Does Blood Flow Through Holmium:YAG Transmyocardial Laser Channels?

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Background. Early reports indicate that transmyocardial laser revascularization improves symptoms in patients with refractory angina. However, there is little experimental evidence of whether blood flow through channels is the mechanism of action.

Methods. Endocardial channels were made in the distribution of the left anterior descending coronary artery in canine hearts (n = 5) using a holmium:yttrium-aluminum garnet laser. Hearts were excised acutely while perfused in a retrograde fashion from a second dog so that the aortic valve always remained closed. The proximal left anterior descending coronary artery was ligated. To measure direct transmyocardial blood flow, colored microspheres were injected into the left ventricular chamber.

Results. The number of spheres per gram of tissue in the channel region was significantly higher than in the control region (low load, 302.5 ± 169.0 versus 41.8 ± 59.4; high load, 208.4 ± 138.3 versus 5.8 ± 11.7; both, $p < 0.05$). However, the estimated regional blood flow through the channels was extremely low (<0.01 mL · g^{-1} · min^{-1}). In the chronic setting (n = 4) (2-week survival), no flow was detected through the channels, and the endocardial entry points were closed.

Conclusions. Transmyocardial blood flow does not appear to occur through channels made with the holmium: yttrium-aluminum garnet laser. It remains to be determined whether this is the case with other types of lasers.

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Transmyocardial laser revascularization (TMLR) is a new procedure under investigation for treating patients with angina that is refractory to conventional therapies [1–3]. It has been hypothesized that TMLR channels passing from the left ventricular (LV) chamber directly into the myocardium allow oxygenated blood to perfuse the myocardium, thus bypassing the epicardial coronary vessels [4]. Further, it has been proposed by some investigators [1, 3, 5] that these channels remain patent and conduct blood indefinitely.

Conflicting experimental results have been obtained concerning the ability of TMLR channels to conduct blood flow in both the acute and chronic setting. These discrepancies do not appear to be related to the fact that different animal species and different types of lasers have been used in studies over the years. For example, some studies [6–8] have demonstrated that myocardial function can be preserved and infarct size can be reduced in regions pretreated with TMLR channels created with either a holmium:yttrium-aluminum garnet (Ho:YAG) laser or a carbon dioxide (CO_2) laser in dogs and sheep; these data suggest that substantial amounts of nutritive blood flow can be conducted through the channels. On the other hand, other studies [9–11] conducted with both Ho:YAG and CO_2 lasers found no substantial benefit with TMLR pretreatment, and using radioactive microspheres, some of these investigators [9, 11] failed to show any evidence of acute myocardial perfusion resulting from these channels. Also, there is little known about the fate of TMLR channels made with either laser and whether they continue to conduct blood over time.

Therefore, the purpose of the present study was to determine whether blood could flow directly from the LV chamber into the myocardium through TMLR channels, and if so, whether this is of a magnitude sufficient to maintain function and viability in the face of an acute occlusion of the supplying epicardial vessels. In addition, we examined this same question 2 weeks after making the channels. In this study, our focus was on defining the blood flow potential and histologic appearance of TMLR channels created with a Ho:YAG laser (rationale discussed later). We employed an isolated, cross-perfused canine heart preparation in which the blood flow from the normal coronary circulation can be separated from blood flow through channels. Measurements of TMLR blood flow were performed in the acute setting and 2 weeks after creation in a region rendered chronically ischemic or infarcted by ligation of the left anterior descending coronary artery (LAD). We show that although blood can pass through acutely created channels, the magnitude of flow is likely to be very small; too small to preserve regional myocardial function or protect from

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infarction. Furthermore, based on microscopic and macroscopic examination, connections between the ventricular chamber and the myocardium completely seal off within 2 weeks, making it unlikely that transmyocardial blood flow can contribute to perfusion in the chronic setting. Potential differences in findings with different types of lasers are discussed.

Material and Methods

All animals were cared for by a veterinarian in accordance 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" published by the National Institutes of Health (NIH publication 85-23, revised 1985).

Acute Study

Laser channels were created in hearts from 5 mongrel dogs weighing 20 to 22 kg, and then the hearts were studied after being isolated and cross-perfused by blood circulating from a second support dog using methods similar to those described previously [12]. The support dog (ie, the animal not given laser treatment) was anesthetized (sodium pentobarbital, 30 mg/kg intravenously) intubated, and mechanically ventilated. The carotid artery was cannulated to monitor arterial blood pressure. The dog was heparinized (5,000 U by intravenous bolus), and both femoral arteries and veins were cannulated and connected to a perfusion system used to supply oxygenated blood to the isolated heart (Fig 1). Total flow to the isolated heart was regulated by a peristaltic pump. An in-line flow probe (Transonic Systems Inc, Ithaca, NY) was used to measure total coronary flow (discussed later).

The heart-donor dog (ie, the dog whose heart will get laser treatment) was also anesthetized (pentobarbital, 30 mg/kg intravenously) and mechanically ventilated. After a median sternotomy, a left lateral thoracotomy was performed to expose the left atrium. To create channels with the pulsed Ho:YAG laser, a fiberoptic cable (400 μm in diameter; CardioGenesis Corp, Santa Clara, CA) within a 10F malleable aluminum guide was introduced into the LV chamber through a pursestring suture in the left atrial appendage. The tip of the fiber was stabilized against the LV endocardial surface, and nontransmural channels were created from the endocardium as has been done in previous studies [7, 8]. Between 23 and 37 transmyocardial laser channels (mean number, 28) per heart were created over the LAD distribution just proximal to the first diagonal branch using the following laser variables: 0.6 J/pulse, 10 Hz, and 20 pulses/channel (LASER-123; Schwartz Electro-Optics, Orlando, FL).

After application of the laser and with the heart still in situ, the LAD was dissected just proximal to the first diagonal branch, and a piece of 3-0 silk was passed around the vessel. All visible epicardial collaterals were ligated with a 5-0 stitch to minimize collateral flow to the LAD territory. The heart was then prepared for isolation and perfusion as follows: The brachiocephalic artery was cannulated to monitor the coronary perfusion pressure of the heart. The arterial cannula of the perfusion system

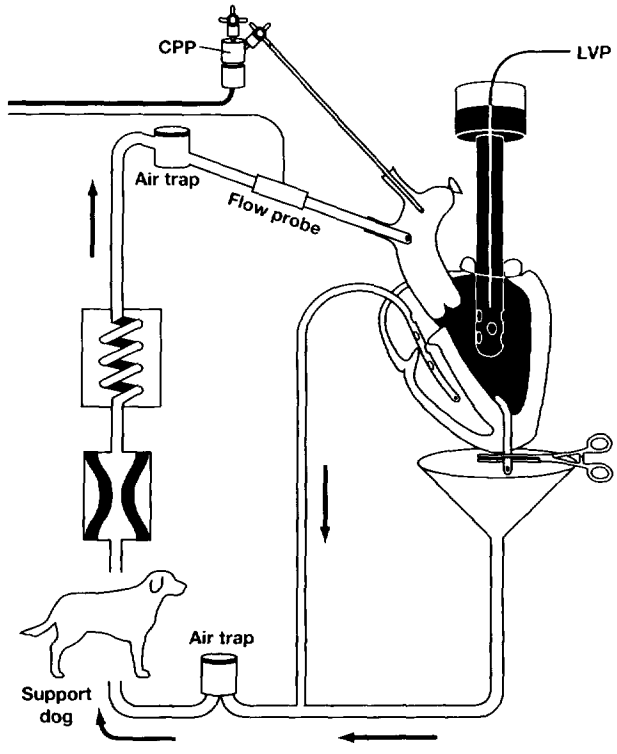

*Fig 1. Isolated heart preparation in which coronary flow could be separated from flow through channels. See text for details. (*CPP = *coronary perfusion pressure;* LVP = *left ventricular pressure.)*

was inserted into the left subclavian artery and the venous cannula, into the right ventricular chamber through the superior vena cava. The descending aorta and the inferior vena cava were ligated simultaneously and cross-perfusion from the support dog was started to maintain the blood supply to the heart. Both hili were ligated, and connections to the lungs were severed. The heart was then excised while metabolically supported by arterial flow from the support dog.

To control the LV volume and pressure, a large-bore cannula (28F) that was connected to a reservoir was introduced into the LV chamber through the left atrial appendage (see Fig 1). In addition, a thin cannula (3 to 4 mm in outer diameter) with side holes was placed through the LV apex and held in place with a pursestring suture; this was used to drain blood from the left ventricle when desired (discussed later). After isolation and preparation of the heart, the LAD was ligated by tying the 3-0 silk, and the heart was allowed to stabilize for approximately 20 minutes prior to the start of the protocol.

Separation of the normal coronary circulation and the transmyocardial blood flow was possible in this preparation because the aortic valve remains closed as long as LV pressure is less than the pressure in the aorta; note that pressure in the aorta also determined the coronary perfusion pressure of the isolated heart. Coronary perfusion pressure was controlled (independent of LV pressure generation) by regulating blood flow from the support dog (see Fig 1). To ensure aortic valve closure, coronary

perfusion pressure was maintained constant at a relatively high level of about 120 mm Hg. Total coronary blood flow measured from the in-line probe averaged 240 ± 40 mL/min and did not vary substantially throughout the course of the experiments. Left ventricular pressure generation was regulated by varying the amount of blood within the ventricular chamber. During LV contraction, the intraventricular blood was ejected through the large-bore cannula in the mitral orifice into the reservoir, which was at atmospheric pressure.

Colored microspheres (15 μm in diameter; ~3 × 10^6 microspheres/mL in a saline suspension with 0.01% Tween 80 and thimerosal; Dye-Trak, Triton Technology Inc, San Diego, CA) were used to estimate both regional coronary artery flow and blood flow through the channels. Calculation of regional blood flow from the coronary circulation required knowledge of the total blood flow, which was obtained from the in-line flow probe already described (see Fig 1). Regional blood flow from the normal coronary vasculature was determined at the start of the protocol by injecting 0.2 mL of white microspheres into the coronary artery perfusion line. The microsphere solution was mixed thoroughly using a vortex and then was drawn into a 1-mL syringe and injected rapidly into the perfusion line approximately 25 cm from the heart. This was considered to provide adequate opportunity for mixing in the blood, as the aortic root (still present in the isolated heart) provides an ample mixing chamber. During this injection procedure, the LV volume was adjusted so that pressure generation was low (≤20 mm Hg) to ensure aortic valve closure; if the aortic valve opened, this could be detected in blood samples taken from the LV chamber by the appearance of white spheres in the blood in the LV chamber.

Next, microspheres were injected into blood placed in the LV chamber under two different loading conditions that were applied in a random order among the hearts. One condition was a low loading condition in which peak LV pressure generation was approximately 20 mm Hg (yellow microspheres), and the other was a high loading condition in which peak LV pressure generation was approximately 60 mm Hg (blue microspheres). These different loading conditions were achieved by varying the amount of blood placed within the cannula-reservoir assembly that was inserted into the left ventricle. Diastolic LV pressures were low and did not vary between the two loading conditions (0.2 ± 3 mm Hg under low loading condition versus 0.4 ± 4 mm Hg under high loading condition).

For each condition, 0.5 mL of the mixed microspheres was injected every 2 minutes for 10 minutes (total of 2.5 mL or 7.5 × 10^6 spheres injected). At the end of each 2-minute period, a 1-mL sample of LV blood was obtained (for determination of microsphere concentration), the left ventricle was quickly drained through the apical vent and then refilled with the same amount of blood, and the next injection was performed. This draining and refilling procedure was performed because preliminary studies in which all microspheres were infused at one time revealed that their concentration decreased over time (despite a constant LV blood volume), a finding suggesting that microspheres sedimented in the LV chamber despite the mixing action caused by blood going in and out of the ventricle with each contraction. After the intraventricular injections were performed at both loading conditions, a final set of red microspheres (0.2 mL) was injected into the coronary artery to measure the collateral blood flow.

At the end of the experiment, the heart was removed from the perfusion system and cut into small (~1 g) samples from the LAD laser channel region and the left circumflex (LCx) region; samples from the border zone between the LAD and other regions were specifically avoided. With the exception of two or three samples from each heart that were submitted for histologic analysis, all other samples were stored at 4°C prior to microsphere analysis (detailed later).

Chronic Study

Four dogs were treated by the Ho:YAG laser. They were sedated with midazolam hydrochloride (0.1 mg/kg), and anesthesia was induced with an intravenous bolus injection of thiamylal sodium (7 to 10 mg/kg). Anesthesia was maintained by 1.0% to 2.0% isoflurane. A left thoracotomy was performed, and the pericardium was opened. In each heart, an average of eight endocardial channels (~1/cm^2) were made over the *distal* LAD distribution. The LAD just distal to the first diagonal branch was dissected and ligated with a 3-0 silk tie. This contrasts with the acute study in which the distribution of the entire (proximal) LAD was laser treated and rendered ischemic; thus, although the absolute number of channels was greater in the acute study, channel densities were the same in both arms of this study. The smaller treatment area was chosen in the chronic studies to limit the size of the ischemic territory to increase animal survival rate. All visible epicardial collaterals were ligated with 5-0 Prolene (Ethicon, Somerville, NJ) sutures. The chest was closed, and each dog was allowed to recover and live for 2 weeks. In addition, 5 control dogs underwent the identical LAD ligation procedure just described except that channels were not created. Two weeks after the operation, the hearts of both laser-treated and control animals were isolated, cross-circulated, and studied in an identical manner as in the acute study.

Microsphere Analysis

Retrieval and quantitative analysis of the microspheres were performed as described previously [13]. Tissue was digested, and the microspheres were retrieved and trapped in a polyester membrane filter (10 μm in pore size, 25 mm in diameter) (Poretics Corporation, Livermore, CA). The dye was then digested from the microspheres using 100 μL of dimethylformamide. The photometric absorption of each 100-μL sample was then measured by a diode array ultraviolet/visible spectrophotometer (model 8452A; Hewlett-Packard Co, Palo Alto, CA). Samples with absorbances higher than 1.3 AU were diluted and analyzed again. The composite spectrum of each dye solution was resolved at the peak frequencies into the contributions from the individual colored spheres using a matrix inversion technique [13].

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The number of spheres in each sample was calculated according to the absorbance of each dye color using a standardization curve generated from known quantities of spheres from the same batch.

Regional blood flow (RBF) from the coronary circulation was calculated by a standard technique modified for the direct coronary injection of the microspheres [14]:

$$RBF = CBF_{total} \cdot N/N_{total} \quad (1)$$

where CBF_{total} is the total coronary blood flow measured from the in-line flow probe, N is the number of microspheres per gram of tissue in the sample, and N_{total} is the total number of microspheres injected into the coronary perfusion line.

Regional myocardial blood flow contributed from the LV chamber through the laser channels was estimated by determining the number of appropriately colored microspheres per gram of tissue in the laser region and comparing this with the number of spheres of the same color in the control region. In addition, we obtained an estimate of absolute blood flow through the chamber from the following equation:

$$RBF = N/(N_{LV,blood} \cdot T) \quad (2)$$

where N is the number of spheres per gram of tissue in the sample, $N_{LV,blood}$ is the number of spheres per milliliter of blood sampled from within the LV chamber, and T is the duration of exposure of the left ventricle to the microspheres, which in our case was always 10 minutes. When estimating regional blood flow with this equation, it is assumed that the flow pattern is from the left ventricle through the myocardium (it does not have a to-and-fro nature but rather goes from the LV chamber into the myocardial capillary network). Because the technique of sphere administration was very different between the intracoronary and the intracavitary injections, it is not possible to obtain an estimate of relative flows from the two sources by directly comparing the respective number of spheres in the myocardium.

Histologic Analysis

Samples removed for histologic analysis were fixed overnight in 10% neutral buffered formalin before dehydration and paraffin embedding. Sections 4 μm thick were stained with hematoxylin and eosin. Myocardial samples were examined on a standard light microscope, and measurements were taken using an ocular micrometer capable of 2.5-μm resolution with a ×40 objective lens.

Results

Acute Study

HOLMIUM:YAG LASER CHANNELS CAN CONDUCT BLOOD. Typical spectra obtained in an acute study from myocardium in the LCx region and the LAD laser-treated region of the same heart are provided in Figure 2. The spectrum from the LCx sample shows two prominent peaks that correspond to the wavelengths of the white and red spheres that were injected into the coronary arteries; no discernible peaks were detected in this sample at the wavelengths corresponding to the yellow and blue spheres injected into the left ventricle.

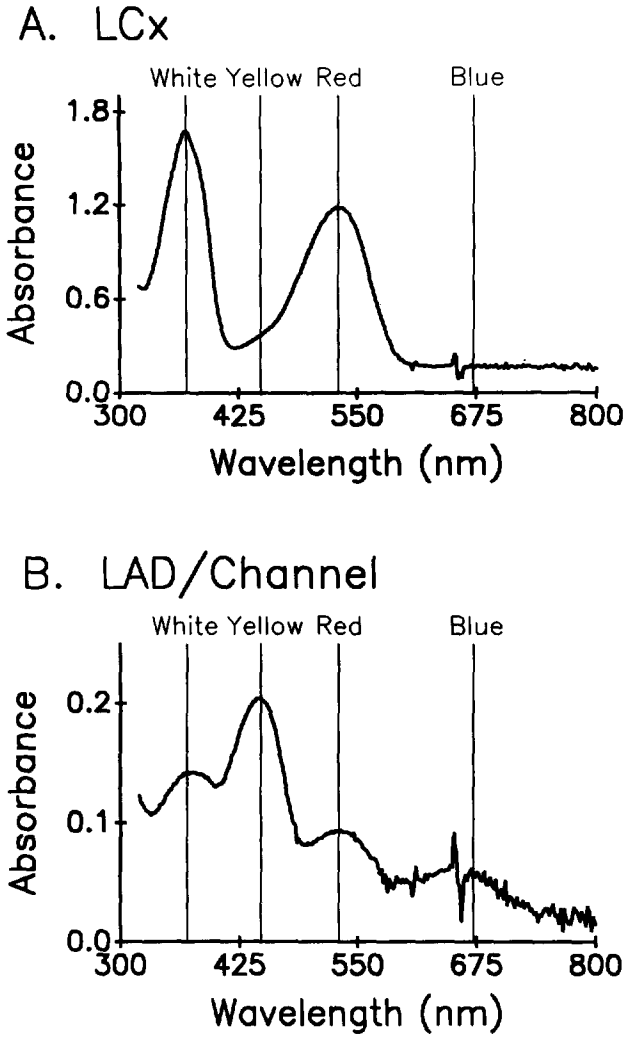

Fig 2. Typical spectra from acute study in which white and red spheres were injected into coronary arteries and yellow and blue spheres were injected into left ventricular cavity: (A) spectrum from left circumflex coronary (LCx) territory revealing no detectable flow from the chamber and (B) spectrum from left anterior descending coronary (LAD) territory, which was ischemic and into which channels were created. This spectrum reveals the appearance of yellow and blue spheres denoting flow from the chamber. Note change in y-axis scale between A and B.

The features of this spectrum contrast sharply with those of the spectrum obtained from the LAD territory to which direct coronary blood flow was reduced by LAD ligation and in which the laser channels were created. First, the heights of the peaks corresponding to the white and red spheres are approximately 10% of those in the LCx territory, consistent with an approximate 10% residual blood flow resulting from native collateral circulation (despite the tying off of all visible *epicardial* collateral vessels). Second, this spectrum exhibits an additional two peaks that correspond precisely to the wavelengths of the yellow and blue spheres injected into the LV chamber. These typical findings indicate that transmyocardial and

Table 1. Summary of Data From Acute Study[a]

Variable/Injection Condition	Coronary Beginning	Left Ventricle		Coronary End
		Low Loading	High Loading	
No. of spheres per gram of tissue				
LAD with channels	972 ± 604[b]	303 ± 169[c]	208 ± 138[c]	409 ± 257[b]
LCx without channels	5,678 ± 2,246	42 ± 59	5.8 ± 11.7	5,229 ± 1,336
Regional blood flow ($mL \cdot min^{-1} \cdot g^{-1}$)				
LAD with channels	0.37 ± 0.24[b]	0.000759 ± 0.000658[c]	0.00125 ± 0.00103	0.15 ± 0.09[b]
LCx without channels	2.14 ± 0.69	0.000096 ± 0.000169	0.000133 ± 0.000265	2.13 ± 0.88

[a] Data are shown as the mean ± the standard deviation. [b] Significance: $p < 0.01$ versus LCx. [c] Significance: $p < 0.05$ versus LCx.
LAD = left anterior descending coronary artery; LCx = left circumflex coronary artery.

native coronary blood flows were separated and that microspheres can reach the myocardium by passing directly through the laser channels.

The results from all 5 hearts studied are summarized in the top portion of Table 1, which compares the mean number of spheres per gram of tissue in the LAD laser-treated territory and in the LCx territory. On average, the concentration of spheres from the coronary circulation was approximately 1,000 spheres per gram at the beginning and 400 spheres per gram at the end of the experiment, which represented approximately 17% and 8%, respectively, of the concentration in the LCx territory. This decline in collateral flow was not significant. However, the mean concentration of spheres from transmyocardial perfusion was significantly greater ($p < 0.05$) in the LAD territory than in the LCx territory, and the magnitude of this effect was not different between the high and low loading conditions. However, the large standard deviation about the mean in sphere concentration was on the order of the mean value itself. This indicates that sphere concentration was very variable even in tissue from the LAD laser-treated territory (implications reviewed in Comment section).

FLOW THROUGH LASER CHANNELS IS VERY SMALL. Quantitation of regional blood flow from the coronary vessels in both the normally perfused and ischemic areas is straightforward using the microsphere techniques (see equation 1). Results, shown in the bottom half of Table 1, indicated that flow to the LCx territory was 2.14 $mL \cdot min^{-1} \cdot g^{-1}$ of tissue at the beginning of the study and 2.13 $mL \cdot min^{-1} \cdot g^{-1}$ of tissue at the end of the study. The calculated collateral flow to the ischemic LAD territory averaged 19.6% ± 14.8% of the flow to the LCx territory at the beginning of the study, and this decreased to 8.4% ± 5.6% at the end of the study (p = not significant). These values are lower than values of collateral flow obtained by other investigators [15, 16] when epicardial collateral vessels were not ligated in the canine model.

Quantitation of transmyocardial flow through the channels is not as straightforward because there are several potential blood flow patterns. Under the assumptions already noted (see equation 2), estimated transmyocardial blood flow was very small (<0.01 $mL \cdot min^{-1} \cdot g^{-1}$) in the channel region, as shown in the bottom portion of Table 1.

Chronic Study

There were no perioperative deaths in either the laser treatment or control studies, and all hearts were examined in isolation after 2 weeks in the same manner as in the acute study. Both control and laser-treated hearts had anteroapical infarcts that grossly appeared to be of similar size. Results of the microsphere analysis are summarized in Table 2. The collateral flow to the infarcted region in the laser-treated hearts averaged 26.0% ± 6.5% at the beginning and 28.0% ± 15.1% at the end of the isolated heart study. For the control group, collateral flow to the infarcted region was 24.8% ± 3.5% at the beginning and 24.6% ± 7.6% at the end of the isolated heart study. Thus, there was no difference between laser-treated and control hearts in regard to the amount of collateral flow into the infarcted region. In addition, the number of yellow and blue microspheres (which were injected into the left ventricle to measure transmyocardial blood flow) per gram of tissue found in the LAD channel region was the same as that found in the LCx region. This indicates that there was no detectable transmyocardial flow after 2 weeks.

Histology

Longitudinal and transverse sections of myocardium treated with the Ho:YAG laser in the acute studies demonstrated typical features of laser channels including a central tissue-ablated channel core, marginal lacunar change immediately surrounding the channel, and a circumferential zone of thermal and thermoacoustic damage (Fig 3) [17]. At the standard laser settings used in this study, there was a broad range in resultant channel dimensions. Results obtained from 13 acute channels over a range of depths through the channel revealed an average (± standard deviation) channel diameter of 477 ± 226 μm (range, 150 to 1,000 μm) and an average thermal damage diameter of 2,292 ± 1,045 μm (range, 500 to 3,750 μm).

In viable myocardium surrounding the laser channels, there was a variable degree of interstitial blood tracking between myofibers and around blood vessels (see Fig 3). This was sometimes present at a great distance (up to 1 cm) from the laser channel and in some instances was extensive. These interstitial red blood cells appeared morphologically normal, which suggests that the vascu-

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Table 2. Summary of Data From Chronic Study[a]

Variable/Group	Injection Condition	Coronary Beginning	Left Ventricle		Coronary End
			Low Loading	High Loading	
No. of spheres per gram of tissue					
Laser	LAD with channels	1,327 ± 533[b]	379 ± 350	667 ± 273	1,165 ± 643[b]
	LCx without channels	4,267 ± 1,682	331 ± 309	485 ± 208	4,727 ± 2,254
Control	LAD without channels	1,019 ± 474[b]	144 ± 272	21 ± 36	908 ± 361[b]
	LCx without channels	3,816 ± 1,543	125 ± 100	40 ± 70	3,513 ± 1,049
Regional blood flow ($mL \cdot min^{-1} \cdot g^{-1}$)					
Laser	LAD with channels	0.46 ± 0.18[b]	0.00152 ± 0.00156	0.00164 ± 0.00146	0.51 ± 0.24[b]
	LCx without channels	1.53 ± 0.62	0.00197 ± 0.00175	0.00110 ± 0.000466	2.11 ± 1.17
Control	LAD without channels	0.42 ± 0.20[b]	0.000117 ± 0.000221	0.000060 ± 0.000125	0.29 ± 0.12[b]
	LCx without channels	1.64 ± 0.66	0.000123 ± 0.000147	0.000104 ± 0.000214	1.24 ± 0.47

[a] Data are shown as the mean ± the standard deviation. [b] Significance: $p < 0.01$ versus LCx.

LAD = left anterior descending coronary artery; LCx = left circumflex coronary artery.

lar extravasation occurred subsequent to the laser treatment. The density of interstitial blood diminished proportionate to the distance from the laser channel, suggesting a directional flow pattern radiating from the channel as the point of origin. From the tissue sections, it is not possible to state whether this blood represents true persistent flow from the channel or whether it is static.

In the chronic studies (animals sacrificed 2 weeks after creation of laser channels and transmural myocardial infarction), only those channels present in infarcted tissue as yet uninvolved by the healing response were recognizable (Fig 4). In these instances, early endothelialization of the channels could be seen, a finding suggesting that under certain conditions, laser channels can persist and be lined by vascular endothelium, at least within the midwall of the myocardium. However, the normal infarction healing process results in a massive wave front of vascular ectasia involving preexisting vessels, and intense fibrovascular proliferation (granulation tissue) extending into the infarcted zone. This reparative response totally obscured the recognition of laser channels; in all likelihood, these tenuous structures are overwhelmed. Although prominent ectatic dilatation of preexisting endomyocardial sinusoids and thebesian vessels was seen, no patent endocardial channel entry or exit sites between the myocardium and the ventricular cavity were identified.

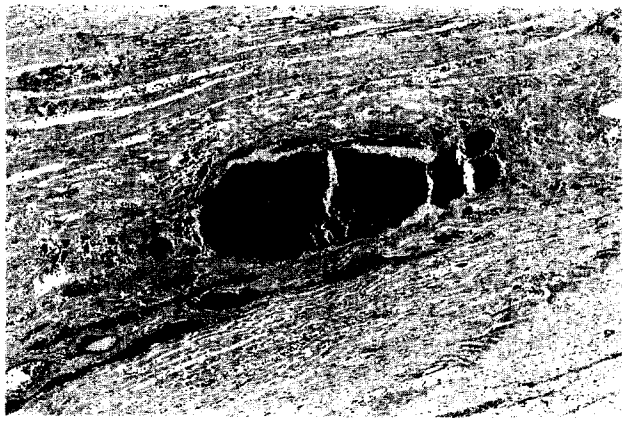

Fig 3. Histologic appearance of acute holmium:yttrium-aluminum garnet laser channel demonstrating typical features of these channels. See text for detailed description. (Hematoxylin and eosin; ×60 before 51% reduction.)

Comment

The results of this study demonstrate that blood can penetrate into the myocardial wall directly through channels made with a pulsed Ho:YAG laser in the acute setting. However, although there are limitations in the quantitation of the flow (discussed later), the results suggest that the magnitude of the flow is small. Further, on the basis of the microsphere analysis, the gross appearance of the endocardium, and the histologic appearance of the myocardium, the channels do not maintain connection with the LV chamber after 2 weeks. The channel remnants were small in caliber, lined with endothelium, and associated with surrounding capillary networks. Yet, despite the neovascularization around the channels, the amount of collateral flow into the channel region was not increased in comparison with control (nontreated) tissue. Finally, the channels did not protect against infarction when the LAD and epicardial collateral vessels were ligated. In the infarcting tissue, the channels appeared to be overcome by the wave of infarction healing that invaded the region from the border zone.

There have been previous reports [9, 11] that failed to show *any* evidence of myocardial perfusion through acute transmyocardial laser channels using the microsphere technique. However, in these previous studies, the microspheres were injected into the left atrium into blood that ultimately perfused both the channels and the coronary circulation. It is likely that with these methods, the microsphere technique is not sensitive enough to detect small amounts of blood flow through the channels, as it could be overwhelmed by collateral flow from the native coronary circulation. In contrast, the techniques used in the present study in which coronary blood and

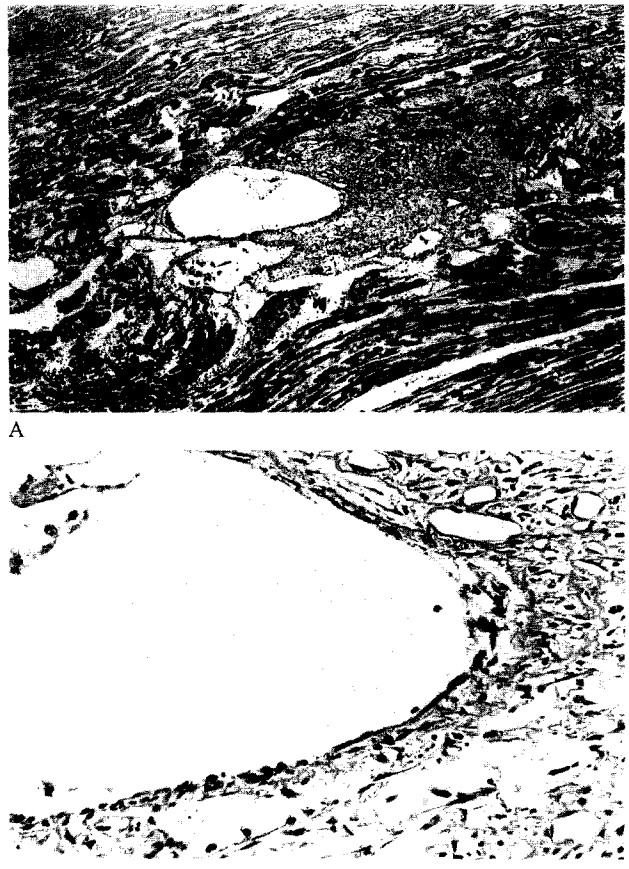

Fig 4. (A) Histologic appearance of a chronic (2-week) holmium: yttrium-aluminum garnet channel, not as yet heavily involved in the healing response to the preceding myocardial infarction, reveals a persistent channel space with evidence of endothelialization. Surrounding the channel remnant is granulation tissue including ectatic, dilated sinusoids and intense fibrovascular proliferation. (B) Magnified view of channel in 4A. (Hematoxylin and eosin; A, ×60 before 51% reduction; B, ×375 before 51% reduction.)

transmyocardial blood were separated provided a high degree of sensitivity for detecting blood flow from the ventricular chamber. As noted in the Material and Methods section, however, the microsphere estimate of flow through the channels depends on the assumption that the blood flows through the myocardium (from the LV chamber, into the myocardium, into the venous circulation). However, if flow was of a to-and-fro nature (in and out of the channel), then spheres would continuously be washing in and out of the myocardium, and their absolute number would not relate to the magnitude of myocardial perfusion.

However, other data suggest that the flow through the acutely made Ho:YAG channels is small. First was the observed variability in sphere number retrieved from laser-treated myocardium (as indexed by a standard deviation that was quantitatively similar to the mean value; see Table 1). This finding indicates that within the treated region, there were some samples that had a high number of spheres and other samples that had a very low number of spheres, a finding implying major variation in physiologic properties of the channels with many channels showing little or no flow. Second, the presence of the channels did not prevent myocardial infarction after LAD ligation, even within a small distance from the channels. Finally, the finding that the channels do not maintain patency further suggests that there is little blood flow through their lumens.

One potential limitation of the present study relates to the fact that the maximum allowed LV pressure was only 60 mm Hg (chosen to ensure that the aortic valve would remain closed) and that if it had been allowed to reach higher levels, a greater amount of myocardial perfusion would have been observed. Several factors suggest that this would not be the case. First, if peak LV pressure were an important determinant of transmyocardial perfusion, then we would have expected to see a greater amount of perfusion at 60 mm Hg than at 20 mm Hg, but no significant difference was observed between these two levels. Second, the expectation that perfusion depends on systolic LV pressure assumes that perfusion occurs during systole. Although some investigators consider this to be the case, it is well established that no myocardial perfusion occurs through coronary vessels during systole; the same pressure gradients and physical forces that regulate myocardial blood flow through blood vessels are also in effect for TMLR channels. One group of investigators [18] concluded that transmyocardial blood flow during systole is a physical impossibility.

In the clinical setting, it is intended that TMLR be applied to underperfused but still viable myocardium supplied by stenotic coronary vessels. In our model, however, we created an abrupt, total LAD ligation that led to infarction. As the maturation process of the channel may be influenced greatly by the environment in which the channel is created, the maturation process of the channels in our canine model with an acute infarction might be substantially different from the process channels undergo in the clinical setting. Although our results are consistent with other results obtained in dogs [11], a recent study [6] performed in sheep using a CO_2 laser provided some histologic evidence of persistence of channels 30 days after channel creation in an infarct area. Because there are species differences in material properties of the myocardium and differences in reparative responses to ischemia and infarction, and because it is difficult to create a chronic animal model that mimics human coronary artery disease, it will be most important to collect data addressing long-term channel patency in hearts from humans. We [19] recently obtained autopsy specimens from a patient who died 4½ weeks after TMLR performed with a CO_2 laser. The histologic findings from that patient were consistent with what we have reported for chronic TMLR channels in canine myocardium: the channels were overcome by granulation tissue, and there was no patent central passage in any channel examined. Thus, although the appropriateness of using animal models to study the histology and physiology of TMLR has been questioned, there is at least some evidence to suggest that such studies do provide pertinent information.

Preliminary clinical experiences with transmyocardial revascularization using a CO_2 laser are promising in

regard to its efficacy in alleviating angina in otherwise untreatable patients [1, 3, 5]. Though it is claimed by some investigators [1, 5] that this procedure provides immediate and direct myocardial perfusion, results of clinical studies with thallium fail to show immediate improvement; rather, 3 to 6 months is required for this technique to show improved regional blood flow [20]. In addition, positron emission tomographic scanning studies have also shown improved regional perfusion at 3 months, but the time course of change in flow has not been studied [21]. The results of the present study and those of previous studies [9, 11] showing that transmyocardial laser channels cannot maintain myocardial viability in the face of an acute LAD ligation are consistent with these clinical observations.

To date, clinical experience with TMLR is confined to studies performed with a CO_2 laser. In contrast, an Ho:YAG laser, with which clinical trials will soon begin, was employed for the present study. Two questions arise: Would the present experimental results be the same if a CO_2 laser had been used, and to what degree do the findings provide information that relates to the clinical experience obtained with the CO_2 laser? First, as already noted, the histologic appearance of CO_2 channels made 4½ weeks after TMLR in a patient with severe angina was similar to that we found with the Ho:YAG laser. Second, preliminary studies [22] in our laboratory suggest that the histologic appearance of chronic TMLR channels made with CO_2 and Ho:YAG lasers in canine myocardium is very similar. Nevertheless, it will be necessary to systematically study TMLR channels created with the CO_2 laser to define their potential for providing nutritive myocardial perfusion and their patency in a variety of settings.

With currently available data, the present results should not be generalized as pertaining to TMLR channels made with other lasers and, in particular, should not be extrapolated to the case of the CO_2 laser with which encouraging clinical results have been obtained. However, if it is ultimately proved to be generalizable between the different laser techniques, our finding that the channels seal off from the LV chamber within 2 weeks suggests that transmyocardial blood flow is not the mechanism that improves blood flow in the chronic clinical setting. Future work should be directed at establishing the clinical efficacy and mechanisms of action of this technique as well as determining the degree to which the clinical benefit depends on the characteristics of the laser used to make the channels.

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