performed in normal myocardium rather than the chronically ischemic conditions under which the technique is applied clinically. Prior work in infarcted human myocardium has shown that higher laser energy was required to produce lesions comparable in size to those in normal tissue (36). Whether this also applies to the chronically ischemic, yet viable, myocardium typically treated with TMR is not known. However, histologic findings, including the degree of collateral thermal damage, after TMR are essentially identical in normal and chronically ischemic myocardium (37), suggesting the functional changes observed in this study might also be expected to occur in chronically ischemic hearts. A second limitation is that the time course of the observed systolic and diastolic dysfunction was not studied. However, one might assume the laser induced injury to be reversible given the period of functional decline observed in human studies has peaked around 24 h postoperatively and improved thereafter (8,13,18).

In summary, TMR with both holmium:YAG and CO₂ lasers induces regional myocardial edema and diastolic dysfunction in the early postoperative period. In addition, TMR with a holmium:YAG laser causes regional systolic dysfunction, possibly secondary to an associated reduction in MBF. These functional changes likely contribute to the adverse cardiac events often observed after the procedure. Aggressive perioperative medical regimens aimed at reducing myocardial edema and ischemia may help limit any associated morbidity occurring as a result of laser induced injury. The impact of these observed functional changes on angina relief and long-term outcome are not known.

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| Table 2. Transmural MBF* in Lased and Nonlased Regions 1 and 6 h post-TMR |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | Nonlased 1 h    | Lased 1 h       | Nonlased 6 h    | Lased 6 h       |
|                  | Post-TMR       | Post-TMR        | Post-TMR       | Post-TMR        |
| Holmium:YAG     | 6.6 ± 1.1 †     | 5.8 ± 1.1 †     | 6.0 ± 0.9 †    | 4.9 ± 0.5 †     |
| CO₂             | 8.0 ± 1.0       | 8.0 ± 0.8       | 8.5 ± 1.3      | 8.1 ± 1.2       |

*Myocardial blood flow expressed as ml/min/g dry tissue weight. †p = 0.02 for lased versus nonlased regions; ‡p = 0.01 for lased versus nonlased regions.

CO₂ = carbon dioxide; holmium:YAG = holmium:yttrium-aluminum-garnet; MBF = myocardial blood flow; TMR = transmyocardial laser revascularization.

| Table 3. Mean MWC in Lased and Nonlased Regions 6 h Post-TMR |
|-----------------|-----------------|-----------------|-----------------|
|                  | % MWC Nonlased Regions | % MWC Lased Regions | % Change | p Value |
| Holmium:YAG     | 80.0 ± 0.3       | 81.5 ± 0.5       | 1.4 ± 0.3    | 0.004 |
| CO₂             | 80.1 ± 0.5       | 81.0 ± 0.5       | 1.0 ± 0.2    | 0.002 |

CO₂ = carbon dioxide; holmium:YAG = holmium:yttrium-aluminum-garnet; MWC = myocardial water content; TMR = transmyocardial laser revascularization.
REFERENCES


