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Is Communication to Endocardium Necessary for Angiogenesis in Transmyocardial Laser Revascularization?

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ABSTRACT

There is evidence of angiogenesis being induced after transmyocardial laser revascularization (TMLR), although the precise mechanism has not been fully elucidated. This study was designed to examine whether or not blood flow from the left ventricle through the channels is essential for angiogenesis following TMLR.

Ten dogs underwent the creation of two types of laser channels in the left ventricle: 1) a transmural channel (TMC), which penetrates the myocardium, and 2) a non-transmural channel (NTMC), which does not open to the epicardium. The animals were sacrificed on the 28th postoperative day and the vascular density was examined. Vessels with smooth muscle media were seen within or around the channel remnant. The vessel density was compared between TMC and NTMC. The outer and inner halves of the myocardium in the TMC region were compared in the same way.

The density of vessels within and around the channel remnants was significantly higher in TMC than in NTMC (1.439 versus 0.685 vessels/microscopic visual field (mvf=40X); p=0.0025). The vascular density was significantly higher in the region adjacent to TMC than in a distant region (>3 mm from the channel center). The vascular density was significantly higher in the outer half than in the inner half of the myocardium (1.730 versus 1.180 vessels/mvf: p=0.0459).

These findings demonstrate that communication to the left ventricular lumen enhances angiogenesis of TMLR, although blood flow in the channel did not exist 4 weeks after TMLR and angiogenesis tended to be more highly enhanced in the outer half than in the inner half of the myocardium.

Key words: *Transmyocardial laser revascularization, Angiogenesis, Blood flow through the laser channels*

Coronary artery disease remains one of the leading causes of death. Although several interventions including percutaneous transluminal coronary angioplasty (PTCA), coronary artery stenting, and coronary artery bypass surgery (CABS) have been improved, these therapies are not suitable for peripheral coronary artery disease. As treatments for chronic cardiac failure, the Batista operation, transmyocardial laser revascularization (TMLR), cardiomyoplasty, and a totally artificial heart have been investigated in addition to pharmacological treatment and heart transplantation.

Among these treatments, TMLR has recently become popular. This method produces an intramyocardial channel by using laser, thus supplying blood from the left ventricular lumen to the ischemic myocardium. A number of clinical studies have clearly shown an improvement of symptoms after TMLR[1,9,14,18,23,24)], and some investigators have reported that TMLR improved even the left ventricular function[8,11,12,19,22,25)].

However, the mechanism of the beneficial effect for symptoms and ventricular function provided by TMLR has not been fully elucidated. While several investigators have suggested that transmyocardial channels remain patent and supply the intraventricular blood into the myocardium[24,27,29,31,32)], others have demonstrated in a variety of TMLR models that the channels created by TMLR are obliterated histologically[4,6,13,20)]. Other groups have concluded that TMLR destroys cardiac nerve fibers and

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reduces the pain of angina pectoris[3,16,17]. It has also been proposed that an injury caused by TMLR could induce neovascularization around the channels[5,13,21,27,29].

Angiogenesis appears to be the most probable mechanism of the beneficial effect of TMLR. The next question is what controls the angiogenesis. So far, angiogenesis after TMLR has been discussed only in terms of myocardial injury. However, it is not known whether or not the patency of the channel and its communication to the ventricular lumen is essential for angiogenesis following TMLR. We had two questions to be solved: 1) does patency of the channel itself induce angiogenesis?; and 2) does the elongated duration of patency enhance angiogenesis? If the answer is yes for both, the best result will be obtained when the channel is kept patent for as long as possible, possibly with appropriate anticoagulation or antiplatelet therapy. Therefore, this study was aimed at solving these questions in an animal experimental model.

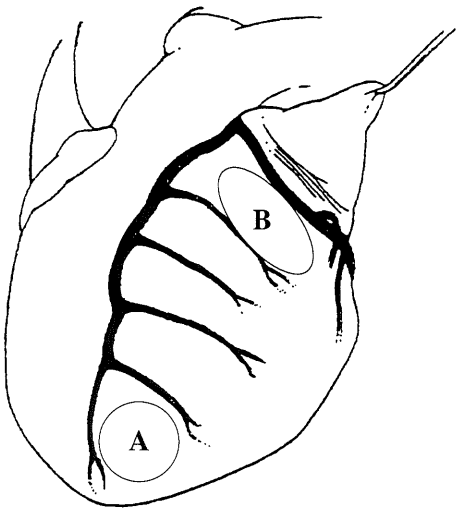

Fig. 1. Illustration showing two regions for creating channels. Two types of laser channels (transmural channel and non-transmural channel) were created in each region (A and B). To eliminate any region-specific bias, the types of channels were alternated in the following animal.

MATERIALS AND METHODS

Animal Care

All animals received human 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 86–23, revised 1985).

Animal Preparation for TMLR

Eleven mongrel dogs were operated on. However, the first dog died 2 weeks after the operation because of pneumonia. The remaining consecutive ten dogs weighing between 11.2 and 15.3 kg (mean, 12.3 ± 1.37 kg) were used in this study.

The animals were premedicated with intramuscular ketamine (30mg/kg). Following establishment of venous access on the right front limb, anesthesia was induced with intravenous administration of sodium thiopental (5mg/kg). They were intubated and mechanically ventilated with a respirator (AM120, AIKA, Chiba, Japan) with 100% oxygen. Anesthesia was maintained with 1.0 to 2.5% isoflurane. All animals received 2 grams of cefmetazole sodium and 60 mg of gentamicin sulfate intravenously before anesthesia induction. Surface electrocardiography was monitored throughout the procedure.

The animals were placed in the right decubitus position and underwent a left anterolateral thoracotomy through the fifth intercostal space. The pericardium was opened and the heart was suspended in a pericardial cradle.

TMLR was applied onto two regions. One was the area at the apex supplied by the left anterior descending artery and the last diagonal branch, which was marked with a 5–0 prolene continuous suture (Fig. 1A). The other was the basal area supplied by the left circumflex artery and the first diagonal coronary artery (Fig. 1B).

Laser System

To create the laser channels, we used a diode laser system (8.5 watts, wave length of 810nm; OSADA electric. Co. Ltd., Tokyo, Japan). The generator is as small as 45 × 30 × 30 cm. Laser is applied by bringing an optical fiber in contact with the object. This optical fiber has an outside diameter of 1.0 mm and an inside diameter of 0.6 mm. This system is smaller and less expensive than the CO_2 laser system and the holmium: yttrium-aluminum-garnet (Ho: YAG) laser system. These two kinds of laser systems (CO_2 and Ho: YAG) have been commonly used for clinical TMLR.

Creation of Channels

In order to examine the influence of channel communication to the left ventricular lumen on angiogenesis, two different types of laser channels were created in each area: 1) transmural channel (TMC), which penetrates the entire layer of the myocardium, and 2) non-transmural channel (NTMC) 7 mm long from the epicardial side, which has no communication to the left ventricular lumen (Fig. 2). While the TMC is subject to injury and channel, the NTMC is subject to injury alone. The influence of the channel was assessed by comparing these two models. The NTMC was created by using a laser optical fiber with a silk string tied at 7 mm from its tip, since the thickness of specimen myocardium was 10.2 mm on average. It took

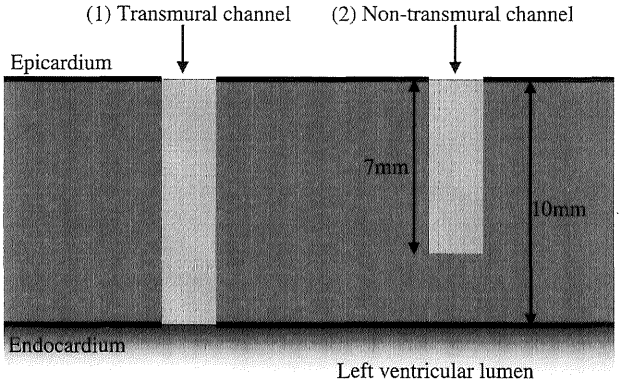

Fig. 2. Schematic illustration of transmural and non-transmural channel (TMC and NTMC).
(1) TMC penetrates the myocardium from the epicardium to endocardium. (2) NTMC does not open to the endocardium and has no communication to the left ventricle. The end of NTMC is 7 mm from the epicardium.

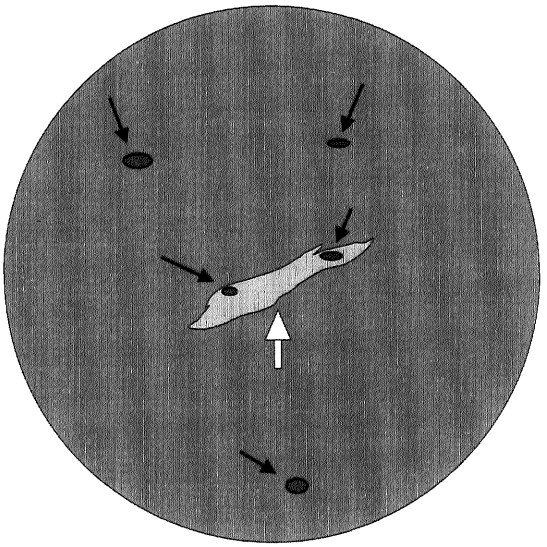

Fig. 3. Schematic illustration showing the method of counting vessels.
Vessels were counted in each microscopic visual field (mvf=40X) with the channel remnant at the center. The white arrow shows the channel remnant. The black arrows show the vessels.

approximately 3 seconds to create TMC and 2 seconds to create NTMC. Approximately ten each of TMC and NTMC were created in two regions of the individual heart. The locations of TMC and NTMC were switched in the next dog, and thus in alternate fashion in ten dogs to eliminate any region-specific bias. The bleeding could be controlled only with digital pressure in most channels. The created channel, however, occasionally caused bleeding refractory to digital compression. In such situations, it was managed with a stitch of 5–0 prolene. The pericardium was closed after bleeding was stopped, and the chest wall was closed in layers.

Preparation of Specimens for Histological Examination

The animals were killed with a lethal intravenous dose of potassium chloride 4 weeks after surgery. The heart regions including channels were sharply excised and fixed in 10% neutrally buffered formalin. To compare TMC and NTMC, specimen was sectioned perpendicularly to the channel axis at a layer 3 mm from the epicardium. The last 4 dogs of this series were used, to examine the vascular gradient along the laser channel. Ten specimens were sampled from each dog at depths of every one tenth of the entire layer of myocardium in the region of TMC.

They were stained with hematoxilin-eosin, trichrome, and elastica van Gieson stain to observe inflammatory infiltration, fibrotic changes and smooth muscle cells of vessel formation, respectively.

Quantitative Assessment of Angiogenesis

The angiogenesis was quantitatively assessed by comparing the density of vessels around the channels with that at the distant region. In the former, the number of vessels was counted in a microscopic visual field (mvf=40X) with a channel remnant situated at the center (Fig. 3). "Vessel" was defined as a cavity with at least a single layer of smooth muscle cells highlighted by elastica staining of the media (Fig. 4A, B). Cavities which did not meet the above-mentioned definition were not counted (Fig. 4A, C). The number of vessels was counted in every region of identified channel remnant. At a distant region that was defined as being more than 3 mm from the center of the channel remnant macroscopically, the number of vessels was counted in every mvf we could observe without any overlap of visual field. The vessels were counted according to the report of Malekan et al[20].

Comparison of Vascular Density between TMC and NTMC

To investigate whether or not communication to the left ventricle enhanced angiogenesis around the channel, we compared the vessel densities between TMC and NTMC. We observed the specimens sectioned perpendicularly to the channel axis and at 3 mm from the epicardium (about half of the entire length of NTMC; 7 mm) to eliminate regional bias of the myocardium. Thus, ten specimens at each TMC and NTMC region were examined from 10 dogs. We counted the number of vessels in all mvf we could identify.

Comparison of Vascular Density between Outer and Inner Halves of TMC

The majority of investigators have reported that

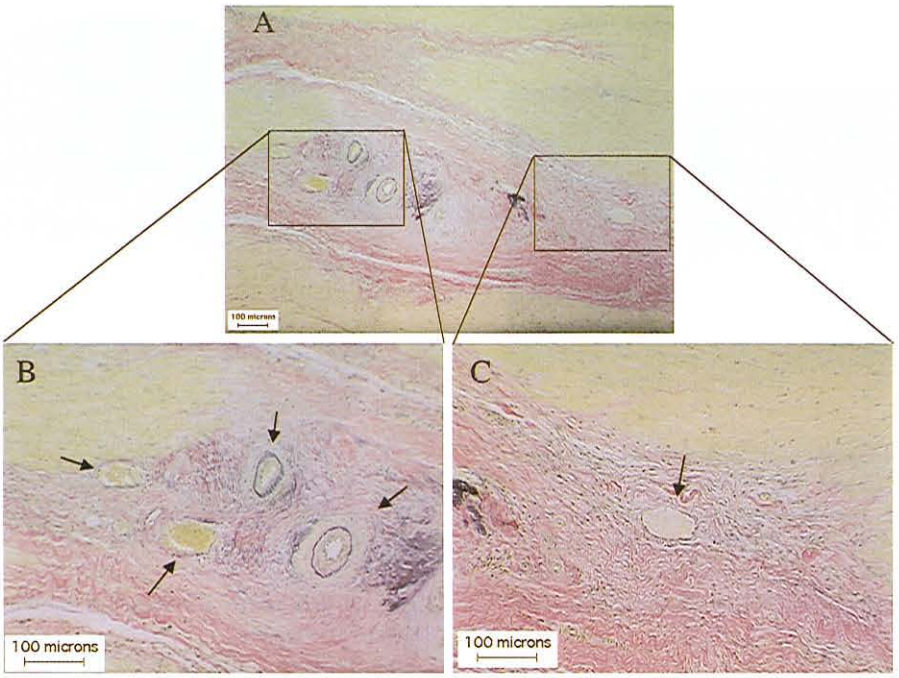

Fig. 4

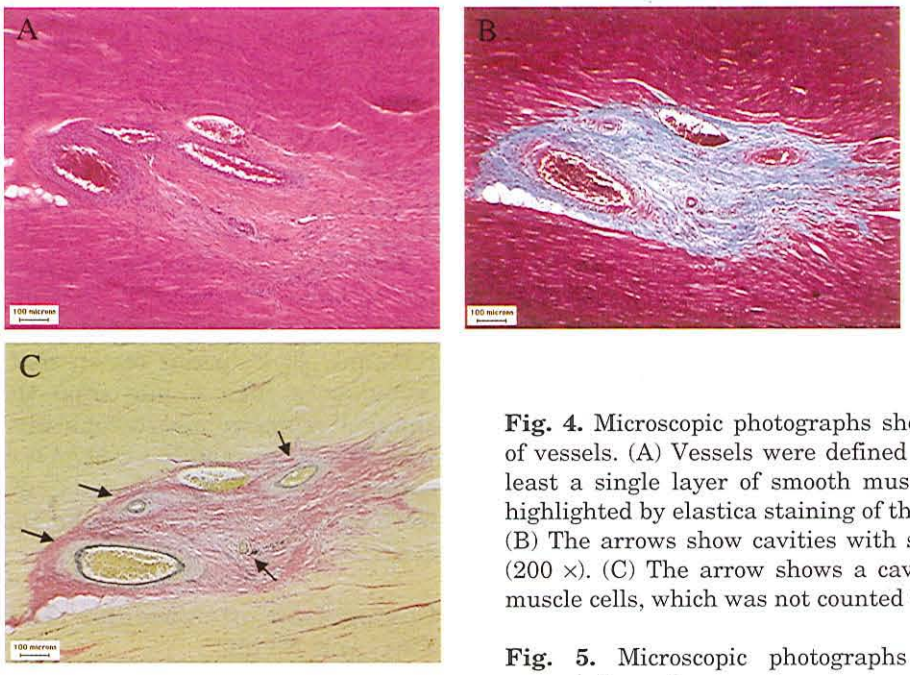

Fig. 5

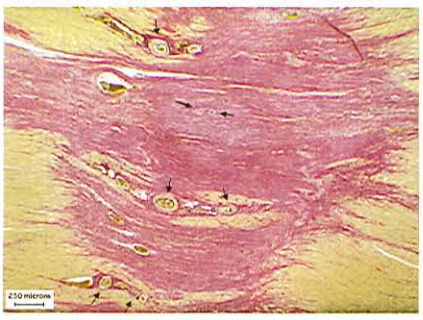

Fig. 6

Fig. 4. Microscopic photographs showing identification of vessels. (A) Vessels were defined as a cavity with at least a single layer of smooth muscle cells, which are highlighted by elastica staining of the media (100 ×). (B) The arrows show cavities with smooth muscle cells (200 ×). (C) The arrow shows a cavity without smooth muscle cells, which was not counted (200 ×).

Fig. 5. Microscopic photographs showing findings around channel remnants. (A) Central channel region is replaced by granulation tissue and fibrosis with scattered chronic inflammatory cells (hematoxylin-eosin, 200 ×). (B) Trichrome stain showing marked fibrosis of channel region (200 ×). (C) Channel region with new vessel formations. The arrows show new vessels with at least a single layer of smooth muscle cells (elastica van Gieson stain, 200 ×).

Fig. 6. Microscopic photographs showing hematoma. The "hematoma" region was larger than the usual channel remnant. Within them there were granulation tissue, fibrosis, a number of vessels, hyalin and hemosiderin (elastica van Gieson stain, 40 ×). The arrows show vessels with smooth muscle cells. Note the difference of scale compared with Fig. 5.

the channel cavity obliterated in about 2 weeks. In the laser channel, it is most probable that obliteration occurs first on the epicardium side and contact between myocardial tissue and fresh blood in the left ventricular lumen lasts longer in the inner half of the myocardium than in the outer half. If prolonged channel patency enhances angiogenesis around the channel, there will be more vessels in the inner half of the myocardium than in the outer half. Thus, the gradient of vascular density along TMC was evaluated. The last 4 dogs were used for this study (see "preparation of specimens for histological examination). We defined "outer half of the myocardium" by 5 specimens from the epicardial side and "inner half of the myocardium" by 5 specimens from the endocardial side. The definition of remote regions and method of the counting vessels were mentioned previously (see "quantitative assessment of angiogenesis").

Statistical Analysis

The results were expressed as means. Continuous variables were compared using the nonparametric Mann-Whitney's U test. The statistical analysis was performed with a software package (StatView J-4.5; Abacus Concepts, Inc., Berkeley, Calif.).

RESULTS

Macroscopic Characteristics of TMLR-created Channels

The regions with channels were readily identified by the landmark of a prolene suture. The laser channels appeared as whitish pin-point sized dots on the myocardium. There was no obvious difference in appearance between TMC and NTMC. However, there were several channels with an unusual appearance in both the TMC and NTMC regions. They were mostly channels which were stitched for hemostasis at the time of surgery, and were identified by a 5–0 prolene suture on the epicardium. They appeared as red spots larger than other channels, with a diameter of about 1 to 2 mm.

Histological Characteristics of TMLR-created Channels

Every original channel lumen was obliterated and totally replaced with granulation tissue and fibrosis with scattered chronic inflammatory cells (Fig. 5A). The channel remnant appeared as an elliptical fibrous scar, which was confirmed by trichrome stain (Fig. 5B). There was multiple vessel formation with or without smooth muscle cells within or around the channel remnants, clearly shown by elastica van Gieson stain (Fig. 5C). Red blood cells were seen within these luminal spaces, which suggested blood flow through them (Fig. 5A, 5B, 5C).

Table 1. Vessel density (TMC versus NTMC)

Region	No. of mvf	No. of Vessels	Density*	
TMC	57	80	1.439	a, b
NTMC	73	50	0.685	
Remote TMC	38	17	0.483	d, c
Remote NTMC	29	14	0.447	

p-value: a=0.0025, b=0.0004, c; not significant, d; not significant
mvf: microscopic visual field (40 ×)
*Density is number of new vessels per mvf.

Vessel Density (TMC versus NTMC)

The total number of detectable channel remnants was 57 for TMC and 73 for NTMC. The vascular density around the channel remnants was significantly higher in the TMC group than in the NTMC group (1.439 versus 0.685 vessels/mvf; $p<0.0025$). In the remote regions, which were defined as being more than 3 mm from the center of the channel remnant, 38 mvfs could be observed in the TMC group and 29 mvfs in the NTMC group. In the TMC group, the vascular density was significantly higher around the channel than in the remote regions (1.439 versus 0.483 vessels/mvf; $p=0.0004$). However, in the NTMC group, the vascular density was not significantly different in the vicinity of the channel and the remote regions ($p=0.3936$). Comparing the remote regions of the TMC and NTMC groups, there was no significant difference in vascular density between them (0.483 versus 0.447 vessels/mvf; $p=0.8943$) (Table 1).

Vessel Density (Outer versus Inner Half of TMC)

The total number of detectable channel remnants was 74 for the outer half of the myocardium and 80 for the inner half. The remote regions were examined at 80 mvfs in the outer half of the myocardium and 60 mvfs in the inner half.

The vascular density around the channel remnants was significantly higher in the outer half of the myocardium than in the inner half (1.730 versus 1.180 vessels/mvf; $p=0.0459$).

The vessel density around the channel remnants was significantly higher than that of the remote region in the outer half of the myocardium (1.730 versus 0.375 vessels/mvf; $p<0.0001$) as well as in the inner half of the myocardium (1.180 versus 0.300 vessels/mvf; $p<0.0001$). There was no significant difference in vascular density in the remote regions between the outer and inner halves of the myocardium (0.375 versus 0.300 vessels/mvf; $p=0.0517$) (Table 2).

Other Specific Findings

In addition to the above-mentioned histological findings, one specific finding was observed. At the

Table 2. Vessel density (outer half versus inner half of myocardium)

Region	No. of mvf	No. of Vessels	Density*	
TMC (outer half)	74	128	1.730	a, b
TMC (innner half)	50	59	1.180	
Remote (outer half)	80	17	0.375	d, c
Remote (inner half)	60	14	0.300	

p-value: a=0.0459, b<0.0001, c<0.0001, d; not significant
mvf: microscopic visual field (40 ×)
*Density is number of new vessels per mvf.

tiny red spots which were observed macroscopically, there was larger granulation tissue, fibrosis, and more vessels than at the common channel remnant, with hyalin and hemosiderin inside them (Fig. 6). Bleeding at the time of laser application probably created these regions. Injury of native vessels by laser could have resulted in hematoma around the channel. Four regions of "hematoma" were observed among all the specimens. In these 4 regions, the vessel density was 10.3 vessels/mvf. This density seems to be higher than usual, though statistical analysis is not feasible because of the small number. We eliminated these regions when comparing the density of vessels between TMC and NTMC and between the outer and inner halves of the myocardium because their appearance was obviously different from that of usual channel remnant.

DISCUSSION

TMLR is a new procedure for treating patients with angina pectoris that is refractory to conventional therapies (pharmacological therapy, percutaneus intraluminal coronary angioplasty, coronary artery stenting and coronary artery bypass surgery). The purpose of TMLR is to decrease clinical symptoms and to improve left ventricular function in patients with ischemic heart disease. However, laser channels or other structures need to be present to supply blood from the left ventricular lumen to the peripheral myocardium.

Angiogenesis around the laser channel remnant, which has been reported by many investigators[5-7,13,17,21,28,30], can provide such structures. However, it is practically unfeasible to examine the relationship between angiogenesis and improvement of clinical symptoms or left ventricular function in the human heart. It is clinically important to enhance angiogenesis for achieving favorable results, but details of the mechanism have not been clarified yet.

On the basis of several reports[5-7,13,17,21,28,30], angiogenesis following TMLR is an inflammatory response against myocardial puncture. Angiogenesis plays a central role in the initial phase of wound healing, and is stimulated by various growth factors which are released as a result of tissue injury and inflammatory cellular infiltration. It results in increased vascular density at the injured area.

The results of our study demonstrated that communication of the laser channel to the left ventricular lumen and blood flow from the left ventricular lumen enhanced angiogenesis around the laser channels at least at the acute phase. In other words, myocardial injury by means of the channel cavity enhanced vessel density more greatly than injury alone, while injury to the myocardium alone was inadequate to promote angiogenesis around the channels (Table 1). Thus, channel patency is essential for angiogenesis at least at the acute phase. Then why does a channel cavity that communicates to the left ventricular lumen promote angiogenesis?

Several investigators have reported on the duration of laser channels. Hardy et al investigated the natural history of channels created with CO_2 laser and those by needle puncture in normal beating heart of dogs. Based on his study, at 24 hr after laser application, serous fluid and red blood cells appeared within the channel lumen. Channel diameter was reduced day by day, and no cavity remained beyond 2 weeks after the operation. In contrast, the channels made by a needle puncture technique were completely occluded at 48 hr post-operation[10]. Bunkhoff et al presented an autopsy result that the channels were not patent in a patient at 4 1/2 weeks after TMLR[4]. On the other hand, Okada et al demonstrated that laser channels of 0.2 mm in diameter were microscopically patent even 3 years after TMLR on canine myocardium[27].

There have been reports on blood "flow" through the cannel in several studies. Kohmoto et al used microsphere analysis, and reported that blood flow can reach the myocardium by passing directly through the laser channels immediately after laser application. But there was no detectable transmyocardial flow after 2 weeks[14]. Perin et al clearly demonstrated blood flow through the channels in left ventricular angiograms just after percutaneous transmyocardial revascularization[29].

These results indicate that the cavity of laser channels remained patent from several days to 2 weeks and that blood flow through the channels from the left ventricle exists immediately following TMLR.

In our study, vessel density was higher in the TMC group than in the NTMC group 4 weeks after laser application in spite of the fact that all TMCs and NTMCs had already been obliterated. Thus the existence of a channel cavity promotes

angiogenesis around the channels if patency lasts for one or two weeks. However, even in TMC with communication to the left ventricular lumen, the channel is a dead-end canal and blood can probably coagulate very quickly. If angiogenesis is enhanced by a longer duration of channel patency, anticoagulation or antiplatelet therapy can be effective and provide better results following TMLR. The design of channels may also be important: for example, 1) a channel with a larger diameter 2) a channel with communication to the native vessel cavity or left ventricular lumen at both ends, avoiding formation of a dead end. However, there is no report on these issues, and further investigations are necessary.

The TMC created in our study had a single orifice to the left ventricular lumen with a dead-end on the other side. It is most probable that the blood stagnates and coagulates at the dead-end (epicardial side) first and this occlusion process propagates toward the subendocardium. If a longer duration of patency enhances angiogenesis further, angiogenesis should be enhanced more in the inner half rather than the outer half of the myocardium. However, the result of our study was opposite to this hypothesis. The vessel density was higher in the outer half than in the inner half of the myocardium (Table 2). There are several possible reasons for this unexpected result. First, the hypothesis that the direction of occlusion processes from the epicardial to the endocardial side is wrong. Second, transient contact of tissue to the uncoagulated blood for a short period is rather advantageous for angiogenesis. Third, the outer half of the myocardium is close to larger coronary arteries and veins, from which newly generated vessels can be supplied and into which they are drained.

Interestingly, the density of vessels in "hematoma" regions was found to be higher than that in the channel remnant without hematoma. Hematoma in the myocardium causes inflammation in reaction to a foreign body, thus leading to angiogenesis in its healing process. Development of hematoma in the myocardium might enhance angiogenesis independent of the channel created by TMLR. If this assumption is correct, it explains the phenomenon of higher vessel density around the laser channel in the outer half of the myocardium. An early obliteration of channel leaves pooled blood in the myocardium, which can enhance like a hematoma.

We used a diode laser system to create the channels. There are several differences between our system and the CO_2 or HO: YAG laser systems which are commonly used in clinical situations. The output energy of this system is lower than that in the CO_2 or Ho: YAG laser system. If a lower output of energy causes an inactive inflammatory response in the myocardium, the vessel density around channels created by the diode laser system should be lower than that created by the CO_2 or Ho: YAG laser system. However, the density of vessels in the adjacent to TMC created by the diode laser in our study was similar to that created by the CO_2 laser[20]. CO_2 and Ho: YAG lasers require a short time to create channel by means of pulsed irradiation, while the diode laser system we used required about 3 seconds by contact application of optical fiber. Thus the injury to the myocardium created by the diode laser system may be more severe than that created by other laser systems.

In our study, we counted the number of vessels in a distant region as a control to assess angiogenesis, because this region is unlikely to be affected by laser injury. Mueller et al reported that there was no new vessel formation in the region, which was 2 mm apart from the channel center[26]. In our laser system, a longer time was required to create the channel than in the CO_2 and Ho: YAG laser systems because it has a lower output of energy than other systems, so we defined the remote region as more than 3 mm apart from the channel center. There was no significant difference in vessel density in the remote regions between the TMC and NTMC groups, or between the outer and inner halves of myocardium. We consider that this definition of "remote region" is valid as the control.

One limitation of this study is that we used normal canine heart. TMLR should have been investigated with ischemic heart after making the model of myocardial infarction because TMLR is a treatment for ischemic heart in a clinical setting. However, it is practically difficult to create myocardial infarction equally in every dog without mortality after myocardial infarction. We used normal canine myocardium, and the ten consecutive dogs were able to survive in our model.

In conclusion, communication of laser channel to the left ventricular lumen is essential to enhance angiogenesis in the myocardium, with higher vessel density around the laser channel in the outer half of the myocardium than in the inner half. The reason for the latter result remains unclarified. Our study has suggested a possible key for promoting angiogenesis of the myocardium: development of hematoma in the myocardium. It can also be created by an injection of autologous blood without TMLR. However, further investigations are necessary to examine these possibilities.

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