Transmyocardial Revascularization Ameliorates Ischemia by Attenuating Paradoxical Catecholamine-Induced Vasoconstriction

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Abstract

The mechanism by which transmyocardial revascularization (TMR) offers clinical benefit is controversial. We hypothesized that TMR ameliorates ischemia by reversing paradoxical catecholamine-induced vasoconstriction. Chronic ischemic cardiomyopathy was created in 11 dogs by placing ameroid constrictors on the proximal coronary arteries and their major branches. Six weeks later, 35 channels were created percutaneously in the left circumflex artery (LCx) region with the left anterior descending artery (LAD) region serving as control. At rest, wall thickening (WT) and myocardial blood flow (MBF) did not change in the treated region, while they deteriorated in the control bed. Contractile and MBF reserve increased in the treated region but deteriorated in the control region. There was diminished ^{123}I-metaiodobenzylguanidine uptake and significant reduction in noradrenergic nerves in the treated region compared to control region, with corresponding reduction in tissue tyrosine hydroxylase activity. We conclude that the absence of catecholamine-induced reduction in MBF reserve and contractile reserve in the TMR treated region with associated evidence of neuronal injury indicates that the relief of exercise-induced ischemia after TMR is most likely due to reversal of paradoxical catecholamine-induced vasoconstriction. These findings may have implications in selecting patients who would benefit from TMR.

Keywords

cardiomyopathy; ischemia; lasers; revascularization

Introduction

Several hypotheses have been put forth to explain angina relief[1–9], improved exercise tolerance, and better quality of life[8,10–13] after trans-myocardial laser revascularization

(TMR). These range from increased myocardial blood flow (MBF) from the left ventricular (LV) cavity via the laser induced-channels[14–16] and/or angiogenesis[17–21] to pain relief from cardiac denervation[22,23].

Unlike normal coronary arteries that exhibit vasodilation during catecholamine stimulation[24], injured or diseased coronary arteries may exhibit paradoxical vasoconstriction[25–28] leading to reduction in MBF and ischemia during stressful situations. We hypothesized that cardiac denervation caused by TMR would lead to attenuation of paradoxical vasoconstriction, thus ameliorating ischemia, which would explain the angina relief and improved exercise tolerance seen in patients undergoing this procedure. We tested our hypothesis in a canine model of chronic ischemic cardiomyopathy previously developed by us [29,30].

Methods

Animal Preparation

The study protocol was approved by the Animal Research Committee. Eleven mongrel dogs were pretreated with aspirin (75 mg daily) for 3 days, which was continued until euthanasia. They were given Gentamicin (80 mg) and Cefazolin (1 g) immediately prior to surgery and Cefazolin was continued through post-operative day 3. Catheters (6F) were inserted into the right femoral artery for pressure measurements and into the left atrium for injection of microspheres. Up to 4 ameroid constrictors (1.5–3.5 mm) were placed around the dissected left anterior descending coronary artery (LAD) and the left circumflex coronary artery (LCx) and their major branches to create multivessel ischemia. Because the first septal perforator artery in the dog usually comes off the left main artery, it was always spared. As a result, the basal interventricular septum (IVS) never becomes ischemic[29,30].

At the completion of procedure, all dogs were treated with buprenorphine (0.15 μg SQ and 0.15 μg IV) and fentanyl transdermal patch (75 mcg·hr^{-1}) for pain control. They were then revived and transferred to the vivarium, where they were examined twice daily and treated appropriately with additional antibiotics or diuretic if infection or heart failure developed. 2D echocardiography (2DE) was performed weekly to assess LV function. All subsequent studies were performed under anesthesia.

Measurement of Regional LV Function and Myocardial Blood Flow

2DE was performed from the right thorax with the dog lying on its left side. Three short-axis views (base, papillary muscle, and apex) were acquired during each study. Care was taken to acquire the same views every time in an individual dog. Wall thickening (WT) was measured offline using a previously defined custom program[31]. Regional MBF was measured using radiolabeled micro-spheres as previously described[32]. Normalized MBF was calculated by dividing the averaged MBF of the region of interest by the averaged MBF of the basal IVS (normal segment).

Assessment of Neuron Density with ^{123}I-metaiodobenzylguanidine Imaging

Labeleing of M-iodine-benzyl-guanidine (MIBG), an analog of guanethidine, that is taken up in the presynaptic vesicles similar to norepinephrine was performed with ^{123}I using solid-phase ammonium sulfate exchange.[33] *Ex vivo* ^{123}I-MIBG imaging (159 kev) of myocardial slices corresponding to the 2DE images was performed by placing them directly on the scan-head of a gamma camera. Regions-of-interest were drawn over the LAD and LCx regions at the 3 levels and counts·pixel^{-1}·min^{-1} were computed and normalized to those at the basal IVS level.

Histopathology and Immunohistochemistry

Samples were stained with hematoxylin and eosin for basic architecture and van Gieson's picric acid for fibrosis and collagen. Samples were also immunostained with a monoclonal antibody against tyrosine hydroxylase for noradrenergic nerve terminals (BIOMOL)[34] and a monoclonal antibody against PECAM-1 for measuring blood vessel density (Santa Cruz). Both tyrosine hydroxylase and PECAM-1 expression were determined by microscopic observation of the diaminobenzidine reaction product (Dako Corp) on the analyzed sections. The regions occupied by fibrosis/collagen (red stain), nerves (dark brown stain), and blood vessels (dark brown stain) were assessed using the following scores based on staining intensity: 0=none, 1=minimal, 2=mild, 3=moderate, and 4=intense. The entire tissue sample (approximately 0.5 cm·0.5 cm) was evaluated for the above changes.

Experimental Protocol

Resting hemodynamic and 2DE data were acquired at baseline and at the time of maximal LV dysfunction (pre-TMR). The pre-TMR evaluation also included MBF and regional WT evaluations at rest and peak dobutamine dose (30–40 $\mu g \cdot kg^{-1} \cdot min^{-1}$) to assess MBF and contractile reserves. For TMR approximately 35 channels were created percutaneously in the LCx region under fluoroscopy guidance using the holmium-YAG laser catheter (Eclipse Surgical Technologies, Inc) via a 9F sheath in the right femoral artery. Laser energy was delivered at 5 J per pulse and at a rate of 5 Hz from the 1 mm diameter optical fiber. Maximum penetration (5 mm) was achieved with 3 consecutive pulses and contact was confirmed by occurrence of premature ventricular contractions. After the procedure, the femoral artery was ligated and the incision was closed. The LAD region served as control in these animals.

Hemodynamic and 2DE data were acquired at rest and peak dobutamine stress at 4, 12, and 24 weeks after TMR. At 24 weeks MBF was also assessed at rest and peak dobutamine stress in order to measure MBF reserve. At this time 5 mCi of ^{123}I-MIBG was injected intravenously, and 30 min later the dog was euthanized, the heart was excised, and confirmation of arterial occlusion was achieved by direct examination of the ameroid constrictors. The LV was divided into 0.5 cm short-axis slices of equal thickness. The slices corresponding to the 2DE images were stained for gross infarction.[35] These slices were then processed for radioactivity measurements (MBF and ^{123}I-MIBG). The remainder of the tissue was processed for histopathology (approximately 0.5 cm·0.5 cm samples in duplicate).

Statistical Methods

Comparisons between stages were performed using repeated measures ANOVA. The paired Student's t test was used to determine the difference between 2 stages. A p-value of <0.05 (two-sided) was considered statistically significant for all comparisons.

Results

Maximum LV dysfunction occurred at 8.6±3.6 weeks after placement of the ameroid constrictors, at which time they underwent TMR. All 11 dogs had follow-up studies at 4.7±1.4 and 12±0 weeks; 10 also had a follow-up study at 22.4±2.3 weeks. The 11th animal died unexpectedly after the 12-week follow-up study, but had histopathology performed.

Table 1 shows hemodynamic data at rest and during dobutamine stress. There was no significant change in heart rate, mean aortic pressure, or double product at rest until 12 weeks post-TMR when they increased, probably indicating an increased catecholamine state caused by heart failure. Dobutamine infusion at 30–40 $\mu g \cdot Kg^{-1} \cdot min^{-1}$ resulted in a significant increase in heart rate and double product when compared to rest at all time points.

WT and MBF at Rest and during Dobutamine Stress

There was no difference in WT between control and treated regions at baseline prior to ameroid constrictor placement ($36.5 \pm 2.1\%$ *vs.* $35.9 \pm 1.9\%$, p=0.36). At the point of maximum LV dysfunction pre-TMR, resting WT was worse in the treated compared to the control bed (Figure 1). After TMR, WT did not change further in the treated bed but continued to deteriorate in the control bed. Whereas, dobutamine resulted in improvement of WT in both beds, the improvement was more marked in the treated region (Figure 2). By 24 weeks, contractile reserve in the treated region was 3.5 fold higher than that of the control region.

Mean resting MBF remained normal in both beds prior to surgery and artery occlusion (baseline) and was unchanged at maximal LV dysfunction (pre-TMR), indicating that the myocardial regions exhibited chronic stunning rather than hibernation. In comparison, MBF at peak dobutamine stress decreased in both the control and treated regions compared to rest (0.66 ± 0.03 versus 0.90 ± 0.67, p<0.05, in the control region; 0.64 ± 0.25 versus 1.06 ± 0.25, p<0.05, in the treated region) indicating the presence of paradoxical vasoconstriction. Resting MBF was not changed 24 weeks post-TMR (Figure 3A); however, dobutamine resulted in a significant increase in MBF in the treated bed (reversal of paradoxical vasoconstriction), but not in the control bed (Figure 3B).

Neuronal Integrity

Figure 4 is a representative ex-vivo image at the papillary muscle short-axis level after injection with ^{123}I-MIBG. There is less MIBG uptake in the treated LCx than in the control LAD region. Figure 5 illustrates ^{123}I-MIBG counts in the basal IVS as well as papillary muscle and apical levels. There was a significant reduction in ^{123}I-MIBG uptake at the apical levels when compared with the basal IVS. ^{123}I-MIBG counts were also lower in the treated when compared to the control regions at both the papillary muscle and apical levels.

Effects of TMR on Histological Changes

Neither ameroid constriction nor TMR resulted in gross infarction as determined by TTC staining. TMR channels were not seen grossly or microscopically in the 1 dog examined at 12 weeks and the other 10 dogs examined at 24 weeks after the procedure. There was significantly more fibrosis in the TMR-treated compared to the control regions. No fibrosis was seen in the basal IVS (Table 2 and Figure 6A).

The untreated basal IVS contained more intact noradrenergic nerve terminals than the 2 lower levels (Figure 6D). The treated regions at both lower levels also exhibited fewer nerve terminals when compared to the control regions at the same levels (Figure 6, E and F). MIBG uptake in the treated regions was also significantly lower than the control region. Capillaries were intact in the basal IVS and were less in the lower 2 levels mostly due to fibrosis. Even within the fibrotic areas, however, less capillaries were seen in the treated compared to the control regions (Figure 6 G, H, and I).

Discussion

The new finding in our study is that paradoxical catecholamine-induced vasoconstriction is reversed after TMR, which may explain the angina relief and increased exercise tolerance reported in patients. We also found evidence of cardiac denervation after TMR, which is very likely to be the proximate cause of the paradoxical catecholamine-vasoconstriction reversal. Furthermore, we confirmed the lack of laser-induced channels or angiogenesis late after TMR, supporting the notion that these are likely not responsible for the clinical benefit seen after TMR.

Our results showing cardiac denervation are consistent with previous reports. One study showed decreased responsiveness of the intrinsic cardiac nervous system at 4 weeks after TMR[36]. Another clinical study reported diminished uptake of ^{123}I-MIBG after TMR with corresponding reduction in angina during a mean follow-up period of 4.3 months[37]. Other experimental studies demonstrated minimal denervation after laser therapy[38,39], but they were conducted in the acute setting (immediately post and 3 days post-TMR) as opposed to ours, where follow-up period was for 6 months, which is the longest reported time in the literature.

When nerves are transected, degeneration occurs via 2 principal mechanisms: Wallerian degeneration[40] and 'dying back' degeneration[41]. In the latter case, degeneration also progresses retrogradely towards the cell body. Thus distally affected nerves also compromise more proximal nerves. Although we did not examine the arterial system for nerve endings, this mechanism seems highly plausible.

Cardiac degeneration also results in upregulation of beta receptors[42], which is responsible for the super-sensitivity of the heart to catecholamines[43]. This super-sensitivity has been documented in cardiac mechanics[44] but not in terms of coronary physiology. Denervation seems to have no effect on autoregulation[45] or chemoreceptor stimulation in non-disease coronary vessels[46]. Furthermore, collateral blood flow is not affected by chronic denervation even in myocardial ischemia[46,47]. Since increase in MBF reserve is not possible in the absence of augmentation of flow through either collaterals or the native coronary arteries, denervation-induced modulation of beta-receptor activity is unlikely to explain our observations.

Other proposed mechanisms

One of the mechanisms proposed early on in the evolution of TMR was that the laser-induced channels were directly responsible for enhancing MBF[14–16]. It was proposed that these channels connected to pre-existing myocardial vessels and increased nutrient flow. There are several drawbacks to this proposal. The myocardium perfuses mostly in diastole because of the pressure gradient between the aorta and the right atrium. The pressure gradient between the LV cavity and the right atrium is minimal in most of diastole and so appreciable flow to the myocardium from the LV cavity is unlikely to occur. In systole the increased myocardial impedance prevents any filling of myocardial vessels whether from the LV cavity or the native vessels. More recently it has been shown that these channels close a few weeks after the procedure and are replaced by fibrosis[48]. Our results confirm these findings.

Another proposed mechanism is angiogenesis[17–21]. It has been postulated that inflammation and/or injury induced by laser stimulates new vessel formation. We not only did not see any new vessels but compared to the control region, we saw a paucity of capillaries in the treated bed, which has significant fibrosis induced by laser.

Critique of our Methods

The dogs were not randomized to treatment of the LAD versus the LCx beds. The LAD region served as the control region in all dogs because the pre-determined curvature of the laser catheter did not allow us to access the LAD bed reproducibly. Rather than performing an incomplete series of treatments in the LAD region, we elected to treat only the LCx region, where we were confident that we could accurately deliver laser therapy to a large area of myocardium.

Prior to TMR, the control myocardium (LAD region) had higher baseline wall thickening compared to the soon-to-be treated myocardium (LCX region) probably because of additional blood flow from collaterals that formed from the first septal perforator to the LAD.

The observed increase in MBF in the control group at 24 weeks post-TMR is probably due to flow from collateral vessels that formed in this region. It is known that the vasomotion of collateral blood vessels differs from the native coronary vasculature. Since the extent of adrenergic innervation is substantially reduced in collateral vessels and the architecture of their smooth muscle is altered during development, they probably do not vasoconstrict in the presence of catecholamine like native diseased vessels

Clinical Implications

We believe that our findings are likely to apply to humans because in the setting of chronic CAD, both coronary circulations are similar in that there is an abundance of collateral vessels. Furthermore, in humans, myocardial denervation has already been demonstrated after laser treatment using PET imaging with [^{11}C]hydroxyephedrine, an inactive norepinephrine analog that is a highly specific tracer of presynaptic nerve terminals[22] and MIBG imaging[37]. Even though the mechanism of paradoxical vasoconstriction has not been fully elucidated, it is plausible that the sympathetic nervous system is involved. When the network of nerves is disrupted, it follows that function would also be lost. Our postulate will need to be confirmed in the clinical setting.

Not all patients benefit from TMR. Our results indicate that relief of TMR is related to reversal of paradoxical vasoconstriction that may or may not be present in all diseased coronary arterial segments. Maneuvers that could elicit paradoxical vasoconstriction (such as hand-grip exercise[25] or cold-pressor test[28]) which in turn could induce regional ischemia (evidenced by inducible regional dysfunction on 2DE) could help select patients that would most likely benefit from TMR. Controlled clinical studies are required to prove this point.

Conclusion

The absence of catecholamine-induced reduction in MBF reserve and contractile reserve in the TMR treated region with associated evidence of neuronal injury indicates that the relief of exercise-induced ischemia after TMR is most likely due to reversal of paradoxical catecholamine-induced vasoconstriction. These findings may have implications in selecting patients who would benefit from TMR.

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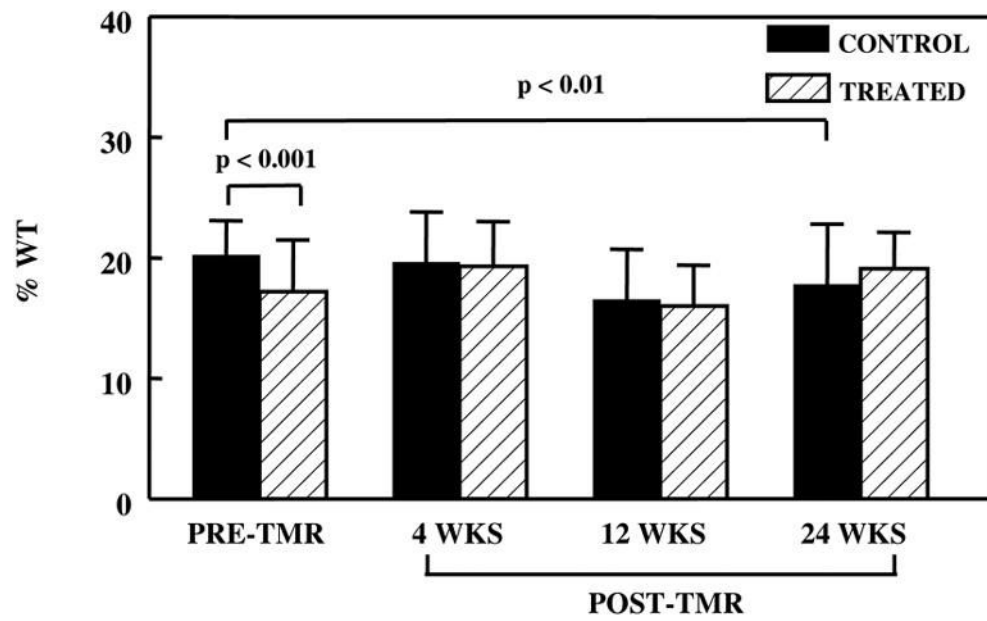
Figure 1.
Resting WT in the control and treated regions before and after TMR. In the control region, there was a reduction in WT at 24 weeks post-TMR when compared to pre-TMR. In the treated region, there was no appreciable change.
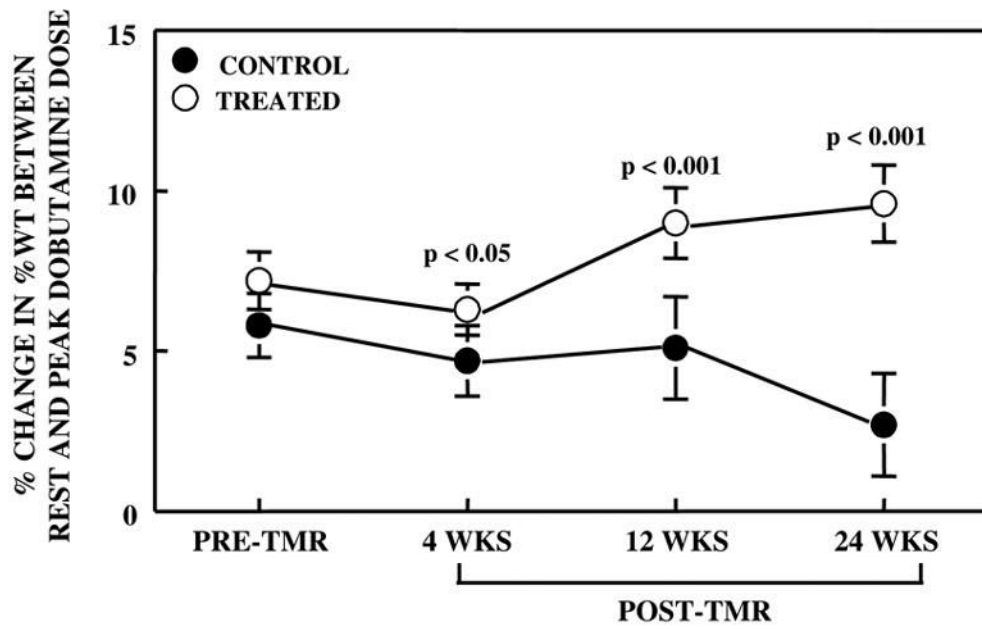
Figure 2.
Difference in the change in WT between rest and peak dobutamine dose in both control and treated regions prior to and after TMR. After TMR, myocardial contractile reserve progressively increased in the treated region and progressively decreased in the control region.
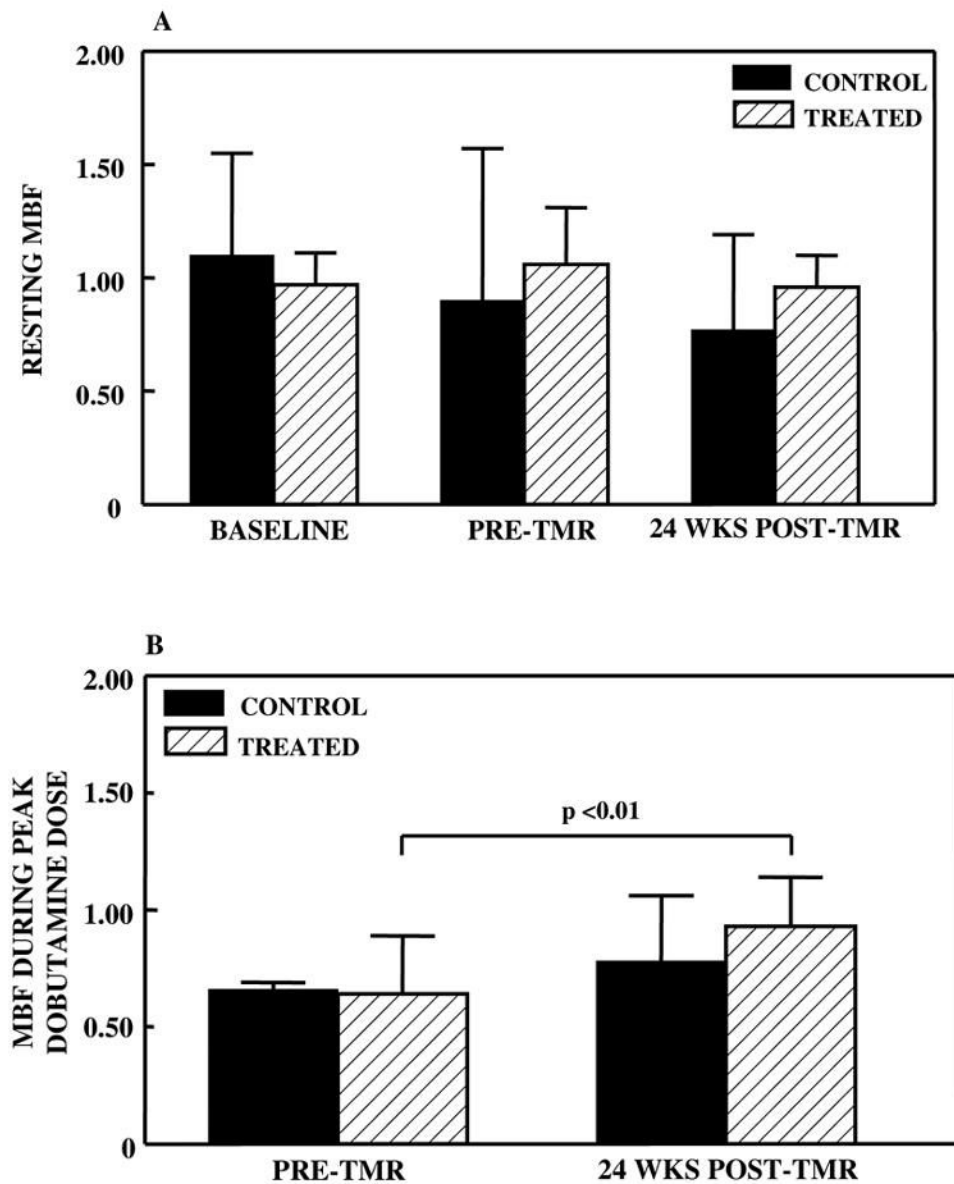
Figure 3.
Comparison of normalized transmural MBF before and 24 weeks after TMR in both the control and treated regions at rest (A) and during peak dobutamine dose (B). There was no change in myocardial perfusion at rest, but during dobutamine, there was increased MBF in the treated region.
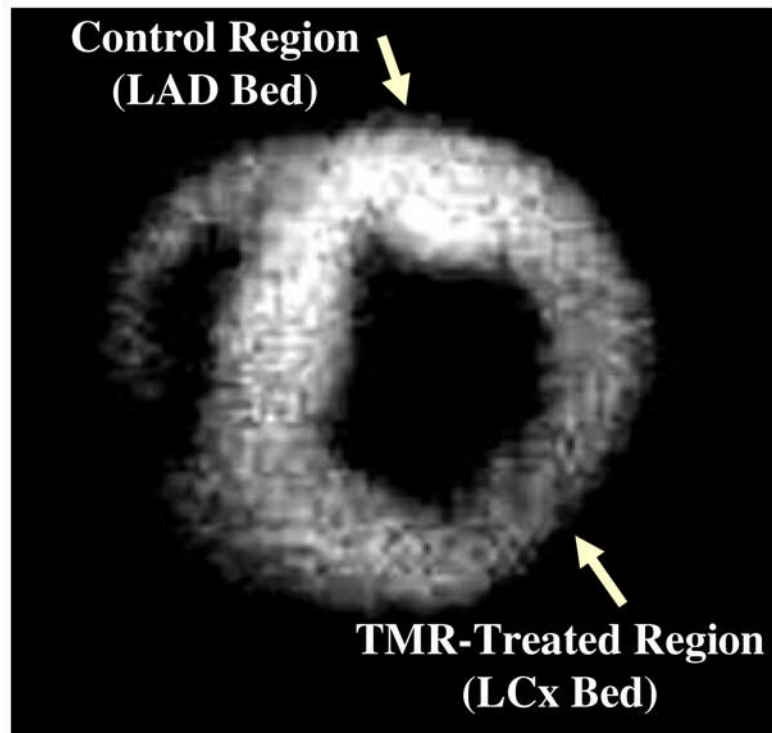
Figure 4.
A representative ex-vivo radionuclide image at the papillary muscle short-axis level after injection with ^{123}I-MIBG, which illustrates that there is less uptake in the TMR-treated (LCx) region than in the untreated control (LAD) region.
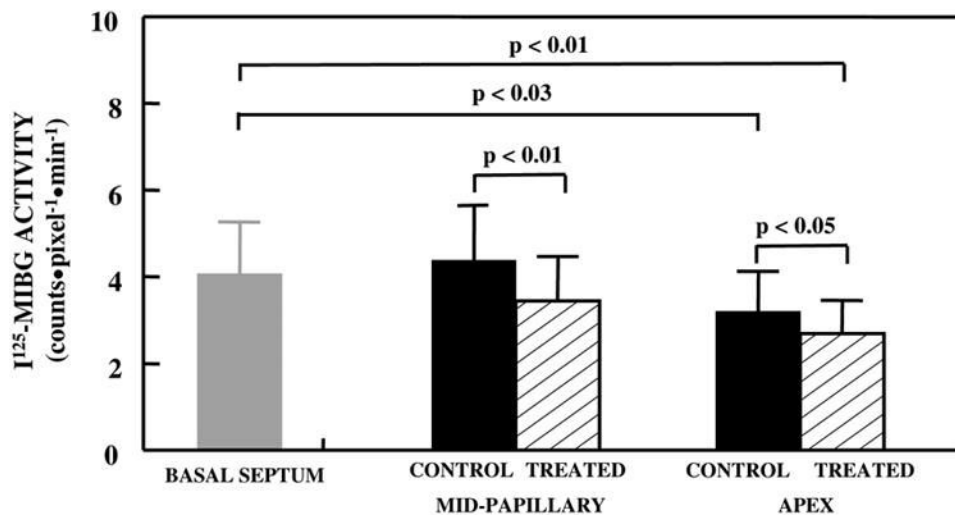
Figure 5.
Quantitative ^{123}I-MIBG uptake in the basal IVS, papillary muscle, and apical levels. At the apex, there were significantly reduced counts in the control and TMR-treated regions compared to the basal IVS. At both the papillary muscle and apical levels, the treated region had significantly lower uptake when compared to the control region.
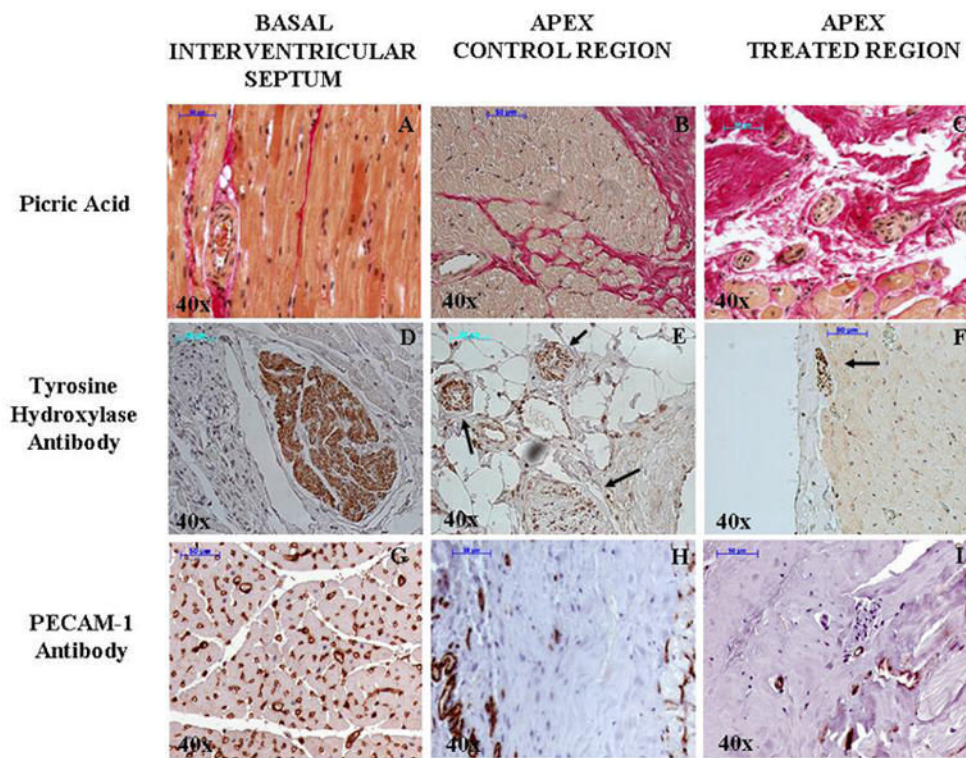
Figure 6.
Representative picric acid (A, B, and C), tyrosine hydroxylase antibody (D, E, and F), and PECAM-1 antibody (G, H, and I) staining of myocardial tissue at the basal inter-ventricular spetum and apical levels (control and treated) at 40x magnification. There is significant fibrosis (red) with corresponding reduction of intact nor-adrenergic nerves (dark brown) and blood vessels density (brown) at the apex compared to the basal IVS. The arrows on panels E and F demonstrate nerve bundles with reduced staining.

Table 1

Hemodynamics at Rest and during Dobutamine Stress$^{\psi}$

Variable	Baseline (n = 11)	Pre-Laser Therapy (n = 11)	4 weeks post Laser Therapy (n = 11)	12 weeks post Laser Therapy (n = 11)	24 weeks post Laser Therapy (n =10)
Rest					
Heart Rate (beats ·min^{-1})	81 ± 27	87 ± 24	93 ± 20	93 ± 20	125 ± 26*†‡
Mean Aortic Pressure (mmHg)	99 ± 19	104 ± 17	102 ± 16	118 ± 17*	121 ± 25*
Double Product (mmHg · beats · min^{-1}) · 100^{-1}	115 ± 48	118 ± 46	127 ± 34	119 ± 54	143 ± 57
Dobutamine (30–40 µg · Kg^{-1} · min^{-1})					
Heart Rate (beats · min^{-1})	—	166 ± 29§	139 ± 48*§	149 ± 32*§	163 ± 23§
Mean Aortic Pressure (mmHg)	—	116 ± 28§	106 ± 12	110 ± 33	117 ± 15
Double Product (mmHg · beats · min^{-1}) · 100^{-1}	—	279 ± 77§	280 ± 74§	249 ± 103§	252 ± 89§

$^{\psi}$Data are presented as mean ± 1SD.

*p < 0.05 *vs.* pre laser therapy

†p < 0.05 *vs.* 4 weeks post laser therapy

‡p < 0.05 *vs.* 12 weeks post laser therapy

§p < 0.05 *vs.* rest

Table 2

Histological results[*]

	Basal IVS	Mid-PM Control Region	Mid-PM Treated Region	Apex Control Region	Apex Treated Region
Picric Acid (Fibrosis)	0.25 ± 0.16	0.25 ± 0.19	$1.68 \pm 0.60^{*}$	01.26 ± 0.61	$1.51 \pm 0.52^{*}$
Tyrosine Hydroxylase (Noradrenergic Nerve Terminals)	2.00 ± 0.43	$0.94 \pm 0.38^{*}$	$0.63 \pm 0.31^{*}$	$0.30 \pm 0.12^{*}$	$0.10 \pm 0.06^{*}$
PECAM-1 (capillaries)	-----	3.50 ± 0.50	1.87 ± 0.68	1.17 ± 0.44	0.67 ± 0.33

[*] Data are presented as mean $\pm$ 1 SEM.

PM=papillary muscle

$p < 0.05$ *vs.* basal IVS