Transmyocardial revascularization aggravates myocardial ischemia around the channels in the immediate phase

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1Departments of Physiology, 2Cardiology, 3Surgery, and 4Research Center for Genetic Engineering and Cell Transplantation, Tokai University School of Medicine, Isehara 259-1193; 5School of Medicine, Keio University, Tokyo 160-8582; 6Shohnan Kamakura Hospital, Kamakura 247-8533; and 7High Energy Accelerator Research Organization, Tsukuba 305-0801, Japan

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Hattan, Naoichiro, Kazunobu Ban, Etsuro Tanaka, Sumihiisa Abe, Taka-fumi Sekka, Yoshinori Sugio, Minhaz U. Mohammed, Eriko Sato, Yoshiro Shinozai, Yozo Onishi, Hisayoshi Suma, Shunnosuke Handa, Shiaki Kawada, Shingo Hori, Atsuo Iida, Hiroe Nakazawa, and Hidezo Mori. Transmyocardial revascularization aggravates myocardial ischemia around the channels in the immediate phase. Am J Physiol Heart Circ Physiol 279: H1392–H1396, 2000.—We examined whether transmyocardial revascularization (TMR) relieves myocardial ischemia by increasing regional perfusion via the transmural channels in acute canine experiments. Regional blood flow during transient coronary ligation (2 min) was compared before and 30 min after TMR, and at the third transient ischemia the mid-left ventricle (LV) was cut and immediately frozen along the short axis for the analysis of NADH fluorescence in the regions around the TMR channels. In low-resolution analysis (2–4 g tissue or 2–3 cm² area), regional perfusion was not significantly altered after TMR, and NADH fluorescence was observed throughout the ischemic region without significant spatial variation. High-resolution analysis (2.8 mg, 1 mm × 1 mm) revealed that the flow after TMR was lower, and NADH fluorescence was higher in the regions close to the channels (1–2 mm) than in the regions 3–4 mm away from them. Creating TMR channels did not improve the regional perfusion and rather aggravated the local ischemia in the vicinity of the channels in the immediate phase.

METHODS

All protocols were in accordance with our institution’s guidelines for animal care and use, which conform to the guidelines set by the American Physiological Society.

Experimental protocol. Eight mongrel dogs weighing 8.4–16.7 kg were anesthetized with morphine hydrochloride (3 mg/kg im) and α-chloralose (80 mg/kg iv) and ventilated with a Harvard pump. After left thoracotomy and pericardiectomy, the proximal left anterior descending artery (LAD) was dissected to allow repeated transient ligation (2 min) with a 30-min interval, a cannula for microsphere injection was placed in the left atrium and another for drawing reference blood in the femoral area. Ten or eleven transmyocardial channels per heart were created after the first ischemia by using a CO₂ laser (20–30 J, The Heart Laser; PLC Medical Systems, Milford, MA). The channels (1 mm in diameter) were aligned along the short axis of the mid LV supplied by the LAD. The channels were 7–10 mm apart from each other and were included in a short axial band zone 15 mm in width (Fig. 1). Before and after the TMR, regional blood flow during

THE MECHANISM OF TRANSMYOCARDIAL laser revascularization (TMR) has not been fully settled, despite its beneficial effects on intractable ischemic heart disease (3). Angiogenesis in the ischemic region can explain its beneficial effects in the chronic phase (9) but not the elimination of anginal pain in the early phase. In addition to cardiac denervation (11), direct blood supply from the left ventricle (LV) through the transmural channels was hypothesized for the immediate relief (17). But the channel patency and direct perfusion through the channel has been almost excluded as a mechanism of action for TMR; that is, in gram order level analysis, regional flow including the channels did not increase after TMR (5, 10, 18). However, Kim et al. (8) visualized that transmyocardial channels with dispersion of contrast into adjacent myocardial tissue during contrast injection of the LV with high-resolution ventriculography immediate after TMR. Therefore it is required to study the precise distribution of flow and metabolism surrounding TMR channels, to settle the discrepancy. In the present study, we evaluated the spatial effects of the creating TMR channels on perfusion and myocardial metabolism with milligram or square millimeter order resolution in dogs subjected to repeated transient ischemia, by using synchrotron radiation-excited X-ray fluorescence spectrometry for heavy element analysis of microspheres (14) and magnified visualization of NADH fluorescence.

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ischemia was measured with microspheres. At the end of third transient ischemia, the beating hearts were rapidly cross-sectioned at the mid-LV level and freeze-clamped for visualization of NADH fluorescence, which become positive sensitively reflecting the change from NAD to NADH induced by ischemia (13). Heart rate and systemic blood pressure were maintained at appropriate levels by cardiac pacing (100–120 min) during both the first and second ischemia (Table 1).

Regional blood flow. Heavy element-loaded (Ba, I, Zr, Nb, Y) microspheres (diameter 15 μm; Sekisui) were injected (1 × 10^7) into the left atrium at 20–80 s after the start of ischemia, while taking reference blood. After the mid-LV was cross-sectioned for evaluation of NADH fluorescence (shown by arrowheads in Fig. 1), the heart was then divided into ischemic and nonischemic regions supplied by the LAD and left circumflex artery (LCx), respectively, for flow analysis of 2–4 g tissue. Ischemic regions were further divided into lased and nonlased region (containing one or more TMR channels shown by “L,” the gray area with oblique lines in Fig. 1), which include the ischemic part of the slice for NADH fluorescence study, and nonlased region (more than 10 mm away from any channel shown by “NL,” the gray area without oblique lines in Fig. 1). Each sample was further divided into endocardial and epicardial region and dissolved in 2N KOH, and the microspheres were extracted and trapped on filter paper. The elemental X-ray fluorescence was determined with a wavelength-dispersive spectrometer (model PW1480, Philips), and regional blood flow was calculated using a following Eq. 1 as described previously (8 dogs) (15)

\[
Q_s = Q_r \times \left( \frac{C_s}{C_r} \right)
\]

where \(Q_s\) is blood flow in the sample, \(Q_r\) is the rate of reference flow, \(C_s\) is the elemental X-ray fluorescence of the tissue sample, and \(C_r\) is the elemental X-ray fluorescence of the reference sample.

The cross-sectioned LV slices were dried after NADH fluorescence analysis, and the regions surrounding TMR channels were subjected to high-resolution (2.8 mg) flow analysis (Fig. 2). By using synchrotron radiation-excited X-ray fluorescence spectrometry, we measured the two-dimensional X-ray fluorescence activity of the heavy element and converted this into absolute flow using Eqs. 2–4 as described previously (563 grid boxes around 7 channels in 4 hearts) (14)

\[
\text{ALF} = \frac{\text{Local XF}_{MC}}{\text{Weighed mean of XF}_{MC}}
\]

where ALF is absolute local flow, and XFMC is the mass-corrected XF, and

\[
\text{RLF} = \frac{\text{Local XF}_{MC}}{\text{Weighed mean of XF}_{MC}}
\]

where RLF is relative local flow, and

\[
Q_{\text{total}} = \text{ALF} \times \text{RLF}
\]

where ALF is absolute local flow, and \(Q_{\text{total}}\) is blood flow of total area applied high-resolution analysis, which is measured with the model PW1480 wavelength-dispersive spectrometer and calculated using Eq. 1.

NADH fluorescence. The beating hearts were cut along the short-axis plane and freeze-clamped bilaterally during 1–2

### Table 1. Regional blood flow, NADH fluorescence intensity at low-resolution, and hemodynamic variables

<table>
<thead>
<tr>
<th></th>
<th>1st Ischemia (before TMR)</th>
<th>2nd Ischemia (after TMR)</th>
<th>NADH-F Intensity, % [3rd Ischemia (cross-sectioning)]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nonischemic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endo</td>
<td>0.86 ± 0.45</td>
<td>0.86 ± 0.43</td>
<td>NS</td>
</tr>
<tr>
<td>Epi</td>
<td>0.63 ± 0.38</td>
<td>0.61 ± 0.34</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Ischemic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lased</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endo</td>
<td>0.14 ± 0.10*</td>
<td>0.10 ± 0.09*</td>
<td>NS</td>
</tr>
<tr>
<td>Epi</td>
<td>0.13 ± 0.10*</td>
<td>0.12 ± 0.13*</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Nonlased</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endo</td>
<td>0.17 ± 0.12*</td>
<td>0.16 ± 0.11*</td>
<td>NS</td>
</tr>
<tr>
<td>Epi</td>
<td>0.16 ± 0.08*</td>
<td>0.18 ± 0.12*</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Mean aortic pressure, mmHg</strong></td>
<td>98 ± 14</td>
<td>95 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Heart rate, beats/min</strong></td>
<td>112 ± 19</td>
<td>107 ± 10</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD; \(n = 8\) dogs. TMR, transmyocardial revascularization; NADH-F, NADH fluorescence; Endo, subendocardial region; Epi, subepicardial region. *\(P < 0.05\) vs. nonischemic (ANOVA). †\(P < 0.05\) vs. first ischemia (ANOVA). NS, no significant difference between first and second ischemia (paired \(t\)-test).
The time required for heart cross-sectioning and freeze-clamping was within 120 ms, which is sufficiently rapid to fix the energy metabolism of myocardium without ischemic artifact. Anatomic configuration was also well preserved, and two-dimensional distribution of the redox state could be visualized by NADH fluorescence. NADH fluorescence evoked by a pair of excitation lamps (360 nm; model B-100A, Ultra-Violet Products) was quantified on a personal computer (Power Macintosh 7600/200, Apple Computer) with Adobe Photoshop (Adobe Systems) and NIH Image (public domain program). The intensity of NADH fluorescence positivity was redistributed in 256 steps between the mean level of the NADH fluorescence-negative areas taken as 0% and that of epicardial adipose areas taken as 100% (8 dogs). Magnified NADH fluorescence images ($\times 50$) were obtained with a xenon excitation light (Supercure-201S, Fibernics) in eight dogs.

**Statistics.** Data are means ± SD, and comparisons were made by paired t-test or ANOVA followed by Tukey's test with a criterion of $P < 0.05$, for statistical significance.

**RESULTS**

Myocardial blood flow in the ischemic region was reduced to 22.9 ± 15.3% and 24.5 ± 16.7% of that in the nonischemic region during transient ischemia before and after TMR, respectively (Table 1). The degree of reduction was not significantly different between the lased and the nonlased region or between before and after TMR in any region. The mean myocardial blood flow in the lased endocardial region was somewhat decreased after TMR, although the difference was not statistically significant. The flow after TMR correlated negatively ($r = -0.54, Sy.x/y = 71\%$) to the channel density, defined as the number of channels per gram of tissue (data not shown).

Correlation and regression analysis applied to high-resolution (2.8 mg) flow of cross-sectioned slices did not show any significant correlation of flow between the first and second episodes of transient ischemia ($r = 0.113, Fig. 3$), in contrast to the high correlation in nonischemic regions ($r = 0.824, Sy.x/y = 31.8\%$) and regression equation near to identical line ($y = 0.87x + 0.09, Fig. 3, inset$). Austin et al. (2) called the significant correlation between the first and second flow in nonischemic region a “temporal correlation.” In this meaning, the present study demonstrated the loss of temporal correlation in the region with TMR. In 388 of total 563 measurements spots (68.9%) in the lased region, the flow after TMR was lower than the flow before TMR (below the line of $y = x$), and in 314 (55.8%) it was $< 50\%$ (below the line of $y = x/2$). The spatial distribution analysis of flow in the regions surrounding TMR channels demonstrated that regional flow after TMR in the regions with a distance of 2 mm or less from a channel was lower than before TMR (paired t-test), and than the regions of 3 mm or more away after TMR (ANOVA, Fig. 4A). The flow ratio (after/before TMR) was calculated in every grid box. The regions with a distance of 2 mm or less from TMR channels were also characterized by decreased flow ratio; that is, the flow ratios were $< 0.5$ in $\sim 70\%$ of grid boxes. The regions of 4 mm apart were characterized by increased flow ratio, that is, the ratio of grid boxes

**Fig. 3.** Temporal variability of high-resolution flow before and after TMR ($n = 563$ grid boxes around 7 channels in 4 dogs). Ischemic region is shown in the main panel, and nonischemic region is in the inset ($n = 392$ grid boxes in 2 dogs).
with flow ratio of more than 2.0 was higher than that of 0.5 (Fig. 4).

NADH fluorescence was noted all over the ischemic region supplied by the LAD, except for the TMR channel sites, and was not noted in any of the nonischemic region supplied by the LCx (Fig. 2A). As summarized in Table 1, comparison among the regions using low-resolution analysis failed to show any statistically significant difference between subendocardium and subepicardium or between lased and nonlased regions supplied by the LAD. High-resolution analysis (1 × 1 mm) in the regions surrounding TMR channels revealed higher NADH fluorescence in the regions next to the channels (1 mm from the channels) than the regions of 3 mm apart (Fig. 5).

**DISCUSSION**

The present results show that 1) TMR abolished temporal correlation of regional flow, 2) decreased the flow, and 3) aggravated metabolic index of ischemia (NADH fluorescence) in the vicinity of the channels. Thus the present study gave the negative solution to functional patency of TMR channels, in other words, the possibility of direct perfusion from the LV cavity. Our microsphere technique could not rule out the perfusion through the microconnection where microsphere cannot pass (18). However, our NADH fluorescence study revealed such perfusion, even if existed, could not work for substantial oxygen supply.

Regional myocardial flow distribution is influenced by hemodynamic state (coronary perfusion pressure, LV pressure, heart rate, etc.) and by the local condition of ischemic tissue and its temporal variation. The increments in tissue pressure in systole probably range from systolic LV pressure beneath the endocardium to near-atmospheric pressure beneath the epicardium (6). As those pressures are added to intravascular arterial pressure, the sum of tissue and intravascular arterial pressure in the subendocardium must exceed the LV cavity pressure. In diastole, the LV cavity and tissue pressures should be quite low, in contrast, intravascular coronary pressure should be maintained at a cer-
tain level due to the arterial elasticity. Our high-resolution analysis revealed that in the regions with a distance of 2 mm or less from the channels, the regional flow and resultant metabolic changes (NADH fluorescence) rather deteriorated after TMR. This flow reduction around the channels might be caused by a tissue pressure increment due to local hemorrhage and/or vasoconstriction induced by laser injury.

Generally, the extent of ischemia following a second occlusion is less than following the first, as Gommell (4) reported that 2-min ischemic preconditioning significantly reduced tissue damage. If we could correct the preconditioning effect, then the flow reduction following TMR might have actually had an even greater and NADH fluorescence might also have become higher. Mueller et al. (16) reported that TMR caused a transient drop of ejection fraction and hypokinesis that were reversed within 30 min. Recently, Al-Sheikh et al. (1) noted that TMR causes sympathetic denervation relieving angina pain without perfusion improvement and possibly silent ischemia. Lutter et al. (12) reported that the process of making the channels caused a 1- to 2-mm rim necrosis and a 1- to 3-mm zone of myofibrillary degeneration and edema in human study. We evaluated the immediate effects of TMR in acutely ischemic heart, whereas in the clinical setting, the involved region is characterized by a composite of various chronic events. The both results indicated that the local deleterious effects of creating channels possibly promote cell death in the vicinity of the TMR channels with a low flow reserve in the immediate phase of the TMR.

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