Effect of transmyocardial laser revascularization on chronic ischemic hearts in sheep

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Abstract

Background: We investigated the effect of transmyocardial laser revascularization (TMR) on myocardial function and regional blood flow in an animal model of ischemic heart disease. Methods: Chronic ischemia was induced in 11 sheep by the application of coronary stenosis on the left anterior descending (LAD) and circumflex coronary artery (LCX). Ten weeks later, in six of them, transmyocardial channels were created in the anterior free wall and in the posterior wall of the left ventricle. Five animals served as controls. The myocardial function was assessed by echocardiography taken at baseline and every 2 weeks after coronary stenosis and after TMR. Myocardial perfusion was measured by colored microspheres, injected at baseline, immediately after coronary stenosis, before and after TMR, and at 20 weeks after coronary stenosis. The hearts were retrieved at 20 weeks for light microscopic examination. Results: The left ventricular end-diastolic and end-systolic cavity area was elevated 20 weeks after coronary stenosis in the control and TMR groups. There was no difference between groups (analysis of variance; ANOVA, non-significant). The wall thickening fraction (WTF) decreased progressively and significantly after coronary stenosis in both groups. The WTF was further acutely reduced by TMR, and recovered gradually to the pre-TMR level. No significant difference in WTF was observed between the TMR and control groups. The resting myocardial blood flow was significantly increased by TMR at 20 weeks ($P = 0.03$). Light microscopic examination revealed channel patency in 49% of the laser scars at 10 weeks post-TMR. A dense capillary network was observed at the edges of the surrounding scar. Conclusions: In an experimental model of ischemic heart disease, TMR developed angiogenesis in the lased channels, but, however, failed to improve myocardial function. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Transmyocardial laser revascularization; Ischemic heart disease; Coronary stenosis; Experimental; Myocardial blood flow

1. Introduction

Transmyocardial laser revascularization (TMR) is a controversial treatment of ischemic heart disease. Clinical reports focus mainly on the improvement of anginal symptoms [1,2]. In these reports, the improvement of myocardial perfusion was documented by $^{99}$Te Setamibi scans. However, cardiac function was not studied. Many fundamental questions remain with regard to the mechanisms contributing to clinical benefits. Frazier and colleagues found that TMR improves anginal status, relative endocardial perfusion and cardiac function in patients who do not have congestive heart failure [3]. On the other hand, Kwong et al. [4] suggested that myocardial denervation as a consequence of laser treatment may contribute to the relief of angina that is observed clinically.

The original concept of transmyocardial laser treatment is based on myocardial perfusion via vessels or channels penetrating the myocardium from the left ventricular chamber, like in the reptilian heart. A second proposed mechanism of transmyocardial laser treatment is angiogenesis. However, data showing improvements in regional myocardial perfusion have been inconsistent [5]. A third factor influencing the effectiveness of transmyocardial laser treatment is the density of the native microvasculature at the time of intervention [6]. TMR would therefore be more appropriate in chronic than acute ischemia.

In this study, we made an experimental model of ischemic heart disease by the induction of coronary stenosis in sheep, and consequently created transmyocardial channels with a holmium: yttrium-aluminum-garnet (Ho: YAG) laser. The objective of the present study was to determine: (1), the
morphological outcome of TMR-induced channels in a model of chronic myocardial ischemia; (2), the effect of TMR treatment on resting myocardial blood flow; and (3), the effect of TMR on left ventricular dilatation and regional cardiac function.

2. Material and methods

All animals were cared for by a veterinarian in accordance with the 'Guide for the Care and Use of Laboratory Animals' published by the National Institutes of Health [7].

2.1. Animal preparation and instrumentation

2.1.1. First procedure

Eleven sheep (54.8 ± 5.8 kg) were fasted for 48 h and premedicated with Ketamine (15 mg/kg), intramuscularly before the induction of anesthesia. After the animal was placed in the right lateral decubitus position, a peripheral intravenous line was placed in the left lower limb using an 18-gauge angiocatheter. Antibiotics (Albipen LA, 15 mg/kg; Mycofarm, Brussels) were given intramuscularly and maintenance crystalloid fluids were given intravenously. Heart rate and rhythm were monitored by electrocardiogram. Anesthesia was induced with a Halothan/oxygen mixture. The animal was intubated and ventilated with a respirator (Engstrom 200). All ventilation parameters were adjusted to keep the blood gas values within a normal range. Anesthesia was maintained with a mixture of 0.5–2% Halothan and oxygen, and bolus doses of Fentanyl as necessary. A fluid filled pressure catheter was inserted into the ear artery. The left chest was shaven, prepared and draped in a sterile fashion. A left thoracotomy was performed through an incision at the third intercostal space. Before opening the pericardium, 100 mg of Lidocain (Astra Pharmaceuticals, Brussels) was administered. The pericardium was incised vertically and retracted to expose the heart. A fluid filled pressure catheter was inserted into the left atrium and a low density (2 cm interval between channels) of transmyocardial channels was created in the anterior free wall of the left ventricle and a low density (2 cm interval between channels) of channels (n = 10) in the posterior wall. Persistent bleeding was either controlled by direct manual pressure, or, on rare occasions, a 6-0 Prolene suture was used. The thoracotomy was closed as in the first surgery and the animals were returned to animal care.

At 20 weeks, all animals were sacrificed. The perfusion fixation of the heart was performed with 3% buffered glutaraldehyde, then the heart was stored in 4% formaldehyde.

2.1.2. Laser procedure

The animals were randomized to a control group (n = 5) or a TMR group (n = 6). In the TMR group, the animals were anesthetized and installed as in the first procedure at 10 weeks after first surgery. The sterile left thoracotomy was performed through the fourth intercostal space. We used a pulsed Ho: YAG laser (Eclipse TMR 2000 Holmium Laser System), which produced multimode radiation at a wavelength of 2.1 μm and a pulse width of 250 μs. The output power at the tip was 400 mJ/pulse and the repetition rate was 5 Hz. TMR was done in such a way that a high density (n = 43 ± 8; 1 cm interval between channels) of transmyocardial channels was created in the anterior free wall of the left ventricle and a low density (2 cm interval between channels) of channels (n = 10) in the posterior wall. The thoracotomy was closed as in the first surgery and the animals were returned to animal care.

At 20 weeks, all animals were sacrificed. The perfusion fixation of the heart was performed with 3% buffered glutaraldehyde, then the heart was stored in 4% formaldehyde.

2.1.3. Hemodynamics

Aortic root pressure, left ventricular pressure, the first derivative of left ventricular pressure and cardiac output were recorded before and after stenosis was applied, during the laser procedure and at termination of the experiment.

2.1.4. Regional myocardial blood flow with colored microspheres

Colored microspheres were administered for measurement of regional myocardial blood flow. Polystyrene microspheres (15 ± 0.1 μm) with a density of 1.09 g/ml (DyeTrak microsphere, Triton Technology, Inc.), labeled with red, violet, white, blue and yellow were injected into the left atrium. Colored microspheres were injected before dissection of the coronary arteries as a baseline flow. A second injection of colored microspheres was given after application of the stenosis to the LAD and LCX. A third injection of colored microspheres was administered before TMR. The fourth color was given after TMR. The fifth color was given when the animal was sacrificed. The heart was cut into five slices perpendicular to the long axis. The third slice was enrolled and 12 different regions of the heart were identified, excised and weighed (three right ventricle and nine left ventricle). The samples were taken from the subendocardium as well as the subepicardium.

2.1.5. Global and regional myocardial function by echocardiography

Echocardiographical examinations were performed...
during sedation of the animal using Ketamine (10–20 mg/kg) intramuscularly. Two-dimensional echocardiograms (Sonotron Vingmed CFM 725 Horten Norway with 2.5 MHz transducer) from the parasternal long and short axes were recorded. The echocardiograms were analyzed by two independent observers. The LV wall thickening fraction (WTF) was calculated as in Eq. (1):

\[
\text{WTF} = \frac{\text{end-systolic wall thickness} - \text{end-diastolic wall thickness}}{\text{end-diastolic wall thickness}} \times 100
\]

The global left ventricular systolic function was measured by calculating the fractional area change (FAC) in the parasternal short axis view. The FAC was calculated according the formula in Eq. (2):

\[
\text{FAC} = \frac{\text{end-diastolic area} - \text{end-systolic area}}{\text{end-diastolic area}} \times 100
\]

All animals had a two-dimensional echocardiogram before and after operation, and every 2 weeks over a period of 20 weeks according to the protocol.

2.1.6. Evaluation of the degree of coronary stenosis by postmortem coronary angiography

After termination of the experiments, the hearts were fixed as described. Then, tissue blocks were cut out, including the left coronary ostium, the main stem and the proximal parts of the LAD and LCX. The block contained the constricting ring and at least 3–5 cm of the coronary arteries distal from the stenosis. A mixture of barium gelatin suspension (1.5 g of gelatin in 40 ml of Micropaque; Guerbet, Brussels, Belgium) was injected by hand into the coronary ostium. Then, X-rays were made from this block at two incidences. The prestenotic and stenotic diameters of the vessel were measured in the two directions. An average value of the diameter was calculated. The percentage reduction in luminal area was calculated by Eq. (3):

\[
\% \text{ reduction of area} = \frac{3R_1^2 - 3R_2^2}{3R_1^2} \times 100
\]

where \(R_1\) is the reference diameter before stenosis and \(R_2\) is the minimum diameter.

The measurements were performed by two independent observers using a micrometer device.

2.1.7. Histological preparation and evaluation

Immediately after explantation, the heart was fixed as described above. After gross pathologic identification of the lased channels, transmyocardial tissue blocks (about 1.5 cm) were taken from both the anterior \((n = 31)\) and posterior \((n = 10)\) walls. The selected tissue blocks were then lamellated parallel to the endocardial/epicardial surface into 3–8 (average, 5.2 ± 1.45) slices, 1.5–2 mm thick, and labeled as a–h, beginning at the endocardial side. One separate tissue block was semiserially cut in a transversal manner to study the channels along its entire course. The number of scars was noted, and the number and size of channels were evaluated as either single or multiple and small or large. In the present study, a channel was considered ‘large’ if its size was comparable to that of the native intramyocardial vessels. As a check, the shortest diameter on transverse section of randomly chosen channels and normal intramyocardial arteries was measured (0.185 ± 0.036 for the channel vs. 0.163 ± 0.031 mm for the control vessels). Morphologically, a channels was defined as a space lined by elongated endothelial cells in an area of lasing. We considered a lumen channel as a patent channel if there was at least one large endothelialized lumen in the scar. Direct morphological evidence of communication with the endocardial or epicardial surfaces was not considered necessary to describe a channel as ‘patent’. Scars with only capillaries or small vessels were considered to be without evidence of patency.

2.1.8. Protocol

The experimental protocol is shown in Fig. 1. In all 11 sheep, a baseline echocardiography was performed after sedation of the animal. After this procedure, the animal was anesthetized and baseline values for hemodynamics and regional myocardial blood flow were obtained before and after induction of coronary stenosis. After recovery, serial measurements of cardiac function (echocardiography) were performed every 2 weeks until 20 weeks after the

![Fig. 1. Experimental protocol. In 11 sheep, the LAD and CX coronary arteries were surgically stenosed. Baseline values were obtained before and after induction of coronary stenosis. Serial echocardiographic measurements of cardiac function were performed every 2 weeks until 20 weeks after initial surgery. After 10 weeks, TMR was performed in six sheep (TMR group). After 20 weeks, the measurements were repeated and the hearts were perfusion fixed and processed for histology. In the control group, the same protocol was used except for the 10 week measurement. W, weeks.](image)
initial surgery. At 10 weeks, full anesthesia was induced and the TMR was performed. After 20 weeks, the baseline measurements were repeated and thereafter the heart was perfusion fixed and processed for light microscopy. In the control group, the same protocol was used, except for the 10 week measurements, which were omitted in this series.

2.2. Statistical analysis

The results are presented as means ± SD. The significance of differences was analyzed by the Student’s t-test. Differences are considered significant if the probability is less than 0.05. Two-factor analysis of variance with repeated measures on one factor was used to analyze the difference in echo-data between the control and TMR groups [8].

3. Results

All 11 animals completed the protocol.

3.1. Changes in hemodynamic parameters

Heart rate, aortic pressure, left ventricular pressure, LV dP/dt max and min, and cardiac output were within the normal range in all experimental animals. In the control and the TMR groups, general hemodynamics remained grossly unaltered during the observation period. There was no statistically significant difference between controls and TMR-treated animals at any point in time (NS).

3.2. Effect of TMR on regional myocardial blood flow

The resting regional myocardial blood flow in the anterior portion of the left ventricle is shown in Fig. 2. The regional myocardial blood flow did not differ between the control group and the TMR group at baseline, after stenosis or 20 weeks later. The situation was exactly the same in the posterior portion of the left ventricle. The density of channels had no influence on myocardial flow.

In the control group, the myocardial blood flow remained unaltered after the induction of coronary stenosis and 20 weeks later (Fig. 2). After TMR, the coronary flow increased. This increase reached significance at 20 weeks compared with the 10 week pre-TMR value (P = 0.03). This same evolution was found in the posterior and anterior regions, and in the subendocardium as well as the subepicardium.

3.3. Effect of coronary stenosis and TMR on regional and global myocardial function

The results of parameters related to global myocardial function are shown in Fig. 3. The left ventricular end-diastolic and end-systolic cavity areas increased progressively during the last 4 weeks in both groups (control group: end-diastolic, P = 0.007, end-systolic, P = 0.03; TMR group: end-diastolic, P = 0.003, end-systolic, P = 0.01). There was no significant difference between the groups (ANOVA, non-significant). Immediately after TMR, the end-diastolic cavity area decreased, as did the end-systolic cavity area, but quickly recovered to the pre-TMR level.

Regional myocardial function was estimated by the WTF. The WTF data in the anterior and posterior walls of the left ventricle in the control and TMR groups are shown in Figs. 4 and 5. The WTF decreased progressively and significantly in the perfusion area of both stenotic vessels in the control group as well as the TMR group. TMR initially induced a

Fig. 2. Regional myocardial blood flow distribution (+ SD) of the subendocardium in the LAD perfusion area. Closed bars indicate the control group. Open bars indicate the TMR group. Myocardial flow was increased at 20 weeks (P = 0.03). The same evolution was found in the subepicardium, as well as in the posterior region of the heart.

Fig. 3. Left ventricular cavity area (+ SD) in the control group (upper panel) and in the TMR group (lower panel) as a function of time. Squares indicate end-diastolic cavity area; circles represent end-systolic cavity area. Note the progressive dilatation of the left ventricle with time, irrespective of the TMR intervention.
sharp drop in WTF in the anterior and posterior walls. There was, however, a gradual increase in WTF during the following days to weeks, and this remained comparable with the pre-TMR values up to 20 weeks of observation. Furthermore, no significant difference was observed in the WTF between the TMR and control groups or between the anterior and posterior walls in the TMR group.

3.4. Degree of coronary artery stenosis

At 20 weeks, postmortem angiography was performed to observe the LAD and LCX stenosis. The degree of coronary artery stenosis (% reduction of luminal area) was 66 ± 10 and 73 ± 7% in the LAD and LCX, respectively. There was no difference in the degree of coronary stenosis between the control and TMR groups (NS).

3.5. Histology

Histologically, lased scars invariably consisted of dense collagenous connective tissue without significant residual inflammation. Altogether, 269 scars were examined histologically (range/heart, from 17 to 58). In 133 (49%), lumen patency was found, mainly due to the presence of one single large vessel (Fig. 6a), or a more or less extensive neovascular response with capillaries especially at the edges of the scar. Scars without any vessels were not encountered. A longitudinal section through a patent channel is shown in Fig. 6b. Its course is rather tortuous and its shape irregular. At the edges of the surrounding scar tissue of this particular channel, a dense capillary network was observed that apparently merged into the native network between the cardiocytes (Fig. 6c).

4. Discussion

Experimental studies on TMR were mainly done in healthy animals or animals in which an acute myocardial infarction was induced. Some studies have shown improvement of regional myocardial function when the infarction was treated by TMR [9–11]. Also, channel patency has been studied histologically in normal animals undergoing TMR [12,13]. The results are, however, controversial; some studies show systematic closure of the channels after 2–4 weeks [12,13], some show patency after 30 days [11], and some show progressive narrowing at 12 months [14]. As it can be questioned whether the results obtained in normal myocardium can provide useful information regarding the benefit of TMR in ischemic heart disease, our study focused on the setting of chronic ischemia. Therefore, we induced stenosis of the LAD and LCX 10 weeks before the TMR procedure. This model is associated with a progressive dilatation of the left ventricle and a reduction in the WTF.

In this animal model, we could show that about 50% of the lased channels remain patent or show some form of neovascularization after 10 weeks. These ‘patent’ channels are very irregular in shape, and follow a tortuous course. This may be due to the combined effect of the complex processes that have led to the formation of a neolumen, i.e. recanalization of thrombus that is initially seen after
lasering [13] and retraction of fibers during maturation of the scar. The interface between a scar and the surrounding myocardium was found to have a variable morphology. As in Fig. 6a, the margin may be sharp. The scar then appears isolated from the surrounding parenchyma and no capillaries are seen. Other scars, as in Fig. 6c, have highly irregular contours, and this condition is associated with a capillary network that apparently interdigitates with the existing myocardial network. This integrates, at least morphologically, the scar within the myocardium. One may wonder whether functional consequences may be attributed to the ‘morphologically isolated’ and ‘morphologically integrated’ scar types. Angiogenesis may play an important role here as suspected by Kohmoto et al. [15]; these authors have indeed shown that TMR leads to local vascular growth as early as 2 weeks after treatment.

In this study, we measured a relative increase in myocardial blood flow immediately after TMR using a colored microsphere technique. This very early increase in flow is associated with a temporary depression of function, induced to the myocardium by the laser treatment. This combination of increased flow and depressed function is suggested for myocardial stunning induced by the laser procedure rather than an increase in nutritional myocardial blood flow. This finding and the histological results provide no certainty that this increased flow is derived from the left ventricular cavity. The patency of the endoluminal side of the channels is difficult to prove. However, Mirhoseini et al. [16] have hypothesized that the lased channels passing from the left ventricular chamber directly into the myocardium allow oxygenated blood to perfuse the myocardium.

We documented the influence of TMR on myocardial function during the weeks following TMR. TMR dramatically depresses cardiac function during the initial period after the TMR procedure. Regional myocardial function recovered after the laser procedure, but never above the pre-laser level. The left ventricular end-diastolic cavity area increased again after TMR, suggesting that the laser procedure did not stop the degeneration of myocardial function induced by chronic coronary artery stenosis.

Extrapolation of the findings of this study to the clinical situation demands some caution. This animal study has its limitations; the obtained state of chronic ischemic heart disease is not simply comparable with human disease, the degree of stenosis is different in each individual animal and the control group did not receive a sham operation after 10 weeks. In addition, the myocardial flow data were obtained in anesthetized sheep at resting condition. These values have a limited importance as they do not reflect myocardial perfusion under stress and the possible presence of ischemia. Finally, the presence of angina, which is the main indication for laser therapy, can not be judged. However, the most objective parameter in this chronic animal study is surely myocardial function. The evolution of myocardial function over time after the TMR procedure indicates that TMR has no value to improve myocardial function in ischemic heart disease.

In conclusion, immediately post-TMR, a severe, but transient depression of cardiac function was noticed. Histological analysis of TMR-induced channels is compatible with

![Fig. 6. (a) Representative histological appearance of a scar with a single large channel, thus classified as ‘patent lumen’ (Masson stain). The scar tissue is of the dense collagenous type, sharply demarcated from the surrounding tissue (‘morphologically isolated scar’). (b) Longitudinal overview of a based channel (Masson stain). In the center of the scar, multiple transsections are seen through a large channel, thus classified as a ‘patent channel’. The contour of the scar is irregular. (c) Detail of the scar edge of Fig. 6b (Masson stain). The interface between scar tissue and myocardium is blurred by a rich capillary network (‘morphologically integrated scar’).]
angiogenesis in the channels and the surrounding myocardium. TMR-induced vascular remodeling in chronic ischemic myocardium, improved myocardial blood flow, but failed to improve cardiac function.

References


Appendix A. Conference discussion

Dr J. Melo (Carnaxide, Portugal): This is a very interesting paper. We have been working with several physical sources of energy for myocardial ablation. Whether we use radiofrequency or microwave, we obtain scars in the myocardium. And we always see patent vessels on the edges and inside the scar. But those vessels are not neangiogenesis. We believe they are native vessels that were not damaged by the heating effects. So, what proof do you have that the vessels that you are showing are neangiogenesis and not the previous vessels that were not damaged by the heating effects of the laser?

Dr Ozaki: It’s a difficult question. I probably saw the new vessels, but I’m not sure, because I don’t know the way to estimate whether this vessel is native or new vessel. So it’s very difficult. I can’t answer.

Mr J.R.L. Hamilton (Newcastle-upon-Tyne, UK): Why did you choose to use the Ho:YAG laser and not compare it with the CO₂ laser? Do you think one is better than the other?

Dr Ozaki: No, no special reason.