Transmyocardial laser revascularization in the acute ischaemic heart: no improvement of acute myocardial perfusion or prevention of myocardial infarction

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Abstract

Objective: Transmyocardial laser revascularization (TMLR) has been used to provide enhanced myocardial perfusion in patients not suitable for coronary revascularization or angioplasty. This study investigates the acute changes in myocardial perfusion after TMLR with a Holmium:Yttrium-Aluminium-Garnet (YAG) laser with a thermal imaging camera in a model of acute ischaemia, and confirms its midterm effects by post-mortem investigation of magnetic resonance imaging and histopathological examination. Methods: Acute myocardial ischaemia was induced by occlusion of the dominant diagonal branch in ten sheep. Perfusion measurements were undertaken first in the unaffected myocardium, then after temporary occlusion of the coronary to obtain a control measurement for ischaemic myocardium. Myocardial perfusion was then evaluated during reperfusion after release of coronary occlusion. Then the coronary was permanently occluded and 20.5 ± 2 channels were drilled with the Holmium:YAG laser and perfusion was measured again. The other four sheep served as control with untreated ischaemia. All animals were sacrificed after 28 days following administration of gadolinium i.v. to serve as contrast medium for magnetic resonance tomography. The hearts were subjected to magnetic resonance tomography and histopathological examination.

Results: Intraoperative perfusion measurements revealed a decreased perfusion after temporary occlusion and an increased perfusion in reperfused myocardium. After TMLR, no improvement of myocardial perfusion above the ischaemic level could be shown. Magnetic resonance images could neither confirm patent laser channels nor viable myocardium within ischaemic areas. On histology no patent endocardial laser channel could be detected. The transmural features were myocardial infarct with scar tissue. Conclusions: In the presented sheep model with acute ischaemia, TMLR with a Holmium:YAG laser did not provide acute improvement of myocardial perfusion above the ischaemic level could be shown. Magnetic resonance images could neither confirm patent laser channels nor viable myocardium within ischaemic areas. On histology no patent endocardial laser channel could be detected. The transmural features were myocardial infarct with scar tissue.

Keywords: Transmyocardial laser revascularization; Holmium:YAG laser; Animal model; Thermal imaging; Magnetic resonance imaging

1. Introduction

Many attempts to perfuse ischaemic myocardium directly from the ventricular cavum were undertaken since Sen et al. [1] proposed direct acupuncture of the myocardium with a needle to relieve myocardial ischaemia. The first application of transmyocardial laser revascularization (TMLR) was done by Mirrheoseini et al. [2] in dogs. In 1983 they reported the first use in humans on a cardioplegical arrested heart [3]. TMLR now is increasingly used as a new operative treatment for patients with so far untreatable diffuse occlusive coronary heart disease, having symptomatic angina pectoris under maximal drug treatment unsuitable for angioplasty or
coronary artery bypass grafting. In these patients TMLR treatment reduces angina significantly in the early postoperative period, but the mechanisms of this clinical effect remains unclear.

Three different types of lasers are used. The high power carbon dioxide (CO₂) laser is creating the laser channel by a single shot and the Holmium:Yttrium-Aluminium-Garnet (YAG) and Excimer lasers are creating the channels with multiple impulses of lower power guided in a fibroptic into the myocardium.

The purpose of this study was to investigate acute and mid-term effects (28 days) of a Holmium:YAG laser revascularization in the acute ischaemic myocardium in a sheep model. First, acute myocardial perfusion changes were measured with a thermal imaging camera. Second, we investigated post-mortem the consequences of TMLR in magnetic resonance imaging and analyzed the macroscopic and microscopic findings.

2. Materials and methods

2.1. Laser

The laser used was a pulsed, solid state Holmium: YAG laser (Eclipse® TMR 2000™, Kiel Court, CA, USA) operating at a wavelength of 2.1 μm in the mid-infra-red spectrum. The output power at the tip was 8 W the pulse width 250 μs and the pulse rate 5 pulses per s. The laser delivery fibreoptic system had an outside diameter of 1 mm.

2.2. Animal model

All animals received human care in compliance with the European Convention on Animal Care. The investigation was approved by the authorities of the administrative district of the country Baden–Württemberg (Germany) on September 1996.

Ten sheep were used, with a mean weight of 37.3 kg (range, 30–42 kg) for the study. After preoperative medication with xylazine (1 mg/kg body weight i.m., Bayer AG, Germany) and atropine sulfate (1 mg i.m., B. Braun Melsungen AG, Germany) the animals were anaesthetized with halothane and oxygen inhalation anaesthesia, intubated and mechanically ventilated. The electrocardiogram was monitored during operation and the right common carotid artery was cannulated for blood pressure monitoring and arterial blood gas analysis. The values were kept within physiologic norms. A central venous line was placed in the internal jugular vein for administration of fluid and drug infusions. Left thoracotomy was performed at the level of the 5th intercostal space, and the heart was exposed and suspended in a pericardial cradle. A bolus injection of intravenous xylocaine® (1.5 mg/kg, ASTRA GmbH, Germany) was given and pacing leads were temporarily sewn on the right ventricular surface and connected to a external pacemaker.

At this point, the animals were radomized to one of two groups: laser treatment or control. In all animals the dominant diagonal branch of the left anterior descending coronary artery was ligated to induce an acute myocardial infarction on the anterior free wall of the left ventricle. After ligation of the coronary artery, the region of interest was easily identified as the acutely ischaemic myocardium appeared to demarcate itself.

One group (n = 4) served as the control group for magnetic resonance imaging and histology with untreated myocardial ischaemia.

The other group (n = 6) was the laser group receiving TMLR treatment after permanent ligation of the coronary artery. Perfusion measurements were undertaken in this group as described below.

At the end of the operation, in all animals chest tubes were put in each hemithorax and the wound was closed in the usual manner. A pericostal infiltration anaesthesia was set with 10 ml Carboestesin® 0.25% (ASTRA GmbH, Germany). After extubation of the animals the chest tubes were removed and after adequate recovery they were allowed to reside to the sheep-pen.

The animals were sacrificed after 28 days with T61® (10 ml i.v., Hoechst, Germany). A single dose of Gadolinium-DTPA (0.1 mmol/kg body weight, Magnevist®, Schering, Germany) was injected 15 min before cardiac arrest of the animals. After death, the hearts were taken to magnetic resonance tomography and to histological examination.

2.3. Perfusion measurements

Perfusion measurements in the region of interest were made with an infrared thermal imaging camera (IVA 2000™, OPGAL, Karmiel, Israel). Perfusion measurements were undertaken only in the TMLR group. The method uses the dynamic infra-red thermography data which accumulates from the thermal camera which is then pelleting out the results in numbers representing the grey levels as defined in arbitrary units. The camera was focused on the region of interest distally of the dominant diagonal branch of the heart and the perfusion was measured – in order to determine the actual perfusion level of the myocardium (normal perfusion). Then the diagonal branch was temporally occluded to obtain a control measurement for under-perfused myocardium in the same animal, providing the grey level measurements corresponding to coronary occlusion (temporary occlusion). After reopening of the diagonal branch, the grey level measurements caused by reperfusion of the previously ischaemic myocardium (reperfusion level) were assessed. After a reperfusion time of 15 min, the diagonal branch was permanently occluded. Then 20.5 ± 2 channels within a minimum distance of 5 mm were drilled with the Holmium:YAG laser in the demarcated area of the myocardium directed from the epicardial surface to the left
ventricular cavity. During creation of the channels, slight pressure was applied to guide the fiberoptic system carefully through the myocardium. Five to eight pulses were required for full-thickness perforation of the myocardial muscle. A loss of resistance was noted when the ventricular cavity was reached. After removal of the fibre, pulsatile bleeding from the cavity was stopped by digital compression for a few minutes until homeostasis was obtained. Another 15 min after completion of the TMLR therapy, perfusion was measured again over the region of interest (TMLR after occlusion).

2.4. Statistical analysis

Grey level dates are expressed as mean ± SEM. When comparing two groups, Student’s two-tailed unpaired t-test was used. A value of $P < 0.05$ was considered indicative of a statistically significant difference.

2.5. Magnetic resonance imaging and image analysis

Magnetic resonance imaging and image analysis The hearts were positioned in a circularly polarized head coil on a 1.5 T whole-body imager (Magnetom Vision, Siemens, Erlangen). A snapshot-FLASH 2D localizer was used to define the intrinsic anatomic axes of the heart. T1- and T2-weighted MR imaging of the entire left ventricle was performed in short axis orientation using three-fold segmented fast spin-echo sequences (T1-weighting: TR 600 ms, TE 12 ms; T2-weighting: TR 3000 ms/TE 90m, 192*256 matrix, 113*150 mm field of view, 5 mm slice thickness, voxel size of 1.7 mm, three signal averages). Additionally, a conventional T1-weighted spin echo sequence (TR 600 ms, TE 15 ms, 320*512 matrix, 94*150 mm field of view, 2 mm slice thickness, voxel size 0.2 mm, two signal averages) was applied in the infarct region to achieve high resolution images of laser channels.

Short axis cuts of the left ventricle were divided into eight regions (45° steps of 360° in a counter clock manner: anterior, antero-lateral, lateral, infero-lateral, inferior, infero-septal, septal and antero-septal) for image analysis. Regions of normal and elevated signal intensities in T1- and T2-weighted fast spin-echo images were defined visually by consensus film reading of two observers (S.M., U.V.). Nor- mal signal intensity of the myocardium was defined to be present at the infero-septal wall, which was not supposed to be involved into regional myocardial ischaemia, caused by ligation of the R diag. II.

2.6. Histopathological examination

After magnetic resonance investigation all hearts were fixed in 4% buffered formalin. The hearts were sliced parallel to the heart base. Tissue samples were taken from the myocardial scar and the adjacent viable myocardium. Sections 4 μm thick were stained with hematoxylin-eosin, Masson trichrome and Elastica-van-Gieson before being reviewed by a pathologist. Specimens were taken from all of the control and the TMLR animals.

3. Results

All animals survived the operation to be subjected to magnetic resonance tomography and histopathological examination after 28 days. In the TMLR group, intraoperative homeostasis of the created laser channels was ensured in all cases with epicardial digital pressure. In this group we noted a considerable incidence of ventricular tachycardias degenerating into ventricular fibrillation despite intravenous application of xilocaine. In two sheep (33.3%) of the TMLR group, five episodes of ventricular fibrillation occurred, all could be terminated with direct internal defibrillation.

3.1. Perfusion measurements

After TMLR no improvement of perfusion could be detected in the lased myocardial areals. Also no improved rewarming could be seen right next to the laser channels leading to the assumption that there would be no detectable blood flow in the channels.

A total of 103 measurements were obtained. Grey level data showed a significant decrease after ischaemia ($P < 0.01$) when compared with normal perfused myocardium and a significant increase after reperfusion ($P < 0.01$) (Table 1). Grey level data after TMLR did not significantly differ from myocardial perfusion after ischaemia ($P = 0.2$) but were also significantly reduced in comparison with normal perfused myocardium ($P < 0.01$).

3.2. Magnetic resonance imaging

In all animals, elevated signal intensity of short axis T1- and T2-weighted images was found in the antero-lateral region consistent with infarcted myocardium. On high resolution, T1-weighted images laser channels were detected directly within or adjacent to the infarct region, presenting as a straight line of increased signal intensity as well (Fig. 5).

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Normal perfusion</th>
<th>Temp. occlusion</th>
<th>Reperfusion</th>
<th>TMLR after occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ($n=103$)</td>
<td>$n=20$</td>
<td>$n=24$</td>
<td>$n=20$</td>
<td>$n=39$</td>
</tr>
<tr>
<td>Grey level data</td>
<td>$393 \pm 67$</td>
<td>$300 \pm 108$</td>
<td>$497 \pm 122$</td>
<td>$284 \pm 83$</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD. Normal perfusion: Temp. occlusion $P < 0.01$; Normal perfusion: Reperfusion $P < 0.01$; Temp. occlusion:TMLR after occlusion $P = 0.2$; Normal perfusion:TMLR after occlusion $P < 0.01$. 

Table 1
1). The appearance of myocardium next to laser channels did not differ from signal behaviour of infarcted myocardium in controls. Signal intensities comparable with the baseline value of normal myocardium, defined at the infero-septal segment, were not recognized. Therefore, magnetic resonance imaging did not show any sign of normal myocardium in regions after TMLR.

3.3. Histopathological findings

On the surface of the heart considerable epicardial adhesions with the pericardium and fatty tissue were found in the TMLR group. Besides focal or complete pericardial fibrosis, the hearts of the TMLR group as well as of the control group, showed a transmural scar distal of the ligature of the diagonal branch. The size of the scar varied individually. Endocardial defects created by the laser could be identified as dot-like retracted tissue. Defects of the visceral pericardium, however, were often obscured by fibrous adhesions.

Microscopically besides small areas of myocardial necrosis the myocardial tissue was replaced by a dense collagenous scar. Within the laser channels could not be detected. The only hint for channels were defects of the elastic fibres of the endocardium and the visceral pericardium (Fig. 2).

4. Discussion

TMLR is a relatively new surgical therapeutic option to treat patients with end-stage coronary artery disease. However, the scientific evidence for this treatment is based only on a small number of animal studies. Therefore, many different results were published in the literature so far.

Weearn et al. [4] first described the myocardial microanatomy with sinusoidal vascular plexus. First application of a laser for TMLR was published by Mirhoseini et al. [2,3] with a CO2 laser. They ligated the left anterior descending coronary artery and found microscopically that the channels were patent and endothelialized after up to 5.5 months. Goda et al. [5] studied TMLR using CO2 laser with acute myocardial infarction. They could not demonstrate any evidence of long-term patency of the transmyocardial channels. Hardy et al. [6] compared transmyocardial channels created by a CO2 laser with needle puncture. Needle puncture channels were fully occluded within 48 h, whereas laser-induced
Channels maintained partial patency for a 2-week period, after which they also became occluded. The only sheep model in TMLR research to our knowledge so far was introduced by Horvath et al. [7]. The sheep heart should therefore be an optimal animal model because of the lack of well developed collaterals. They created infarcts by coronary occlusion and examined the short- and long-term (30 days) effectiveness after TMLR with a CO2 laser. After 30 days, histologic analysis of the laser-treated infarcts revealed patent channels surrounded by viable myocardium. This successful investigation led us to choose this sheep model too for our investigation with a similar set up, but to use a Holmium:YAG instead of a CO2 laser.

Less studies had been performed with a Holmium:YAG laser so far. Whittaker et al. [8] failed to increase blood flow to ischaemic tissue using a Holmium: YAG laser in a model of acute ischaemia after 6 h of permanent coronary artery occlusion. These findings in myocardial perfusion after TMLR with injected microspheres were similar to our findings after TMLR with a Holmium:YAG laser detected by thermal imaging perfusion measurements. Our perfusion measurements could be obtained in an acceptable range to compare the different perfusion states. Images of lased channels after TMLR with the thermal camera were reported by Maas in 1996 [9]. In contrast to his findings, we could not detect patent laser channels and signs of improved rewarming of the myocardium surrounding the laser channels. Limitations of this measurement method with the thermal imaging camera are obvious. Obtained perfusion data represent arbitrary units and can only be interpreted when compared with reference data. Reference data must be obtained from the same area of the heart at the beginning of the investigation. The heart must be well exposed for the measurements and it is advantageous when it is not covered by fatty tissue, and perfusion measurements must be obtained from a relatively large area to reduce influences of the beating and moving heart.

Our perfusion measurements support the findings of Kohmoto et al. [10]. They created TMLR channels using a Holmium:YAG laser. To measure direct transmyocardial blood flow, coloured microspheres were injected into the left ventricular chamber. No significant transmyocardial blood flow could be observed through channels made with the Holmium:YAG laser. In the chronic setting with 2-week survival of the animals, no flow was detected through the channels, and the endocardial entry points were closed. The lack of improvement of regional myocardial blood flow in acute ischaemic myocardium after TMLR seemed not to be laser-type depended and was also found by Hardy et al. [11] after TMLR with a CO2 laser.

This stands in contrast to the findings of Yano et al. [12]. The difference between laser-revascularized and control dogs, in contractility assessed from regional preload recruitable stroke work in the ischaemic region was significant. In their series, induced arrhythmias were well tolerated in the non-ischaemic heart. In our study, intraoperative problems with ventricular fibrillation were remarkable. They were induced by lasing the transmural channels with the non-ECG triggered laser as also reported in human treatment [13]. Laser systems synchronized with the R-wave are reported to be significantly less arrhythmogenic [14].

The obtained myocardial magnetic resonance images of our study were comparable with the late wash out phase of ischaemically injured myocardium in vivo [15]. Regions of myocardial infarction can be expected to present with increased signal intensity in T1- and T2-weighted MR images. The intravitally applied gadolinium was washed out only of viable myocardial cells and enriched in malperfused and fibrotic areas of the heart. After explantation of the heart the left ventricular cavum was filled with saline to regain the anatomic configuration of a resting heart. Images showed a sharp borderline between viable myocardium in black and infarct area in white. In some images, longitudinal white lines were visible, believed to be scars from fibrosed TMLR channels. No viable myocardium in the infarcted areas could be detected in any of the examined hearts. Magnetic resonance findings from hearts of the control group did not differ in signal intensity and appearance from hearts after the TMLR procedure. This was confirmed by our histopathological examinations. TMLR treated animals had no numerical increase of vessels in the borderline zone between infarct tissue and viable myocardium compared with the control group. Therefore, no visible signs of angiogenesis induced by TMLR channels could be seen.

Angiogenesis was first examined by Fleischer et al. [16]. Transmyocardial revascularization alone resulted in a significant injury response, including increased vascularity without patent channels. Vascular endothelial growth factor increased surrounding inflammation without improving vascularity or patency. They claimed that the clinical benefit of TMLR may result simply from a non-specific histologic response to injury and that molecular interventions appear to stimulate more inflammation but no additional angiogenesis. Mueller et al. [17] examined TMLR in a model with acute ischaemia using a Holmium:YAG laser. They found signs of neovascularization only around the channels in non-ischaemic myocardium. Macroscopic and microscopic findings were similar to our findings. On examination of the endocardial surface, no open channel could be detected and on histology, in the laser-infarction group the channels were embedded in the infarction scar. In contrast therefore, Yamamoto et al. [18] found in a canine model of chronic ischemia, that TMLR significantly should enhance angiogenesis as evidenced by the increased number of vessels, markedly increased vascular proliferation and increased blood flow capacity during stress.

These findings could be dependent on the model with chronic ischemia leading to the main limitation of our and previous studies dealing with acute ischemia. Although the animal model with acute ischemia is discussed not to be the
optimal model for TMLR, it should be mentioned that introdu-
cution of TMLR with a CO2 laser in human treatment was
based on results in even this animal model with acute ische-
mia. In the past, unfortunately no standard animal model
was established and therefore results are difficult to com-
pare. In addition, the assessment of results is further comp-
icated by the use of three different laser types, the CO2,
Holmium:YAG and Excimer lasers. So far, it has not been
clearly shown the reason for occlusion or patency of laser
channels and in this case, how long patency is present. Our
results indicate an immediate occlusion directly after lasing.
Nevertheless, promising results in the chronic ischaemic
animal model stands in contrast to several post-mortem
examinations in patients after TMLR.

Not many data from examined hearts of patients who died
after TMLR were published so far. The first anatomic evi-
dence of long-term channel patency in one patient who died
3 months after TMLR with a CO2laser was reported by
Cooley et al. [19]. Burkhoff et al. [20] presented autopsy
results from a patient obtained 4.5 weeks after TMLR that
showed that the channels do not maintain patency. Gassler
et al. [21] examined three hearts from patients who died
without clinical evidence of a persistent therapeutic effect,
up to 150 days after TMLR. They found no evidence of
patent and endothelialized laser-created channels. In con-
trast therefore, Schweitzer et al. [22] examined hearts of
patients who died during the first 18 days after TMLR and
could verify open intramyocardial channels in all patients.
These channels communicated with intramural vessels, but
in no instance with chamber lumen, so that additional blood
could not be delivered directly from the left cavum.

From our investigation and the above discussed different
results from animal investigations, a clear underly-
ing mechanism of TMLR could not be presented so far, con-
tributing to the observed clinical benefits [23,24]. Inconstant
findings of patent laser channels and angiogenesis led to
the suggestion that mechanisms other than blood flow
through the channels or angiogenesis triggered not by the
inflammation reaction of the surrounded tissue should be
considered. Neural destruction leading to symptoms relief
in patients should be further examined in clinical trials. At
least it seemed unclear, whether the different laser types led
to the same or different results in myocardial changes and if
the terminus ‘Transmyocardial Laser Revascularization’
should be the correct description for the practised treatment.

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Appendix A. Conference discussion

Dr B. Walpoth (Bern, Switzerland): Your presentation has two major limitations. First, in the acute phase, all the channels must be thrombosed; otherwise you would have continuous bleeding. Thus, you will not have increased perfusion signals on your thermal camera. Second, you created an acute infarct, and as you well know from the literature, that is just not suitable myocardium to perform TMR. So I think you should revise some of your conclusions.

Dr Eckstein: All animal studies performed and published so far except one investigated laser channels in acute ischaemic myocardium. Only Yamamoto et al. (J Am Coll Cardiol 1998) published a canine model with a hibernating myocardium proving enhanced angiogenesis after TMLR. All published models had varying animal species to investigate with a hibernating myocardium proving enhanced angiogenesis after TMLR. So I think you should revise some of your conclusions.

Dr Eckstein: The measuring depth of the used thermal imaging camera is a few millimetres, approximately half of the thickness of the myocardium. The received data are in arbitrary units, no absolute flow capacity data are available so far. It is only possible to measure temperature changes of the investigated myocardium related to undertaken reference measurements.

Dr Kanellopoulos: Do you think that this methodology could detect shunting of blood from the left ventricular cavity into post capillary venous spaces? What is the sensitivity of the methodology that you used?

Dr Eckstein: We have not investigated this.

Dr R. Seitelberger (Vienna, Austria): I have to agree with Dr. Walpoth that I think it is the wrong model that you used, because everybody knows that the laser does not treat myocardial infarction. That is why I am asking for the border zone. You mentioned histological investigations not only in the core ischaemic zone but also in the border zone, and then my question actually points to this area. Did you quantify or could you quantify angiogenesis in terms of developing new collaterals in the border zone, because even in this model, this would be the only interesting area?

Dr Eckstein: We could not find enhanced vascularization in the border zone of the TMLR group.

Dr Seitelberger: You mentioned in the talk that you did see more collaterals or more vessels in the border zones.

Dr Eckstein: In the border zone of both groups was enhanced vascularization due to normal repair mechanisms after infarction. In the TMLR group, laser channels in the border zone were occluded and not surrounded by enhanced collateralization.