Transmyocardial laser revascularization preserves regional myocardial perfusion: an MRI first pass perfusion study

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Abstract

Objective: It is controversial whether transmyocardial laser revascularization (TMLR) improves myocardial perfusion. Therefore, we assessed myocardial perfusion before and after TMLR with quantitative magnetic resonance perfusion imaging (MRPI) in an animal study.

Methods: One week after partial occlusion of the left circumflex artery (LCx) in 12 pigs, resting perfusion (ml/g/min), perfusion reserve (PR) with adenosine, regional wall thickening (RWT), cardiac output (CO) were quantified with MRI in the LCx (lateral) and LAD (septal) dependent myocardium. Subsequently, six animals were treated with TMLR of the lateral left ventricle (LV). Six animals were left untreated. A final MR was performed 8 weeks later. MRPI data were compared to microsphere-derived blood flow and % LV necrosis (TTC). ‘Normal’ myocardial perfusion was assessed with MRPI in 12 non-instrumented animals.

Results: Resting perfusion prior to TMLR (0.7–0.9 ± 0.3) in the LV-lateral myocardium was preserved after TMLR (1.0 ± 0.3) and decreased without TMLR (0.3 ± 0.1, P < 0.05). There was a significant difference (P < 0.01) between the TMLR treated and untreated group. Compared to ‘normals’ (1.2 ± 0.2) perfusion of the LV-lateral wall was not different after TMLR but reduced (P < 0.02) without TMLR. PR was not different between TMLR-treated (1.4 ± 0.9) and untreated (1.9 ± 0.6) group but was reduced (P < 0.04) compared to PR of ‘normals’ (2.7 ± 0.8). MRPI data and microsphere-derived perfusion were significantly correlated (P < 0.01). RWT in the LCx-dependent myocardium improved (P < 0.02) after TMLR. CO decreased (P < 0.02) and TTC-staining indicated more LV-necrosis without TMLR (6.6 ± 1.6 vs. 3.7 ± 1.5, P < 0.01).

Conclusion: TMLR preserves regional myocardial perfusion and improves function as shown with MRPI.

Keywords: Cardiovascular surgery; Coronary circulation; Infarction; Microcirculation; NMR; Regional blood flow; Stunning

1. Introduction

Bypass surgery or angioplasty is routinely applied to restore blood supply to ischemic myocardium. Nevertheless, many patients with end stage coronary artery disease continue to suffer from disabling angina. This has led to increasing interest in alternative revascularization methods such as transmyocardial laser revascularization (TMLR). Recent clinical trials have demonstrated symptomatic relief in patients with refractory angina after TMLR. However, it remains controversial whether this symptomatic relief is accompanied by improved myocardial perfusion [1–4]. Conventional nuclear imaging techniques which are often used in clinical trials, have lower spatial resolution compared to magnetic resonance imaging (MRI) [5]. Furthermore, these techniques assess perfusion only semiquantitatively.
tively, with the exception of PET-imaging and might have limited capability to demonstrate absolute changes of myocardial perfusion, i.e. in patients with multi-vessel coronary artery disease [6]. On the other hand, MRI offers absolute quantification of myocardial perfusion with excellent spatial resolution [5]. In addition, it also allows quantification of regional and global myocardial function [7].

In our study we used MRI to assess changes of myocardial perfusion and function after TMLR in a porcine model. We hypothesized that quantitative MRI can be used to monitor changes in function and perfusion after TMLR. To induce dysfunctional myocardium we created a coronary stenosis with subsequent occlusion by placing a hollow-bead into the left circumflex artery (LCx). This model formerly introduced by Gewirtz et al. [8] has the advantage to avoid open-heart surgery prior to therapeutic interventions in an animal model.

2. Methods

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

One week after partial occlusion of the left circumflex artery (LCx), 12 pigs (27±2 kg, range 24–29 kg) underwent a baseline MRI to measure myocardial perfusion and function. The animals were then randomized to a treatment (n=6) and an untreated group (n=6). The treatment group underwent TMLR immediately after the baseline MRI. That was 1 week after partial occlusion of the LCx. Eight weeks after the baseline MRI, a second MRI was performed in both groups and microspheres were injected to determine myocardial blood flow. The animals were then euthanized and necrotic myocardium was determined by histochemical analysis. MRI was also performed in a group of non-instrumented, weight-matched pigs (n=12) to measure ‘normal’ myocardial perfusion and function.

2.1. Induction of ischemia

The animals were premedicated with ketamine (25 mg/kg; Keta Ved, Vedco, MO) and anesthesia was maintained with pentobarbital (35 mg/kg, Ambro Pharmaceutical, CA). An 8F-catheter (Bard-USCI, Billerica, MA) was advanced fluoroscopically into the LCx via a right carotid access. A custom made (University of Minnesota, Biomedical Engineering Institute) hollow bead (outer diameter: 1.36–1.85 mm; inner diameter: 1 mm, length: 4 mm) was advanced in to the proximal LCx over a 0.018-inch guide-wire following a previously described protocol [8]. Bead diameter was predetermined according to the diameter of the LCx and the appropriate bead (1.36–1.85 mm) was implanted causing an immediate stenosis of approximately 50% lumen reduction. Heparin i.v. (300 IE/kg, Elkins-Sinn Inc., Cherryhill, NJ) was administered over a period of 7 days following bead implantation. We previously found that the beads close approximately 10 days after implantation when treated with heparin (unpublished data). According to a study by Weaver et al. [9] and the exclusion of coronary abnormalities by the initial angiogram the LCx region covers about 25% of the porcine left ventricle.

One week after implantation of the bead the instrumented animals underwent anaesthesia (as above) and a coronary angiogram was performed preceding the baseline MRI. A left ventricular (LV) and aortic catheter were placed via the femoral artery for pressure monitoring during the MRI. A central catheter in the jugular vein was used for administration of drugs and venous access. The non-instrumented group of animals underwent the same protocol for anaesthesia and MRI imaging.

2.2. TMLR procedure

TMLR was performed in six of the 12 instrumented animals immediately after the baseline MRI. As previously described [10], a left lateral thoracotomy through the fifth intercostal space was performed in six of the instrumented animals. A modified high-powered 1000 W (40±5 J pulse energy) CO₂ laser (PLC Medical Systems, Franklin, MA) was used to create 30±5 laser channels over the LV lateral wall. Transmural penetration was confirmed by transesophageal echocardiography. Hemostasis was obtained by manually applied gentle pressure and the thorax was closed in layers.

2.3. Image acquisition

The imaging protocol was identical for baseline and the 8-week follow-up MRI. Anatomical landmarks were used to match slices and regions from two different measurements. A 1.5-T MRI scanner (Siemens Vision, Germany) and a phase-array body coil were used for imaging. Two scout images determined the short and long axis view of the heart. For cine-imaging an ECG-gated, non-breathhold, segmented cine sequence was used. TR, TE and flip angle were 33, 6 ms, and 25°, respectively. The spatial resolution was 2 mm×1.4 mm² and the slice thickness was 8 mm with an increment of 10 mm. The temporal resolution was 50 ms. The heart was imaged from base to apex with eight to ten LV-short axis slices. Perfusion imaging was performed in three slices each matched to a cine slice using the coordinates given by relative position of the heart in the scanner. After rest images were acquired, imaging was repeated under adenosine (Adenoscan; Fujisawa, IL), which was infused over the central line increasingly titrated from 70 to a maximum dose of 140 µg/kg/min. After stable circulatory conditions were reached image acquisition was started. Perfusion was determined using a
single shot saturation-recovery FLASH sequence with linear k-spacing. TR, TE and flip angle were 2.4, 1.2 ms, and 18°, respectively. The slice thickness was 10 mm at a temporal resolution of 1 image/heart beat/slice and a spatial resolution of 2–3 mm. Three heart beats after initiation of the sequence, a compact bolus of 0.03 mmol/kg bodyweight gadolinium-DTPA (Magnevist, Schering AG, Germany) was injected over the central venous line at a rate of 9 ml/s using a power injector (MedRad, PA). A total number of 40 images/slice were acquired.

2.4. Image analysis

The image analysis was performed under blinded conditions. For cine analysis (MASS software, Leiden, Netherlands [11]) endo- and epicardial borders of the LV were defined each in end diastolic (ED) and end systolic (ES) frame in contiguous slices. Cardiac output (CO, ml/min/kg) was determined from the stroke volume (SV, ml) and the heart rate (beats/min) and adjusted to the body weight (kg). Regional wall thickening (RWT, mm) was determined using the centerline method [12]. According to this method the myocardium is partitioned in 100 cords aligned perpendicular to the endo- and epicardial border. Thickening in septal and lateral myocardium was averaged over 25 cords in the according region.

Perfusion studies were analysed (Argus Software, Siemens, NJ) by manually applying endo- and epicardial contours to one image. An automated algorithm matched the contours to the remaining images. Spatially averaged signal intensity (SI) values were used to plot SI–time curves of the septal myocardium (between the anterior and posterior juncture of the right ventricle) and the lateral wall (between the anterior and posterior papillary muscle) of the LV short axis. Model-constrained deconvolution was used to calculate the maximum amplitude of the impulse response function from the SI–bolus curves [13]. Based on the central-volume theorem and the input function of the bolus, the maximum amplitude of the impulse response function can be interpreted as a measure of flow in ml/g/min [14].

2.5. Determination of blood flow with microspheres and histopathology

Histopathologic and microsphere blood flow data were acquired in all TMLR treated and un-treated animals. Myocardial blood flow (MBF, ml/min/g) was determined using radiolabeled microspheres, which were injected at rest (strontium) and hyperaemia (caesium) as described earlier [15].

The amount of LV necrotic tissue was determined using triphenyltetrazolium chloride (TTC). The necrotic area was determined by planimetry in slices of 10-mm thickness according to the double oblique short axis orientation of the LV obtained by CMR. The volume of the necrosis was determined by the product of the average area of the TTC-unstained area at the basal and apical surface of each slice times the thickness. In each animal no more than one or two slices contained necrotic (TTC-unstained) myocardium.

2.6. Statistics

Data were analysed using MedCalc for Windows Version 5 (MedCalc Software, Belgium). A paired t-test was used for testing rest vs. hyperaemic data. Data at 1 or 8 weeks, and between two regions in one group, were tested with one factor ANOVA. Follow-up data were tested using two-factor ANOVA with repetition (treatment and time). All data are mean±standard deviation (S.D.). A P value of <0.05 was considered to be significant.

3. Results

3.1. Angiography

One week after bead implantation, the angiogram showed a patent LCx with delayed anterograde filling in all instrumented animals. No detectable collateral flow was noted.

3.2. Regional myocardial perfusion with MRPI

In non-instrumented animals ‘normal’ resting and hyperaemic perfusion were 1.2±0.2 and 3.3±1.2 ml/g/min and perfusion reserve was 2.7±0.8.

Fig. 1a and b show myocardial perfusion data of all instrumented animals. Bead placement resulted in a significantly (P<0.05) lower resting perfusion in the lateral vs. the septal wall. However, neither lateral nor septal myocardial perfusion of the instrumented animals was significantly different compared to the regionally matched ‘normal’ perfusion of the non-instrumented animals. Hyperaemic perfusion was reduced in both lateral (1.1–1.3±0.3 ml/g/min) and septal (1.8±0.3 ml/g/min) myocardium in instrumented vs. the non-instrumented animals (P<0.01). With TMLR of the lateral wall perfusion did not change in this region at rest (see Fig. 1a) or hyperaemia (1.3±0.3 ml/g/min). However, without TMLR resting (see Fig. 1a) and hyperaemic (0.5±0.2 ml/g/min) perfusion decreased significantly (P<0.02) in the lateral wall and was significantly lower at rest and hyperaemia compared to the TMLR-treated group (P<0.01) and to ‘normal’ myocardium of the non-instrumented group (P<0.01). With or without TMLR-treatment adenosine failed to show a significant effect on perfusion in the lateral wall but significantly increased perfusion in the septum (P<0.05). The perfusion reserve was not different between regions or groups of the instrumented animals.
(1.2–1.8) at any time and never reached the levels seen in 'normal' myocardium of non-instrumented animals.

3.3. Microsphere-derived myocardial blood flow

Microsphere-derived MBF data are included in Fig. 2. The data correlated significantly (P<0.01) with the MRI perfusion data.

3.4. Histopathology

The beads were found occluded in all animals 9 weeks after implantation. TTC-staining revealed small 'patchy' TTC-unstained areas (see Fig. 3) with significantly higher amount of TTC-unstained myocardium in the TMLR-untreated vs. the treated group (6.6±1.6 vs. 3.7±1.5% of total LV volume, P>0.01).

3.5. Regional myocardial function

Data of regional myocardial wall thickening (RWT) is shown in Table 1. RWT in the lateral wall of all animals was significantly (P<0.01) lower compared to septal myocardium and RWT in the group of non-instrumented animals (2.1±0.1 mm). RWT improved significantly (P<0.02) after TMLR and was significantly (P<0.05) higher compared to RWT in the group of untreated animals. At 8 weeks, the RWT of the TMLR-treated lateral myocardium was not significantly different from 'normal' RWT of non-instrumented animals. RWT in the septum of all instrumented animals was significantly (P<0.01) higher compared to the non-instrumented animals.

3.6. Global function and volume

SV and CO decreased and was significantly (P<0.01) reduced without TMLR (SV: from 1.4±0.3 to 0.9±0.2 ml/kg, P<0.01 and CO: from 120±20 to 73±18 ml/kg/min, P<0.01) but was preserved with TMLR (SV: from 1.4±0.3 to 1.3±0.2 ml/kg, P=n.s. and CO: from 116±23 to 118±12 ml/kg/min, P=n.s.).
4. Discussion

In this controlled animal study a beneficial effect of TMLR on myocardial perfusion and function was demonstrated using cardiac MRI. Perfusion was preserved after TMLR of stenosis-dependent lateral left ventricular myocardium with subsequent LCx occlusion. The preserved perfusion was accompanied by improved regional and preserved global left ventricular function. In addition, the amount of myocardial necrosis developing during the course of the study was less after TMLR. Compared to a group of normal, un-instrumented animals perfusion at rest and hyperaemia deteriorated with occlusion of the coronary artery in the untreated group but not after TMLR.

After occlusion of the LCx, antegrade flow was not restored in our model. Therefore, the perfusion in the lateral wall completely depends on collateral perfusion. TMLR is believed to improve myocardial perfusion by

![Fig. 2. Linear correlation between myocardial perfusion determined with MR and myocardial blood flow (open circles) based on microsphere measurements in treated and untreated group at rest and hyperemia.](https://academic.oup.com/cardiovascres/article-abstract/57/1/63/375852)

![Fig. 3. The upper row of pictures shows MR perfusion images of the left ventricular (LV) short axis in an untreated control animal with a lateral perfusion defect (arrow) 8 weeks after induction of stenosis and the matched pathologic TTC-stained sample. The pictures below are taken from an animal 8 weeks after TMLR treatment and the matched pathologic TTC-stained sample. The white 'patchy' stains in the TTC samples indicating necrotic myocardium.](https://academic.oup.com/cardiovascres/article-abstract/57/1/63/375852)
induction of neovascularization and collateral growth [16]. Thus, the differing perfusion values between treated and untreated group in the LCx-dependent myocardium can be seen as a result of TMLR.

In the present study, MR analysis demonstrated impairment not only of myocardium distal to an occluded coronary artery but also of myocardium remote from the occluded artery. This finding is in agreement with previous studies demonstrating that perfusion [17] and function [18] in regions remote from a flow-limiting stenosis is indirectly affected [19] by the stenosis. An increasing workload and mechanical tethering [19] in the compensating [20] remote region have been identified to cause these changes.

A wall thickening of 51–93% found in our study appears high considering the published literature on normal myocardial wall thickening assessed with ultrasonic microspheres (48%) [21], echocardiography (35%) [22] or MRI (41%) [23]. Part of it might be explained by a compensatory function in the remote myocardium. As demonstrated in a study by Buda et al. [24], percent wall thickening in the non-ischemic remote region was 76% measured with echocardiography. This is close to the values we found in compensating remote myocardium. The following methodological limitations further explain overestimation of wall thickening with MR. First, the employed analysis software does not correct for through-plane motion of the myocardium and, second a reduced in- and through-plane blood flow in the dysfunctional left ventricle can cause a decreased blood-endocardium contrast and an overestimation of the end-systolic thickness [25].

4.1. Relationship of perfusion and function

In the TMLR-treated group myocardial function recovered despite a complete occlusion of the artery. Thus, TMLR treatment prevented the deterioration of myocardial perfusion and helped to re-establish myocardial function. The situation where function improves without changes in normal baseline perfusion, as it is seen after TMLR in our study, is also observed in stunned myocardium. However, we are unable to distinguish between necrotic and ischemic myocardium with the employed MR method and we cannot conclude that stenosis-dependent myocardium in our study resembles stunned myocardium. Although, our model might create myocardial stunning in a non-classical way [26,27].

In our study we found near normal perfusion and reversible myocardial function following implantation of the bead. A mixture of normal and reduced perfusion in stenosis-dependent myocardium explains the slight but persistent reduction in perfusion in our model as it was demonstrated by Hughes et al. [28].

Responsibility for the decreasing perfusion without recovery of function in the lateral wall myocardium of the untreated group is a more extensive amount of necrotic tissue with a reduced amount of microvasculature [29] and an increase of fibrosis [30] as shown in the TTC analysis (see Fig. 3).

4.2. Perfusion reserve in stenosis-dependent and remote myocardium

One important finding of our study is that perfusion reserve failed to reflect the benefit of TMLR in this study. The perfusion reserve showed neither a difference between the lateral and septal myocardium in one group nor in these regions between the treated and untreated group.

In the LCx-dependent lateral myocardium with subsequent vessel occlusion, perfusion reserve did not improve despite an improvement in function after TMLR. This finding is in contrast to the direct relationship between function and perfusion [31] described in antegrade perfused myocardium. However, in collateral-dependent myocardium a persistent reduced perfusion reserve [31] along with a weak correlation to regional function [32] has been demonstrated.

A mild but non-significant increase in regional resting perfusion and a significantly blunted response to adenosine compared to ‘normal’ myocardium, cause a reduction in perfusion reserve also in the remote region of our animals. Several authors described a similar phenomenon of reduced perfusion reserve in remote myocardium. First, Traverse et al. found an impaired microvascular function of the remote myocardium supplying an adjacent collateral zone which likely causes a reduced perfusion reserve in remote myocardium [33]. Second, it has been hypothesized that one single vessel supplying two vascular regions is a reason for a reduced perfusion reserve in remote myocardium [34]. Third, changes in regional vascular resistance and hemodynamic changes [35] as well as mechanical overload [36] are shown to parallel a diminished perfusion reserve in the remote region. This is in agreement with the increased thickening seen in the septum of our instrumented compared to the non-instrumented animals of our study.
4.3. Aspects of quantification of myocardial perfusion with MRI

In ischemic myocardium re-circulation or interstitial leakage are often seen [37] and cause a well-known problem for fitting the tissue signal curves with a γ-variate function. Therefore, MR-imaging and Fermi model constrained deconvolution employed for quantification of myocardial perfusion are of particular advantage in this study. The Fermi model is sensitive to the early phase of the contrast signal increase in the myocardial tissue. Therefore, it is not necessary to separate the first-pass signal from the re-circulation or interstitial leakage [13]. Under low-flow conditions, the contrast material has already exited before it all has reached the ROI. This causes a problem applying the ‘central volume theorem’, which assumes that all contrast agent passing through a ROI is entirely contained in the ROI at some time during the first-pass signal [38]. By deconvolution of the measured tissue curve a response curve was obtained to which the ‘central volume theorem’ can be applied.

4.4. Study limitations

One limitation of the study was the lack of a sham-operated control group demonstrating a possible effect on thoracotomy alone on myocardial perfusion in the present study. Therefore, we cannot differentiate the effect of TMLR and thoracotomy on myocardial perfusion. However, Hughes et al. [39] have shown no effect of thoracotomy alone on myocardial perfusion and function using echocardiography and PET imaging in a very similar porcine model.

A further limitation is that the occlusion of side branches cannot be controlled during placement of the bead. Although care was taken to avoid occlusion of major branches during implantation of the hollow bead, there was a possibility of occlusion of the minor branches. However, invasive and non-invasive data 1 week after bead placement did not indicate a systematic bias induced by possible occlusion of minor side branches before randomisation to surgery. The follow-up angiogram did not show any local displacement of the bead 1 week after the study or at the 8-weeks autopsy.

Perfusion reserve in our groups of animals, even in the non-instrumented group never reached a value greater than three. Fallavollita et al. [40] reported perfusion reserve values of 3–4 in a porcine model using a different hyperaemia protocol. Two modifications explain these differences in our protocol compared to the protocol published by Fallavollita et al. [40]. First, the dose of adenosine was much lower in our study (0.14 vs. 0.9 mg/kg/min), and second, we did not use phenylephrin to maintain blood pressure during adenosine infusion. Overall we believe that our protocol was safer to apply since the risk of arrhythmias induced by phenylephrin was abolished. It might be argued that the hyperaemic protocol used in our study might contribute to the failure of perfusion reserve to show a difference between remote and the affected LV-lateral myocardium. If blood pressure would have been maintained during adenosine infusion, e.g. with phenylephrin, the perfusion reserve in the remote zone probably would have further increased. This could result in a more pronounced difference between remote and stenosis-dependent myocardium. However, the use of phenylephrin would have not affected the perfusion reserve differentiation between the TMLR and the control group since the same drug dosage protocol would have been applied to both groups.

4.5. Conclusion

We demonstrated preserved resting perfusion after TMLR applied to stenosis-dependent myocardium with subsequent occlusion. This was in accordance with the functional changes seen from cine-MRI and microsphere flow measurements. Histopathologic findings further demonstrated significant less patchy necrosis in animals, which underwent TMLR. Quantitative resting and hyperaemic perfusion demonstrated significant regional changes between a TMLR-treated and an untreated group of animals, while relative perfusion reserve did not differentiate between the groups or regions after TMLR. This demonstrated the importance of absolute measures in this study. In addition to demonstrate improved function and perfusion after TMLR, our data give further insights into the relationship of function and perfusion of stenosis-dependent and remote myocardium following TMLR.

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