Digital Radiographic Quantification of Myocardial Blood Flow Around a Transmyocardial Laser Channel in Rabbit Hearts

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Background  A mechanism underlying the benefits of transmyocardial laser revascularization (TMLR) has been presumed to be improvement in perfusion. We evaluated myocardial blood flow around a laser channel using digital radiography combined with a ³H-labeled desmethylimipramine ([³H]DMI) deposition.

Methods and Results  A laser channel was created in the left ventricular wall using a YAG-laser in 6 non-ischemic rabbit hearts. After 8 weeks, [³H]DMI(1.11 MBq) was injected into the left atrium and the TMLR-treated myocardium was sectioned. Another 6 hearts were examined as controls. We measured [³H]DMI density in arbitrary units with digital radiography in the channel remnant, the surrounding area and a remote area. Flow distribution was quantified by the coefficient of variation of flows (CV). The surrounding area had the highest density (p<0.001) and the lowest CV (p<0.001), and had higher density (p<0.001) and lower CV (p<0.001) than the controls. There was no transmural difference in the density in all domains. The CV increased with depth in the remote area, as well as in controls (p<0.001), but there was no transmural difference in the surrounding area.

Conclusions  The TMLR increases myocardial blood flow and decreases flow heterogeneity in the surrounding area. The disappearance of transmural difference in flow heterogeneity might indicate the remodeling of microcirculation to improve regional oxygen delivery. (Circ J 2005; 69: 488–492)

Key Words: Angiogenesis; Digital radiography; Microcirculation; Perfusion; Transmyocardial laser revascularization

Transmyocardial laser revascularization (TMLR) is a technique that uses a laser to create channels through the myocardial wall. It has received a great deal of attention because it has been shown to provide significant relief from angina. The original idea of this therapy was to restore blood flow to ischemic myocardium with oxygenated blood delivered directly from the left ventricle through the created channels. However, results of experimental studies have indicated that laser channels do not remain patent, but are rapidly occluded by granulation tissue. Accordingly, investigators have searched for other possible mechanisms through which clinical benefits could be achieved. Although histological, immunohistochemical and molecular studies in animal models have demonstrated the presence of angiogenesis in the myocardium surrounding the treated region, the controversy continues as to whether or not TMLR can enhance myocardial perfusion in clinical settings.

Various modalities have been employed to assess myocardial perfusion in the TMLR-treated heart such as the microsphere technique, positron emission tomography and Tc-sestamibi scintigraphy. However, perfusion around an individual laser channel has not been analyzed because the required spatial resolution cannot be achieved through these methods. Digital radiography combined with the technique of tritium-labeled desmethylimipramine ([³H]DMI, an α-adrenergic antagonist) deposition is a novel method for quantifying regional blood flow. Using this technique, which enables the assessment of flow distribution with a resolution of 100-µm, we evaluated myocardial blood flow around a laser channel in non-ischemic rabbit hearts.

Methods

Animal Preparation

All experimental animals were cared for in accordance with institutional guidelines and the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Science and published by the National Institutes of Health (NIH Publication 86-23, revised 1985). Six male Japanese white rabbits weighing 3–3.5 kg were anesthetized using an intramuscular injection of ketamine (5 mg/kg) and chlorpromazine (3 mg/kg). After oral endotracheal intubation, each animal was artificially ventilated with a tidal volume of 10 ml/kg, and anesthesia was maintained with inhaled 0.5 to 2.0% isoflurane. Each rabbit was placed in the right lateral decubitus position and the thoracic area was prepared in a sterile fashion. The heart was exposed via a left lateral thoracotomy through the fourth intercostal space. A laser channel was created in the...
lateral left ventricular (LV) free wall using a holmium: YAG laser (Eclipse Surgical Technologies, Inc, Sunnyvale, CA) with a wavelength of 2.1 μm, pulse length of 250 μs, and maximum average power of 6–8 W. The transmural penetration of laser channels was confirmed by the visible flow of blood from the channel. Hemostasis was obtained by manual compression. The pericardium was left open. The chest wall was closed and the animals were extubated.

Eight weeks after creation of the channel, each rabbit was re-anesthetized. A polyvinyl catheter was introduced into an ear vein for drip infusion of heparinized saline (10 units/ml) and infusion of pentobarbital sodium when needed. The heart was exposed via a median sternotomy and a pericardial cradle was made to suspend the heart. Body temperature was maintained at 37–38°C on a heating blanket. A flexible Teflon catheter was inserted into the LV for blood sampling and monitoring. Arterial blood pH and gases were adjusted to lie within physiological limits (pH 7.35–7.45; PCO2, 35–45 mmHg; PO2, 90–140 mmHg). After stabilization, [3H]DMI (1.11 MBq) was injected into the left atrium using a 0.1-ml glass syringe over a period of 4–5 s. One minute later, the heart was arrested by infusing potassium chloride. After harvesting the heart, the coronaries were washed out with 50 ml of an isosmotic cardioplegic rinsing solution (1.0 mmol/L ethylene glycol-bis (2-aminoethyl ether)-N,N',N'-tetraacetic acid and 0.2 mg/L nifedipine) and subsequently with 50 ml of 2,3-butanedione monoxime (1.5 g/L) to prevent myocardial contracture.15,16,18 The myocardium that included the TMLR-treated area was excised. The samples were sandwiched in aluminum sheets and immediately placed in a –80°C freezer. The frozen samples were then processed into 40-μm-thick slices parallel to the epicardial surface.16,17 Another 6 normal untreated rabbit hearts were examined as controls. After the injection of [3H]DMI, tissue slice samples were prepared in a similar manner as described above.

**Imaging of Myocardial Blood Flow**

The slices were exposed to an imaging plate (IP-TR2040, Fujix, Japan) for 72 h. Two-dimensional radioactive images (ie, relative flow distributions) were constructed using a bio-imaging analyzer (model HE, Fujix, Japan), which can convert the distribution of regional tissue radioactivity recorded on the imaging plate ([3H]DMI density in AU) into a 100×100-μm² pixel image in 256 levels of black and white graduation (Fig 1).16,17 The mean density of the background was less than 10% of that of a region overlying the tissue. For the analysis of regional myocardial blood flow, 3 slices were randomly selected from each of the 3 layers of different depths (subepicardium, mid-myocardium and subendocardium). As shown in Fig 1, a line was drawn 2 mm from the outer margin of each channel remnant. The area inside the line, excluding the channel remnant, was defined as the surrounding area. The area far from the channel remnant was defined as the remote area. The relative [3H]DMI densities inside the channel remnant, in the surrounding area and in the remote area were estimated after correction for background activity using NIH image (Version 1.62, National Institutes of Health, MD). In addition, a normalized index, the coefficient of variation (CV = standard deviation/mean), was used to evaluate the microheterogeneity of myocardial blood flows in the defined domains.15,19

**Statistical Analysis**

The Mann–Whitney test was used for comparisons between groups. Differences between groups were determined by one-way analysis of variance (ANOVA), followed by Bonferroni/Dunn test for multiple comparisons. The level of statistical significance was set at p=0.05. The results are given as mean ± standard deviation and NS indicates statistical insignificance.

**Results**

On the digital radiograms, all laser channels were closed and appeared as a low-density areas (Fig 1A). The surrounding area was identified as a black area of high [3H]DMI density (Fig 1B), and extended approximately 2–3 mm from the outer margin of the channel remnant. The [3H]DMI density was highest in the surrounding area and lowest in the channel remnant (surrounding area: 77.7±5.6 AU/pixel; remote area: 64.1±3.7; channel remnant: 33.4±8.2, p<0.001) (Fig 2). Although there was no difference between the density in the remote area and control myocardium, the surrounding area had a higher density than found in the controls (77.7±5.6 vs 63.1±3.2 AU/pixel, p<0.001). There was no transmural difference in [3H]DMI density among...
the 3 layers in all domains (Fig 3).

The CV was lowest in the surrounding area and highest in the channel remnant (surrounding area: 0.12±0.02; remote area: 0.20±0.02; channel remnant: 0.54±0.03, p<0.001). The surrounding area had a lower CV than in the control myocardium (0.12±0.02 vs 0.20±0.03, p<0.001), while there was no difference in the CV between the remote area and the controls (0.20±0.02 vs 0.20±0.03, NS). The decreased perfusion with a markedly increased CV in the channel remnant was probably due to fibrous tissue ingrowth. A comparison of CV differences between the layers was performed in the surrounding area, remote area and control tissue. The CV increased with depth of the left ventricle (from subepicardium to subendocardium) in the remote area as well as in the control, while no transmural difference in the CV was found in the surrounding area (Table 1).

Discussion

In the present study, we measured transmural myocardial blood flow with a resolution of 100×100㎛² pixels, and computed CV (related to global heterogeneity) of flow distribution in the channel remnant, the surrounding area and the remote area 8 weeks after TMLR in normal rabbit hearts. Our findings were as follows: 1) myocardial flow...
increased in the surrounding area, but decreased in the channel remnant; 2) transmural flow differences were not noted in any of the 3 regions; and 3) regional flows were distributed less heterogeneously in the surrounding area than in the normal myocardium.

Digital radiography combined with a molecular deposition technique enabled us to visualize and accurately quantify regional blood flow in smaller myocardial regions than previous methods. Local \[^{[3H]}\text{DMI}\] deposition occurs in small segments of tissue in proportion to the flow because it is a molecular flow marker. Furthermore, \[^{[3H]}\text{DMI}\] is delivered to tissue in proportion to the local blood flow, is nearly completely extracted during a single pass and is stably deposited mainly in the endothelial cells and endothelial lining of capillary-tissue units. Stochastic and methodological errors are considered to be insignificant because a large number of \[^{[3H]}\text{DMI}\] molecules can be perfused without vascular embolization, which is the major error source inherent in the use of microspheres.

The present study showed 20% lower \[^{[3H]}\text{DMI}\] density in the remote area than in the surrounding area, but no difference in the density between the remote area and control myocardium. Kohmoto et al demonstrated a 2-fold increase in the number of vessels with increased proliferating vascular cells in the surrounding area 2 to 3 weeks after TMLR in normal canine hearts. Yamamoto et al created 14 channels in ischemic canine hearts. Two months after TMLR, they found an increased number of vessels and vascular proliferation in the surrounding area. Furthermore, they noted increased blood flow capacity during stress in the ischemic territory. The highly perfused surrounding area in this study corresponded to the region with angiogenesis in the histological studies. These histological findings coupled with our results suggest that angiogenesis in the surrounding area could, at least in part, contribute to the increase in myocardial blood flow after TMLR.

Regional blood flow in the normal myocardium shows marked spatial heterogeneity (region-to-region flow variability) even within a single myocardial layer. Possible factors contributing to myocardial flow heterogeneity are coronary anatomy, cardiac mechanical effects on coronary circulation and variations in coronary vasomotor tone. Austin et al studied the relative contributions of these factors and have shown that coronary vascular tone plays an essential role in determining the distribution of myocardial blood flow. Spatial flow distribution may depend to a considerable degree upon \(\text{O}_2\) extraction at the capillary level because vascular tone is largely controlled at the arteriolar level and is closely coupled to myocardial \(\text{O}_2\) supply and demand. In the present study, the decreased flow heterogeneity noted after TMLR in the surrounding area might be explained in part by alteration of the coronary anatomy due to angiogenesis and/or by changes in coronary vascular regulation due to regional myocardial denervation. The finding of a decrease in CV with the disappearance of transmural difference is similar to the response of regional myocardial blood flow in the normal left ventricular wall to hypoxemia. Thus, we speculate that TMLR might result in more uniform myocardial perfusion and more effective \(\text{O}_2\) delivery in each myocardial region in the surrounding area.

Despite approval of TMLR for clinical use, continued controversy exists as to whether or not TMLR is capable of enhancing perfusion to ischemic myocardium. Although we created single laser channels in non-ischemic rabbit hearts and evaluated regional blood flow around the channels, the region of increased \[^{[3H]}\text{DMI}\] density was limited to the vicinity of the channel remnant on digital radiograms. A recent experimental study demonstrated a dose–response relationship between channel number and perfusion enhancement using \(^{99m}\text{Tc}\)-sestamibi perfusion scans in chronically ischemic hearts. The total channel number might be of significance when evaluating the efficacy of TMLR in improving blood flow to ischemic myocardium because a single laser channel induces neovascularization and an increase in regional perfusion in a relatively small area.

In this study, normal untreated myocardium were used as controls, but a sham procedure was not performed. Although it is possible that the thoracotomy might have an effect on density and the heterogeneity of subepicardium, the superficial epicardial layer including coronary arteries were not used for the digital radiographic analysis. We believe the normal untreated myocardium could be a control group in this study. Another limitation of this study is that we used normal myocardium. Despite the 20% increase in \[^{[3H]}\text{DMI}\] density that was found in the surrounding area in normal myocardium, the response to TMLR treatment with the increase in perfusion is not clear in ischemic myocardium. It is uncertain whether or not perfusion in the surrounding area is greater than that in the non-ischemic region when a laser channel is created in ischemic myocardium. In addition, there have not been any studies regarding myocardial blood flow heterogeneity in ischemic myocardium. The blood flow heterogeneity response to channel creation in the surrounding area is not clear in ischemic myocardium. Further studies using an ischemic model should be sought to elucidate the mechanism of improvement in perfusion in the TMLR-treated heart.

In conclusion, digital radiography combined with the \[^{[3H]}\text{DMI}\] deposition technique enabled quantitative evaluation of regional myocardial blood flow around a TMLR channel. Results of the present study showed that TMLR increases regional myocardial blood flow and decreases the heterogeneity of flow distribution in the myocardium surrounding the channel. The disappearance of transmural differences in flow heterogeneity might indicate remodeling of microcirculatory units to improve regional oxygen delivery.

### Table 1 Transmural Difference in Coefficient of Variation

<table>
<thead>
<tr>
<th>Domain</th>
<th>Subepicardium Mean±SD</th>
<th>Mid-myocardium Mean±SD</th>
<th>Subendocardium Mean±SD</th>
<th>ANOVA</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control tissue</td>
<td>0.19±0.01</td>
<td>0.20±0.01</td>
<td>0.21±0.02</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>Remote area</td>
<td>0.18±0.01</td>
<td>0.20±0.02</td>
<td>0.21±0.02</td>
<td>&lt;0.001**</td>
<td></td>
</tr>
<tr>
<td>Surrounding area</td>
<td>0.11±0.02</td>
<td>0.12±0.03</td>
<td>0.12±0.02</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Channel remnant</td>
<td>0.45±0.13</td>
<td>0.56±0.15</td>
<td>0.60±0.19</td>
<td>NS</td>
<td></td>
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</tbody>
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*Subepicardium vs Subendocardium, p<0.001; **Subepicardium vs Subendocardium, p<0.001.
Acknowledgement

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References