Transmyocardial Laser Revascularization Remodels the Intrinsic Cardiac Nervous System in a Chronic Setting

R.C. Arora, MD; G.M. Hirsch, MD; K. Hirsch, MD; J.A. Armour, MD, PhD

Background—Prospective trials have demonstrated that transmyocardial laser revascularization (TMLR) imparts symptomatic relief to patients with refractory angina. Because peak clinical effectiveness of TMLR is usually delayed by several months, it has been proposed that ventricular denervation is one mechanism whereby TMLR imparts symptomatic relief. We have demonstrated that TMLR does not denervate the heart in the acute setting, nor does it modify the intrinsic cardiac nervous system (ICNS) in the acute setting. However, the long-term effects of TMLR on the ICNS remain unknown.

Methods and Results—A holmium:yttrium-aluminum-garnet laser created 20 channels through the anterolateral left ventricular free wall of 10 dogs. Four weeks later, the function of cardiac sensory inputs to the ICNS was studied by applying veratridine (7.5 μmol/L) to ventricular sensory fields. Chronotropic and inotropic responses elicited by cardiac sympathetic or parasympathetic efferent neurons stimulated electrically (10 Hz, 4 V, 4 ms) or chemically (nicotine 5 to 20 μg/kg IV) were also assessed. Chemical activation of epicardial sensory neurites with veratridine elicited expected ICNS excitatory responses. Electrical stimulation of sympathetic and parasympathetic efferent neurons induced expected altered cardiac responses. In contrast, the responsiveness of the ICNS to systemically administered nicotine was obtunded.

Conclusions—Although chronic TMLR does not affect cardiac afferent or extracardiac efferent neuronal function, it does “remodel” the ICNS so that its responsiveness to a known potent chemical agonist (ie, nicotine) becomes obtunded. Remodeling of the ICNS may account in part for the delayed symptomatic relief that TMLR imparts to patients with refractory angina. 

Key Words: nervous system, autonomic \ angiotensin \ conduction \ contractility

Patients with anatomically diffuse, end-stage coronary artery disease not amenable to standard revascularization procedures typically suffer from intractable angina (Canadian Cardiology Society [CCS] functional class 3 to 4) despite maximal medical therapy. Several prospective studies have demonstrated that the creation of multiple transmural channels in the heart by means of a laser (transmyocardial laser revascularization, TMLR) provides symptomatic relief of refractory angina in such patients. Channels produced by TMLR in the wall of an ischemic ventricle were initially thought to remain patent after the procedure, thereby permitting blood from the chamber to reach underperfused cardiomyocytes by forming direct communications between the chamber and ventricular blood vessels. Recently, this theory has been challenged with the demonstration that such channels are only transiently patent. An alternate hypothesis proposed is that nonspecific laser-induced myocardial damage may involve local neuronal tissue. It has been proposed that such injury damages local ventricular sensory neurites and autonomic efferent axons, thereby producing local cardiac denervation and thus anginal relief. However, we have shown that TMLR does not destroy cardiac afferent or efferent neuronal function in an acute setting.

TMLR imparts symptomatic relief of ≥2 CCS anginal classes in 25% to 76% of patients 1 year after TMLR. Such symptomatic relief usually is evidenced by a reduction in nitrate usage, increased exercise tolerance and higher quality-of-life scores. Although TMLR does not modify the cardiac nervous system on a short-term basis, it remains to be established whether TMLR induces delayed effects on this system. This is particularly relevant with respect to its effects on the intrinsic cardiac nervous system (ICNS).

Intrinsic cardiac neurons are situated in ganglionated plexuses located in discrete atrial and ventricular regions. In the canine and human models, these ganglionated plexuses contain afferent, efferent, and local circuit neurons that allow for “fine tuning” of cardiac function on a beat-to-beat basis. As such, the ICNS acts as the final common regulator of regional cardiac function.

To determine whether TMLR imparts delayed effects on the function of the cardiac nervous system, the effects of...
TMLR were studied in a chronic, nonischemic canine model. The nonischemic model was investigated to avoid the confounding effects that ischemia has on the ICNS.16,17 This was done by (1) activating local sensory neurites associated with intrinsic cardiac afferent neurons with the topically applied ion-modifying agent veratridine; (2) electrical activation of sympathetic and parasympathetic efferent neurons that modulate regional ventricular dynamics; (3) chemically activating sympathetic and parasympathetic efferent neurons via systemic administration of nicotine; (4) chemically activating sympathetic efferent neurons via systemically administered angiotensin II and; (5) testing the capacity of laser-treated ventricular muscle to respond to an exogenously applied β-adrenergic receptor agonist. All interventions were performed 1 month after the TMLR procedure or sham operation. In this manner, we assessed the long-term effects that TMLR exerts on not only local ventricular cardiomyocyte function but also the cardiac nervous system.

Methods

Animal Preparation

Adult mongrel dogs (n = 14) of either sex, weighing between 25 and 30 kg, were used in this study. All experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (1996) and were approved by the institutional animal care and use committee of Dalhousie University. The dogs were sedated with a mixture of morphine (0.5 mg/kg IM), atropine (0.2 mg/kg IM), and acepromazine (0.1 mg/kg IM). After endotracheal intubation, general anesthesia was maintained to effect halothane. Four animals underwent a sham operation consisting of a thoracotomy and pericardotomy as described below.

Laser Therapy

With sterile surgical technique, 10 dogs had the pericardium exposed via a limited left-sided thoracotomy. After a pericardial incision to expose the left ventricle, 20 separate channels ~1 mm in diameter were created that penetrated through the ventral-lateral wall of the left ventricle in a 4X4-cm epicardial region. This was done with a holmium:yttrium-aluminum-garnet laser (λ = 2.1 μm; pulse length = 250 μs) (Eclipse Surgical Technologies, model TMR 2000). These channels were created in the distribution of the terminal left anterior descending and diagonal coronary arteries as in our short-term study.12 An average of 7 to 9 pulses over 2 to 4 seconds was required to traverse the left ventricular wall. Digital pressure was applied to the epicardial openings of transmural channels sites, when required, to achieve homeostasis. If ineffective, a figure-of-8 suture was placed around the epicardial opening. The creation of transmural channels was noted intraoperatively by the change in the pitch of the channels was noted intraoperatively by the change in the pitch of the channels was noted intraoperatively by the change in the pitch of the

Electrical Stimulation of Autonomic Efferent Neurons

Stellate ganglia were decentralized and bipolar electrodes were placed around them so that they could be stimulated electrically later in the experiments. Acutely decentralized right and left stellate ganglia were stimulated individually (10 Hz, 5 ms, 4 V) for 20 seconds. The distal ends of the sectioned right or left cervical vagi were stimulated individually for 10 seconds (20 Hz, 5 ms, 4 V). The bipolar stimulating electrodes (electrode tips 5 mm apart) were connected to a Grass SD-9 square wave stimulator, the output of which was monitored on a Telequipment D-54 oscilloscope.
Alterations in Recorded Variables by Various Interventions in the Chronic TMLR Model

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<tr>
<th>Intervention</th>
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<td>HR, bpm</td>
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<td>Neurons, impulse/min</td>
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| All 0.01 | 116.3±12.0 | 113.3±10.5 |
| All 5    | 111.0±8.2  | 137.0±13.9 |
| Isoproterenol | 110.5±5.0 | 164.0±7.3 |
| RSG      | 117.5±7.1  | 210.0±11.3 |
| LSG      | 121.0±7.8  | 186.0±11.3 |
| RV       | 117.8±7.0  | 72.2±7.5  |
| LV       | 119.4±7.6  | 58.3±6.8  |

HR indicates heart rate; LVP, left ventricular pressure; LVIMP, left ventricular intramyocardial pressure; All 0.01, angiotensin II 0.01 mg/kg; All 5, angiotensin 5 µg/kg; RSG, right stellate ganglion; LSG, left stellate ganglion; RV, right ventricle; and LV, left ventricle.

*P<0.05

Administration of Pharmacological Agents

Veratridine 7.5 µmol/L was applied for 30 seconds to epicardial loci overlaying the affected left ventricular TMLR zone with the use of 2×2-cm gauze squares soaked with 2 mL of the chemical. After removal, the epicardial locus was washed with normal saline for 30 seconds (~2 mL/s). At least 10 minutes was allowed to elapse before the next intervention. Gauze squares soaked with room-temperature normal saline were applied to the same epicardial sensory fields to determine whether neuronal responses elicited by epicardial chemical application were due to vehicle effects or the mechanical effects elicited by gauze squares.

The following 3 chemicals were then administered individually into the systemic circulation: (1) nicotine (5 to 20 µg/kg IV), a chemical that binds to cholinergic nicotinic receptors, thereby activating sympathetic and parasympathetic efferent postganglionic neurons; (2) angiotensin II (0.01 to 5 µg/kg IV), a chemical that binds to AT1 receptors associated with intrinsic cardiac neurons; and (3) the β-adrenoceptor agonist isoproterenol (5 µg IV), which acts directly on the myocyte. These agents were then administered to determine whether neuronal responses elicited by gauze squares were related to the chemical stimulation.

Echocardiographic Analysis

Echocardiographic assessment of left ventricular wall motion was performed in 2 animals before and during stellate ganglion stimulation with an Ultramark 8 Ultrasound Systems echocardiographic apparatus (model UM-8-OPO1, Advanced Technology Laboratory). The cross-sectional midpapillary view of the left ventricle was obtained via an Access C ultrasound probe placed on the free wall of the left ventricle.

Data Analysis

Heart rate, left ventricular chamber systolic pressure, and intramyocardial systolic pressure in the normal and TMLR-treated zones of the left ventricular free wall were monitored for 1 minute before and after each of the interventions described above. The activity generated by identified right atrial neurons was analyzed for 1-minute periods before and during each intervention. Cardiac, vascular, and neuronal data obtained before and during each intervention are presented as mean±SEM. One-way ANOVA and paired t test with Bonferroni’s correction for multiple tests were used for statistical analysis. Significance values of P<0.05 or P<0.01 were used for these determinations.

Results

Laser-Treated Animals

Left ventricular dynamics responded in a normal fashion to systemically administered isoproterenol, including the zone that had undergone TMLR. Echocardiographic analysis performed in 2 dogs demonstrated normal left ventricular transverse diameters in diastole and systole during control states. These dimensions were reduced during stellate ganglion stimulation in a manner consistent with that found in normal dogs. The ventricular free wall zone that had undergone TMLR did not display any detectable wall motion abnormality.

However, the heart did not appear to respond to stress in a normal fashion. Indeed, the left ventricular systolic pressure progressively diminished throughout the experiment (during exposure of the heart and its instrumentation, left ventricular systolic pressure fell from 132±6 to 96±8 mm Hg; P<0.01). Additionally, dysrhythmias developed in 2 animals. The heart of 1 dog fibrillated during the dissection of pericardial adhesions to expose the ventricular epicardium. After heart defibrillation, baseline hemodynamic variables returned to control values. In another animal, atrial pacing was required because of the development of a third-degree heart block. In this animal, only responses to stellate ganglion stimulation and systemic administration of the chemicals were studied.

The sodium channel modifier veratridine, when applied to the epicardium over the laser-treated zone, modified the activity generated by identified right atrial neurons (increase in activity in 7 animals, decreased in 2 animals; delta neuronal change=19.0±3.1 impulses per minute; P<0.01). Reapplication of veratridine to the same epicardial region induced similar neuronal responses.

Electrical stimulation of acutely decentralized stellate ganglia enhanced all monitored cardiac indices in a normal fashion (Table). Specifically, both the laserized and nonlasered regions responded in a similar fashion to this intervention (Figure 1 and Table). Electrical stimulation of parasympathetic efferent neurons suppressed intramyocardial systolic pressure in both regions of the left ventricle to similar degrees (Table).

On the other hand, nicotine, which normally activates the ICNS, failed to alter recorded neuronal activity (Figure 2). Neuronal activity was unaffected even when supranormal doses of nicotine were administered (Figure 3). Minor alterations in recorded cardiovascular variables occurred only...
when the highest dose of nicotine (20 \( \mu g/kg \) IV) was studied. Angiotensin II, when administered in doses that normally induce intrinsic cardiac neuronal and cardiac responses in normal hearts (ie, 0.01 to 5 \( \mu g/kg \) IV), failed to elicit neuronal responses overall. When the largest dose of angiotensin II was studied (5 \( \mu g/kg \)), cardiac indexes increased a little (Table). Isoproterenol not only enhanced both inotropic and chronotropic responses but also increased ICNS activity. In this chronic model, isoproterenol enhanced recorded cardiac indexes, including left ventricular intramyocardial systolic pressures in the TMLR zone; it failed to enhance intrinsic cardiac neuronal activity (Table).

Phenol destroys epicardial nerves. To demonstrate that ventricular myocyte responses elicited by autonomic efferent neurons can be eliminated, phenol was applied in 2 animals to the epicardium surrounding the intramyocardial pressure sensor located in the treated (lasered) ventricular zone. After epicardial application of phenol, stimulation of either stellate ganglion failed to produce regional inotropic responses that were previously inducible (Figure 4A, laser). The unaffected ventricular region (not surrounded by epicardial phenol) responded to stellate ganglion stimulation in a fashion that was similar to that induced before phenol application (Figure 4A, control). To test myocyte viability independent of the ICNS, isoproterenol was exogenously administered. Both the phenol-treated and untreated ventricular regions responded similarly to this intervention (Figure 4B).

Sham-Operated Animals

Unlike the laser treated animals, the performance of the thoracotomy did not alter hemodynamics in the 4 sham animal. Inotropic and chronotropic changes elicited during stellate ganglia stimulation were similar to those in the chronic TMLR animals (right stellate ganglion: left ventricular intramyocardial pressure, control versus stimulation, 50 ± 11.3 versus 134 ± 22.5 mm Hg; heart rate, control versus stimulation, 140 ± 7.1 versus 163.7 ± 6.6 bpm [mean ± SEM]). In contrast to laser-treated animals, neuronal responses elicited with nicotine (5 \( \mu g/kg \) IV: delta neuronal change = 67.5 ± 35.0) and angiotensin II (0.01 \( \mu g/kg \) IV: delta neuronal change = 27.5 ± 11.1) were similar to those previously reported for normal controls. 12,16,20,21

Discussion

The results of the present study indicate that TMLR remodels the ICNS over time, thereby affecting its capacity to influence regional cardiac function. This occurs despite the fact that TMLR does not affect the function of cardiac sensory neurons or alter the capacity of sympathetic and parasympathetic efferent neurons to modulate regional ventricular dynamics.

Prospective studies indicate that TMLR relieves angina of cardiac origin.1–3 The mechanisms whereby this occurs remain unknown. Although up 85% of patients attain significant symptomatic improvement after TMLR, peak anginal relief is usually delayed 3 to 6 months after TMLR, regardless of the type of laser used.1–3 It has been suggested that cardiac denervation may be one mechanism whereby TMLR exerts its salutary effects.10 Kwong et al, 10 by applying high doses of bradykinin to the ventricular epicardium before and after TMLR, concluded that TMLR renders sensory neurites in the affected myocardium nonfunctional. However, direct assessment of cardiac afferent neuronal function before and after acute TMLR indicated that this is not the case.12 The cellular sodium channel modifier veratridine modifies ventricular sensory neurites associated with cardiac afferent neurons in a consistent fashion.22 The effects of veratridine on ventricular sensory inputs to intrinsic cardiac neurons after chronic TMLR were similar to those induced in normal prepa-
tions. These data indicate that the function integrity of intrinsic cardiac afferent neurons is preserved in this model of chronic TMLR.

It has been suggested that cardiac tyrosine hydroxylase immunoreactivity, an indirect measure of sympathetic efferent postganglionic axonal density, becomes reduced in ventricular regions subjected to TMLR, which equates with functional sympathetic efferent neuronal denervation. However, direct assessment of the function of cardiac sympathetic efferent neurons demonstrated that TMLR does not affect their capacity to influence ventricular tissues in an acute, nonischemic canine model. Neither did TMLR modify the
capacity of cardiac adrenergic efferent neurons to enhance regional ventricular dynamics in a chronic setting when activated electrically (Figure 1 and Table). Similarly, electrical stimulation of parasympathetic efferent preganglionic axons suppressed regional intramyocardial systolic pressures in treated and untreated regions of the left ventricular free wall to comparable degrees (Table). These data demonstrate that TMLR does not obtund the influence of extracardiac autonomic efferent neurons (with intracardiac axonal projections) on ventricular function in a chronic setting.

In contrast, regional denervation occurs after the topical application of phenol, which negates the ability of electrical activation of sympathetic efferent postganglionic neurons to enhance regional ventricular contractility. In confirmation of that, ventricular regions treated with phenol failed to respond to electrical activation of sympathetic efferent neurons even though adjacent untreated ventricular regions elicited normal responses to the stellate ganglion stimulation (Figure 4A). On the other hand, systemic administration of isoproterenol enhanced intramyocardial systolic pressure in both regions similarly (Figure 4B). These data indicate that one can functionally destroy the sympathetic efferent innervation to a region of the heart without causing detectable cardiomyocyte injury.

Despite the normalcy of cardiac afferent and extracardiac efferent neurons, intrinsic cardiac neuronal function was not normal after chronic TMLR. As previously stated, the ICNS is an important regulator of regional cardiac function. In extensive experience with the canine model in our laboratory, the canine preparation used was a robust model, capable of tolerating manipulation without preparation compromise. However, 4 to 5 weeks after the TMLR procedure, it was apparent that the animals did not tolerate surgical stress in the usual fashion. Two animals suffered significant dysrhythmias on exposing of the heart. No dysrhythmias or hemodynamic instability was seen in the sham animals. Additionally, the ICNS proved to be nonresponsive to the potent chemical agonists nicotine and angiotensin II.

Nicotine activates both parasympathetic and sympathetic efferent postganglionic neurons (cell bodies) of the ICNS, thereby inducing bradycardia (parasympathetic efferent neuronal effects on atrial tissues), followed by enhancement of
cardiac indices (via activating sympathetic efferent neurons). In other words, both populations of autonomic efferent neurons on the heart become activated after systemic administration of nicotine. Nicotine continued to enhance cardiac indices immediately after TMLR was performed in a canine model and in sham-operated animals in this study. In the chronic TMLR model, however, nicotine not only failed to enhance intramyocardial pressures in the treated zone but also failed to alter the other monitored cardiac indices monitored (Figure 2). This occurred despite the fact that the maximum dose of nicotine studied was 4 times that required to elicit a maximal response in normal preparations. Angiotensin II actively AT1 receptors associated with the ICNS. Normally, angiotensin II activates this neuronal system to enhance cardiac indexes. A lack of effect occurred when angiotensin II was administered to this chronic TMLR model (Table). Taken together, these data indicate that the global functioning of intrinsic cardiac efferent neurons becomes obtunded within a month after TMLR. This correlates with the animals’ inability to tolerate the usual manipulation required during the course of experimentation.

On the other hand, TMLR did not affect the capacity of ventricular myocytes to respond to the exogenously administered β-adrenergic agonist isoproterenol. This agent enhanced intramyocardial systolic pressure by 47% in the treated region and by 42% in the untreated area (Table). That the treated zone retained its normal contractile responsiveness to such a challenge was confirmed in 2 animals by echocardiography (data not shown).

In conclusion, data derived from this study indicate that the function of the ICNS is substantively altered within a few weeks of TMLR. TMLR does not denervate cardiac afferent or extracardiac efferent neuronal function in ventricular regions undergoing the procedure in a chronic setting. This is in accordance with the lack of TMLR effects on a short-term basis. Thus, the beneficial effects that TMLR affords patients with ischemic heart disease cannot be ascribed to local ventricular denervation. Rather, alteration or “remodeling” of the ICNS, the final common regulator of regional cardiac function, may occur within a month of TMLR. Remodeling of the ICNS has been shown to occur in autotransplanted dogs, chronically decentralized dogs, and dogs undergoing spinal cord stimulation. It therefore appears that some surgical procedures and therapeutic interventions have the ability to alter the functioning of this important neuronal system. As such, remodeling observed chronically after TMLR may represent a novel mechanism whereby such therapy imparts symptomatic relief. However, remodeling the ICNS in this manner may exert deleterious effects on cardiac function.

Acknowledgments

This work was supported by the Medical Research Council of Canada (MA-10122), Nova Scotia Heart and Stroke Foundation, and QEII Foundation, Halifax, Nova Scotia. We gratefully acknowledge the technical assistance of Richard Livingston.

References

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_Circulation_. 2001;104:I-115-I-120
doi: 10.1161/hc37t1.094901

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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