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Evaluation of the indirect revascularization method after 3 months chronic myocardial ischemia☆

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Abstract

Objective: The long-term effectiveness of transmyocardial laser revascularization (TMLR) was evaluated in the setting of a severe left anterior descending artery (LAD) stenosis. **Methods**: To employ the chronic ischemic model, pigs underwent three operative procedures over a 13-week period. In the first operation, an operative stenosis of the LAD was created. One week later, the animals were studied at baseline by analyzing different parameters of perfusion (microspheres), function and ECG changes. Afterwards, pigs were randomized into one of three different experimental groups: animals in laser group 1 received one laser channel ($n = 9$) and laser group 2 two channels per cm^2 ($n = 6$) in the LAD territory (using a CO_2-laser). Animals of the ischemic group ($n = 12$) underwent the same procedures without TMLR-treatment. Twelve weeks later, the animals were re-studied (third operation) and killed. Additional analysis of myocardial water content and histochemistry was performed. **Results**: Chronic myocardial ischemia and regional myocardial blood flow (RMBF) in laser group 2 revealed relatively higher RMBF values compared with the ischemic group ($P = 0.015$), after 3 months, but no absolute improvement of perfusion at rest compared with baseline was observed in all experimental groups. Left ventricular stroke work index (LVSWI) at rest and under stress did not show any improvement compared with initial values in all study groups (P not significant). However, laser group 1 demonstrated relatively higher $LVSWI_{max}$ values in comparison with the ischemic group ($P = 0.013$) as did laser group 2 ($P = 0.017$). Regional contractility of the laser groups recovered after 3 months (which was deteriorated shortly after TMLR, $P < 0.001$) and increased under stress compared with baseline (laser 1: $P = 0.015$, laser 2: $P = 0.017$). In contrast, the ischemic group did not show any difference from initial values (P not significant). The lased pigs of group 2 were less prone to intractable ventricular fibrillation ($P = 0.036$ vs. ischemic group), and showed a significant smaller area of necrosis in the area at risk ($P = 0.012$ vs. ischemic group). **Conclusions**: This model of chronic regional ischemia demonstrates that CO_2-laser revascularization significantly ameliorates microperfusion and regional contractility, and diminishes the incidence of ventricular fibrillation and necrosis in the area at risk. However, it does not change the overall perfusion and global LV function.

Keywords: Coronary artery disease; Transmyocardial laser revascularization, Chronic ischemia; Myocardium

1. Introduction

We previously demonstrated that transmyocardial laser channels failed to acutely increase regional myocardial blood flow [1]. Nevertheless, this method is being used to treat patients with endstage coronary artery disease who are not amenable to conventional procedures. It has become increasingly evident that there is a growing number of patients suffering from severe angina with small, diffuse or peripheral vessel disease and impaired collateral blood flow in the coronary arteries [2]. Transmyocardial laser revascularization (TMLR) creates transmural channels in the myocardium via laser ablation [3]. The clinical experience of single- and multi-institutional studies with TMLR [4,5] indicates that angina is relieved significantly, perfusion and treadmill tolerance improved and hospital admissions are decreased. Other centers report similar results excepting a significant improvement in regional perfusion and metabolism [6,7]. The exact mechanism which facilitates these subjective and objective TMLR-related improvements remains as yet unknown [8,9].

Therefore, this study was initialized to evaluate the long-

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term effectiveness of TMLR in the setting of chronic ischemia in porcine hearts.

2. Material and methods

Animals received humane care as approved by the Center for Experimental Animal Research at Freiburg University and in compliance with the ‘Principles of Laboratory Animal Care‘ and the ‘Guide for the Care and Use of Laboratory Animals‘ published by the National Institutes of Health (NIH publication 85-23, revised 1985).

To accurately mimic the clinical coronary artery disease, we employed a model of chronic myocardial ischemia, which is described elsewhere [10]. For this experiment, the animals underwent three operative procedures over a 13-week period. In the first operation, an operative stenosis of the left anterior descending artery (LAD) was created. One week later, the animals were studied by analyzing different parameters (see parameters below). Afterwards, pigs were randomized into one of three different experimental groups. At 12 weeks after the second operation, the animals were restudied (same parameters as before) and killed.

2.1. Animals and anesthesia

Pigs of the ’German Landrace’ weighing 28–34 kg were premedicated and anesthetized after ear vein cannulation, as previously described [1,3]. The same anesthetic regimen was used for each of the three different surgical procedures that the animals underwent. Cefazolin (0.5 g i.v.) was given preoperatively. Continuous electrocardiographic and pulse-oximetric monitoring was used throughout the procedure to ensure stable cardiac rhythm and adequate oxygenation. Sinus rhythm and all types of rhythm disturbance were registered.

2.2. Experimental groups

Before randomization of the pigs into one of the experimental groups 1 week after the first operation, three animals died due to myocardial infarction (mostly 1 or 2 days after creation of the LAD stenosis) and four additional animals were excluded due to the lack of a severe LAD stenosis in the angiography.

2.2.1. Ischemia group

In 12 pigs a LAD stenosis was created during the first operation. After re-minithoracotomy during the second operation, the animals were merely observed. No laser treatment was performed in these cases.

2.2.2. Laser group 1

Nine pigs received the same treatment as the ischemic group and additional treatment by TMLR after the animals were studied at the second operation. They were treated by one laser channel (of 1 mm diameter) per cm^2 in the ischemic LAD area. To define this area at risk LAD had been occluded for 10 s prior to laser treatment. Transmural channels were created in a distribution of one channel per cm^2.

2.2.3. Laser group 2

Six pigs were treated with the same regimen as laser group 1. However, in contrast to laser group 1 these animals received two laser channels (of 1 mm diameter) per cm^2.

2.3. Operation 1

After left anterolateral minithoracotomy, under sterile conditions, pericardium was incised and suspended to reveal the anterior free wall of the left ventricle (LV). After thoracotomy 150 IE/kg heparin and 1 g acetylsalicylic acid were given i.v. to all pigs. Lidocaine was administered at 40–60 μg/kg per min in a continuous drip. The LAD was carefully dissected, isolated immediately distal to the bifurcation of the first diagonal branch (D1) over 1–2 cm to accept an ultrasonic transit time (UTT) flow probe (Transonic Systems, Inc., Ithaca, NY) recording downstream flow through the LAD. A severe LAD stenosis (immediately distal to D1) was created proximal to the flow probe by the arterial puncture needle technique, as described by Chen and coworkers [10], to produce an area at risk of about 20–30% of the LV anterior free wall.

Baseline measurements of the heart rate, coronary flow, and oxygen content were obtained under stable conditions. Stability was defined as three consecutive measurements at 5-min intervals with a mean heart rate difference < 5/min, an oxygen content difference $< 4\%$, and a coronary flow difference < 3 ml/min. LAD flow was reduced to $\approx 50\%$ of baseline, and the reduction was maintained throughout the study. The stability of the coronary stenosis was verified by serial measurements of coronary flow at 15 and 60 min and 7 days under the same conditions of anesthesia. All recordings were stored on a computerized system (Pent.II-Lab, IBM) from which they were retrieved for further processing. After maintenance of a stable LAD stenosis for > 60 min, the flow probe was removed and the chest was closed in layers with the pericardium partially closed. A control angiography was performed to verify the high-grade LAD stenosis. The pigs were then allowed to recover in their intensive-care cages.

The animals were monitored daily by a veterinarian and his staff as well as the surgical team. Antibiotics were administered intramuscularly for 1 day postoperatively. Pain medications were also given intramuscularly until the animals were ambulating without difficulty and exhibited normal levels of activity. Until the third operation 100 mg acetylsalicylic acid was administered daily.

2.4. Operation 2

Through a re-minithoracotomy, the pericardium was opened and reexposed 7 days after the setting of chronic ischemia under the same conditions as described above. The monitoring was performed, as reported elsewhere [1].

Once UTT flow probe data confirmed the presence of chronic ischemia (LAD flow reduction, see above) baseline measurements (parameters see below) of segmental myocardial shortening (SMS) by ultrasonocrystals, heart rate, LV pressure, pulmonary pressure, right and left atrial pressure, H^+ concentration, and oxygen content were obtained under stable conditions (see above). Left ventricular stroke work was calculated as stroke work index in mJ/g, as already described [3], and normalized for body weight. For comparison between the experimental groups stress was induced and the maximal achieved left ventricular stroke work index ($LVSWI_{max}$) and SMS (SMS_{max}) was used [3].

After investigations were performed, the LAD territory (area at risk) was treated with TMLR, as reported elsewhere [3,9]. Drilling took an average of 18 min/animal (laser group 1) and 29 min/animal (laser group 2) to complete. Various numbers of laser channels were performed: 14.7 ± 3.4 and 25.7 ± 4.3 in laser groups 1 and 2, respectively (laser group 1 vs. 2, $P < 0.001$).

2.5. Operation 3

Twelve weeks later, the animals underwent a sternotomy. As per the protocol described for operation 2, measurement of all parameters was repeated. Histochemical assessment and myocardial water content analysis were performed, as previously described [9].

2.6. Parameters

2.6.1. Regional myocardial blood flow

Regional perfusion of the LAD and left circumflex artery (LCx) territory was measured based on the radioactive microsphere and arterial reference sample technique, as published previously [3]. Retrieval of the radioactive microspheres were performed at rest in all animals at the second (before TMLR) and third operation. The microsphere suspensions were injected into the left atrium under stable hemodynamic conditions so that no differences between the experimental groups were revealed.

2.6.2. Hemodynamic measurements

Hemodynamic measurements of LVSWI at rest and stress were performed, as reported elsewhere [1,3].

2.6.3. Regional contractility

SMS was calculated as follows:

$$\%SMS = \frac{EDL - ESL}{EDL} \times 100$$

where EDL and ESL are end-diastolic length and end-systolic length, respectively. Results were expressed as percent of systolic myocardial shortening relative to control values (baseline at 1 week) to allow comparison between the animals and avoid bias relative to differences in heart size and crystal placement [11].

2.6.4. Histochemical assessment and myocardial water content

Measurements of the myocardial water content and computerized, planimetric analysis to determine the total area of the LV, the area of ischemia and necrosis by the triphenyltetrazolium chloride technique were performed, as described elsewhere [9].

2.7. Statistical analysis

Data of all experimental groups were analyzed based upon analysis of variance for normally distributed and Kruskal–Wallis test for non-normally distributed data, as appropriate (SPSS version 9.01). In case of significance, post hoc comparisons were performed using single Mann–Whitney tests with Bonferroni correction. Furthermore, Fisher's exact test was used for the comparisons of the incidence of ventricular fibrillation and mortality. Results are expressed as mean value ± standard deviation. A P-value less than 0.05 was considered statistically significant. Only data were used from animals who survived the entire 13-week observation period.

3. Results

The results are shown in Table 1 and Figs. 1–5.

3.1. Procedural outcome

Lasing was well tolerated in all animals. There was no

Table 1
Incidence of ventricular fibrillation (VF) and mortality

	Incidence of VF (%)	Intractable VF (%)	Overall mortality (%)
Ischemic group ($n = 12$)	75 ($n = 9$)	42 ($n = 5$)	42 ($n = 5$)
Laser group 1 ($n = 9$)	44 ($n = 4$)	22 ($n = 2$)	33 ($n = 3$)[a]
Laser group 2 ($n = 6$)	17 ($n = 1$)	0 ($n = 0$)	0 ($n = 0$)[b]
Before randomization ($n = 3$)			($n = 3$)

[a] One animal in laser group 1 died of a pressure pneumothorax.
[b] $P = 0.036$ vs. ischemic group.

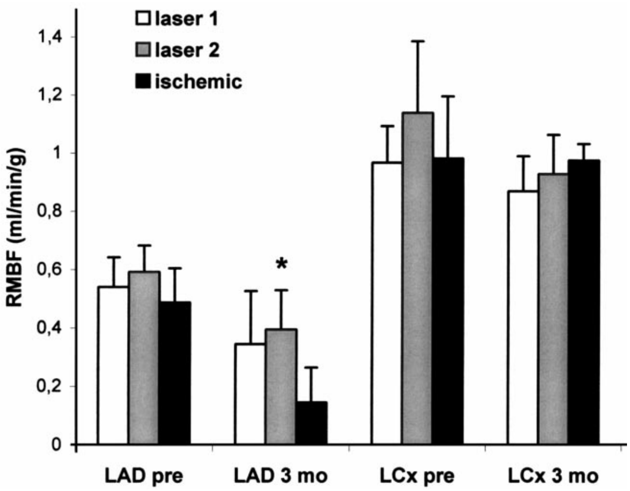

Fig. 1. Radioactive microsphere measurements of regional myocardial blood flow (RMBF) at rest into the ischemic anterior wall (LAD distribution) demonstrated that blood flow was reduced ($P < 0.001$) in all experimental groups compared with blood flow in their normally perfused circumflex territory (LCx distribution) 1 week after the chronic ischemic setting (LAD pre and LCx pre, pre-TMLR). After 3 months (3 mo), blood flow decreased ($P = 0.034$) only in the ischemic group, reflecting the 'normal process of coronary artery disease' that occurs in this chronic model in response to the fixed stenosis and consecutive intima proliferation. Nevertheless, analysis indicated that relative blood flow in laser group 2 was greater than in the ischemic group ($^*P = 0.015$), mirroring the angiogenetic process that was enhanced by TMLR. Values (regional transmural perfusion of the LV free wall) are mean ± SD.

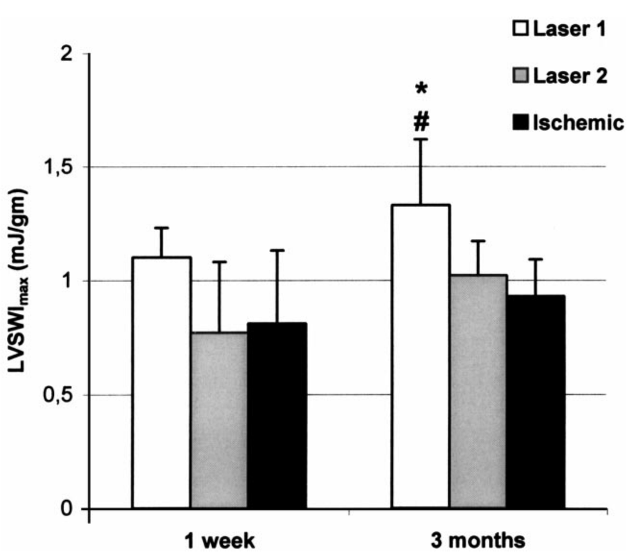

Fig. 2. The maximal left ventricular stroke work index (LVSWI $_{max}$) is demonstrated after a 1-week and 3-month observation period in all experimental groups. Animals of all groups showed comparable reduction of $LVSWI_{max}$ at the time of randomization into each experimental group (P = n.s.). In the chronic (3-month) setting, global contractility did not change compared with the 1-week $LVSWI_{max}$-values in each experimental group (P = n.s.). However, laser group 1 revealed relatively higher values compared with the ischemic group ($^*P = 0.013$) and laser group 2 ($^\#P = 0.017$) at 3 months. Mean values (column height) and SD are indicated.

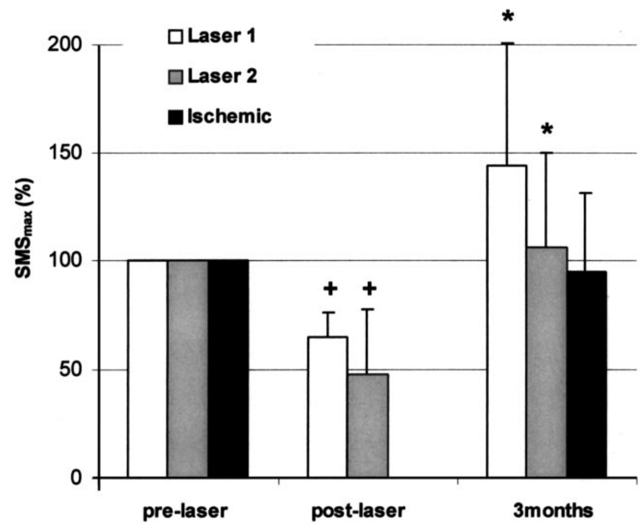

Fig. 3. Segmental myocardial shortening under stress (SMS_{max}) in the LAD region were normalized to resting values of 100 (pre TMLR). Shortly after TMLR, a deterioration of the regional contractility compared with baseline values was observed in the laser groups 1 and 2 ($^+P < 0.001$). After a 3-month observation period, regional function recovered in the laser groups (1 and 2) and demonstrated higher SMS_{max} values compared with baseline ($^*P < 0.02$). In contrast, the corresponding ischemic group values were not different from initial values (P = n.s.). Values are mean ± SD, pre-, post-laser (1 week) and after 3 months in all experimental groups.

ventricular tachycardia or fibrillation associated with channel creation. Transmyocardial penetration could be confirmed by the presence of a limited number of air bubbles seen within the ventricular cavity by echocardiogram. Bleeding was easily controlled by manual compression.

After randomization into the experimental groups at the second operation seven pigs died of intractable ventricular fibrillation and one of pressure pneumothorax (Table 1). Intractable ventricular fibrillation occurred less often in laser group 2 compared with the ischemic group ($P = 0.036$). All other subsequent animals survived until euthanasia without postoperative complications and were analyzed.

Measurements of heart rate, left ventricular end-diastolic pressure, mean arterial and left atrial pressure, ECG changes and all additional parameters revealed no statistically significant difference between the three study groups during the second operation (P = n.s. (not significant)).

3.2. Regional myocardial blood flow (RMBF)

Data indicated that (1 week after ischemia) RMBF at rest in the LAD territory was reduced compared with the LCx territory ($P < 0.001$) in all study groups (Fig. 1). No differences between the experimental groups were revealed after 1 week of chronic ischemia (P = n.s.). Stenosis of the LAD caused a profound and persistent reduction in transmural blood flow to regional myocardium [10] supplied by the LAD in the ischemia- and laser groups compared with the remote myocardium until 3 months of chronic ischemia

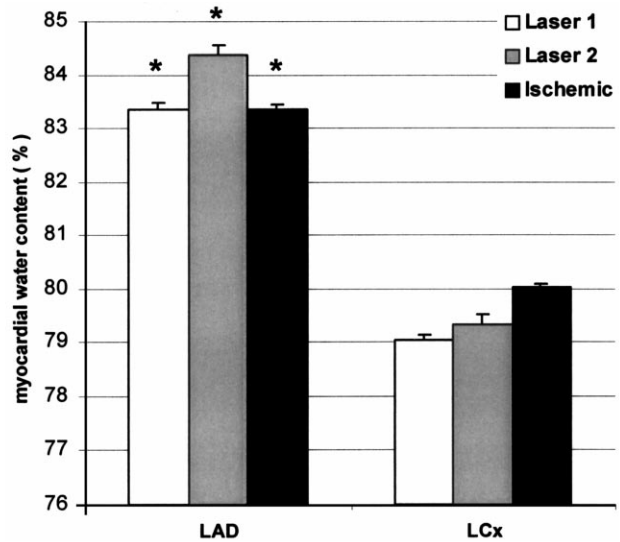

Fig. 4. Myocardial water content (MWC) in the analyzed LAD and LCx territory of chronic ischemic myocardium after TMLR treatment and an observation period of 3 months for the ischemic, laser groups 1 and 2 (in percent). MWC of all experimental groups differed significantly in the LAD compared with the LCx territory (*$P < 0.01$). Mean values (column height) and SD are shown.

($P < 0.001$ vs. LCx territory). Nevertheless, after 3 months, laser group 2 demonstrated higher perfusion values compared with the ischemic group ($P = 0.015$). The ischemic group itself indicated lower RMBF values compared with baseline ($P = 0.034$). In contrast, normal RMBF in remote myocardium supplied by the LCx was assessed in all study groups after 3 months. No differences

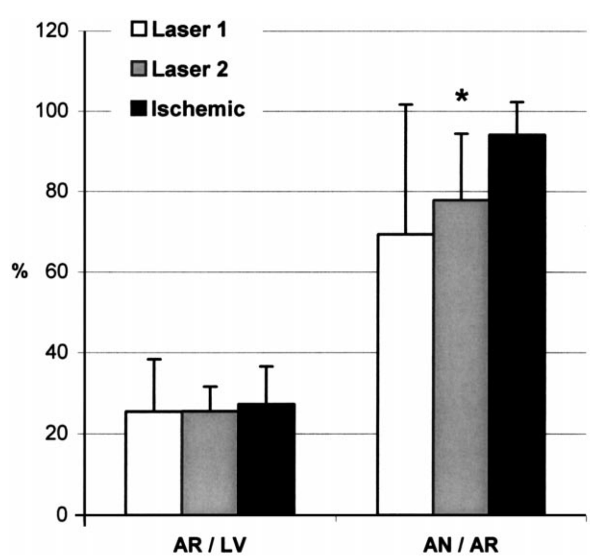

Fig. 5. Percentage of area of necrosis (AN) in the area at risk (AR) for the ischemic, laser 1 and 2 groups. Mean values (column height) and standard deviation (error bars) are shown. Note: (a) the area at risk in the ischemic and laser groups did not differ significantly, but (b) the area of necrosis in the area at risk of laser group 2 were smaller compared with the ischemic group (laser 2 vs. ischemic group, *$P = 0.024$). Mean values (column height) ± SD are shown.

between the endomyocardial and epimyocardial layers in the LAD and LCx territories were observed after 3 months ($P =$ n.s.).

3.3. Systemic hemodynamics

The maximal LV stroke work index ($LVSWI_{max}$) at baseline (1 week) and end of study (3 months ischemia) are summarized in Fig. 2 for all study groups. After 1 week LVSWI (at rest) and $LVSWI_{max}$ (under stress) in the ischemia group and laser groups 1 and 2 were reduced (Fig. 2) compared with baseline values of healthy porcine hearts (not demonstrated, Ref. [3]). No differences between the experimental groups were observed at 1 week ($P =$ n.s.). At the end of the study LVSWI and $LVSWI_{max}$ were not different from initial values in all study groups ($P =$ n.s.). However, laser group 1 revealed relatively higher $LVSWI_{max}$ values compared with the ischemic group ($P = 0.0013$) and laser group 2 ($P = 0.017$) at 3 months.

3.4. Regional contractility

Percent systolic fiber shortening in the LAD region is demonstrated in Fig. 3. SMS of the remote myocardium did not reveal differences between baseline and 3 months values ($P =$ n.s.). Shortly after laser channel creation, a reduction of the regional contractility compared with initial values was observed in the laser groups 1 and 2 ($P < 0.001$). After a 3-month observation period, the laser groups' regional function recovered and demonstrated higher SMS values under stress compared with baseline (laser 1: $P = 0.015$; laser 2: $P = 0.017$), whereas SMS values at rest did not change ($P =$ n.s.). In contrast, the corresponding ischemic group values were not different from initial values ($P =$ n.s.).

3.5. Water and histochemistry

At the end of the study, myocardial water content (MWC) of all experimental groups differed significantly in the LAD compared with the LCx territory after 3 months ischemia ($P < 0.01$). No differences between the study groups were detected ($P =$ n.s.).

Histochemical analysis indicated that the area at risk in all study groups did not differ significantly ($P =$ n.s.), but the area of necrosis in the area at risk of laser group 2 was smaller compared with the ischemic group (laser 2 vs. ischemic group, $P = 0.024$) at 3 months.

4. Discussion

Various acute animal models have provided contradictory results that have led investigators to differing conclusions as to patency, perfusion and global function after TMLR [1]. The reason for these discrepancies may be that these models did not reproduce accurately the complex anatomy and pathology of the human atherosclerotic heart disease [2].

Clinically, the only current indication for the application of CO_2 laser channels is a chronically ischemic and viable myocardium (myocardial hibernation). Therefore, this chronically ischemic model [10] was used to evaluate the TMLR method in pig hearts.

This experimental study demonstrated that CO_2 laser revascularization in chronically ischemic myocardium improved relatively regional myocardial blood flow (RMBF) in the area at risk (laser 2 vs. ischemic group, $P = 0.015$), whereas in all experimental groups an absolute improvement of perfusion compared with baseline (1 week ischemia) was not observed at rest (Fig. 1). At the end of the study, global left ventricular function (LVSWI and $LVSWI_{max}$) were not different from initial values ($P =$ n.s.) in all study groups. However, laser group 1 revealed relatively higher $LVSWI_{max}$ values compared with the ischemic group ($P = 0.013$) and laser group 2 ($P = 0.017$, Fig. 2).

In the laser groups regional function recovered after 3 months (which was deteriorated shortly after TMLR, $P < 0.001$) and increased only under stress compared with baseline (laser group 1: $P = 0.015$, laser group 2: $P = 0.017$). In contrast, regional contractility of the ischemic group was unchanged from initial values ($P =$ n.s.; Fig. 3). After 3 months, the myocardial water content (MWC) of all experimental groups did not differ in the area at risk ($P =$ n.s., Fig. 4). However, histochemical analysis revealed differences in the area of necrosis in the area at risk. The amount of necrosis of laser group 2 was significantly smaller compared with the ischemic group (laser 2 vs. ischemic group, $P = 0.024$; Fig. 5).

4.1. Experimental studies

RMBF analysis indicated that relative blood flow in the laser group 2 was greater than in the ischemic group ($P = 0.015$), mirroring the angiogenetic process that is enhanced by TMLR [12,13]. These results are consistent with the planimetric analysis of the area of necrosis in the area at risk. The laser-treated group 2 demonstrated a smaller amount of necrosis in the area at risk [1,2] compared with the ischemic group ($P = 0.024$). There is little experimental information in the literature as to whether TMLR improves long-term transmural perfusion and LV function in chronically ischemic myocardium. In a long-term study conducted in porcine hearts, Hughes and coworkers [14] found an improvement in perfusion and regional contractility [15], after subtotal coronary artery occlusion and subsequent TMLR.

4.2. Study limitations

The main limitation of this study is that measurements of the regional myocardial blood flow (RMBF) were only performed at rest, whereas functional parameters were taken at rest and under stress. Therefore, the improvement of regional contractility under stress could not be mirrored by an improvement of RMBF at stress which was observed by Yamamoto et al. [13]. Further measurements of RMBF under stress would have provided additional information, especially concerning the quantity of laser channels.

4.3. Channel density

The number of channels that should be drilled is a topic that remains controversial [2,3]. We drilled one and two CO_2 laser channels per cm^2 in laser groups 1 and 2, respectively ($P < 0.001$). Regarding the parameters of perfusion, regional contractility, myocardial water content and histochemistry, we did not observe any difference between laser groups 1 and 2, whereas laser group 1 indicated higher $LVSWI_{max}$ values at the end of the study compared with laser group 2. In contrast, intractable ventricular fibrillation occurred less often in the laser group 2 compared with the ischemic group (Table 1, $P = 0.036$). This is consistent with the results of our short-term studies [1] and Okada et al., who observed in the acute setting of a myocardial infarction significantly more intractable ventricular arrhythmias in the control (80%) than in the laser group (0%) [16]. However, one channel per cm^2 is the most widely used channel density in clinical and experimental studies [1–9,15], and a general threshold of channel density should be defined for clinically treating myocardial ischemia, because carbon dioxide laser channels significantly decrease global heart function shortly after TMLR in healthy porcine myocardium [3].

4.4. Mechanical versus laser revascularization

Laser-induced angiogenesis is based on the observation that significant inflammatory reaction is consistently present in the vicinity of myocardial punctures. This angiogenic response is stimulated by various growth factors released as a result of tissue injury and inflammatory cellular infiltration. This nonspecific response to tissue injury may be created by a variety of methods. Simple needle punctures of myocardium have also been shown to induce an angiogenic response in a chronically ischemic porcine model. In this long-term study [17] it could be demonstrated that transmyocardial mechanical needle revascularization is effective in stimulating intrinsic expression of several different angiogenic growth factors, and these findings were fundamentally indistinguishable from other studies of TMR using laser devices [17].

4.5. Clinical studies

Our results are in agreement with preliminary clinical results obtained by Cooley et al. [18] and Naegele et al. [7]. In addition, our data are supported by the results of Rimoldi et al. [19]. They found no statistically significant improvement in regional myocardial blood flow after TMLR based upon PET with $H_2^{15}O$. Furthermore, Schofield and colleagues in their randomized, controlled trial (TMLR vs. medical management) did not observe an improvement in perfusion compared with baseline in their TMLR patients, but they demonstrated an increase in irreversible ischemic sites in the medical group [20].

In contrast, other investigators such as Horvath and coworkers [5] reported a significant improvement of regional perfusion [4] in the lased areas of reversible ischemia over time (12 > 6 > 3 months) in their single- and multicenter study. In addition, Frazier and colleagues [21] observed in their randomized, controlled multicenter trial (TMLR vs. medical management) significantly improved perfusion following TMLR and Allen et al. [22] significantly improved exercise tolerance over a 12-month period follow-up.

All of the above-mentioned study groups used the same CO_2 laser regimen to perform the TMLR operation, but it remains unclear why the long-term results of these studies determined by the objective parameter of perfusion are very different. Despite its increasing surgical use, careful step-by-step experimental and clinical validation of TMLR is required to prove its effectiveness in treating regional myocardial ischemia [1–3,8,9].

In conclusion, we demonstrated that CO_2 laser channels significantly improve microperfusion and regional contractility at stress after 3 months of chronic ischemia. In contrast, transmural perfusion, LV function at rest and stress, and regional contractility at rest were not affected by TMLR.

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Appendix A. Conference discussion

Dr H. Barner (*St. Louis, MO, USA*): The LAD was ligated, or was it stenotic?

Dr Lutter: It was stenotic. It was high-grade stenotic. So we used the technique of Chen and colleagues from New York, which was described in 1996 in *Circulation*.

Dr Barner: Did you reassess the patency of the LAD late?

Dr Lutter: It is known that it is very difficult to create a high-grade LAD stenosis. Regarding the structure of our chronic ischemic model, we firstly performed a severe LAD stenosis under on-line ultratransit time flow control. Secondly, after 1 week, a coronary angiography and ultratransit time flow were performed again. After creation of this severe LAD stenosis, some animals died as a result of an acute infarction. Therefore, we had a dropout rate before randomization (at 1 week) into these three experimental groups of about 35% which, in comparison with others, is quite good.