Transgenic and transmural revascularization: regional myocardial tissue pressure during chronic ischemia

Tim Attmann\textsuperscript{a}, Claudia Heilmann\textsuperscript{b}, Matthias Siepe\textsuperscript{b}, Juergen Martin\textsuperscript{b}, Florian Jentzmik\textsuperscript{b}, Patrick von Samson\textsuperscript{b}, Friedhelm Beyersdorf\textsuperscript{b}, Georg Lutter\textsuperscript{a,}\textsuperscript{*}

\textsuperscript{a}Department of Cardiovascular Surgery, Medical School, University of Kiel, Arnold-Heller-Straße 7, 24105 Kiel, Germany
\textsuperscript{b}Department of Cardiovascular Surgery, University of Freiburg, Freiburg, Germany

Received 7 April 2003; received in revised form 7 October 2003; accepted 21 October 2003

Abstract

Channel patency and a cavito-myocardial pressure gradient are prerequisites for one potential mechanism of transmyocardial laser revascularization (TMLR), namely indirect (non-coronary) myocardial perfusion. We assessed the effect of TMLR combined with vascular endothelial growth factor (VEGF) on the myocardial tissue pressure (MTP) in chronic ischemia questioning firstly, whether transmural pressure allows perfusion of laser channels, and secondly, whether additional application of VEGF improves channel patency. One week after creation of an operative left anterior descending artery stenosis (2nd operation), pigs were designated to untreated ischemia (\textit{n} = 7), TMLR (\textit{n} = 8) or TMLR + VEGF-cDNA (2 mg intramyocardially, \textit{n} = 6). MTP and left ventricular pressure (LVP) were recorded simultaneously in the endo-, mid-, and epimyocardium before and after stenosis (1st operation), before and after therapy (2nd operation), and 12 weeks later (3rd operation). Myocardial samples were subjected to immunohistochemistry. Endo- and epimyocardial MTP exceeded LVP in all groups throughout the study, whereas midmyocardial MTP was constantly below LVP (\textit{P} < 0.05). Immediately after combined TMLR + VEGF, the endo-MTP decreased from 246.5 ± 44.2 to 176.7 ± 20.7 mmHg (\textit{P} = 0.043), remaining higher than LVP. After 12 weeks, it increased to 225.6 ± 31.8 mmHg (\textit{P} = 0.04), but did not reach baseline values (\textit{P} = 0.04). Histological examination revealed occluded channels with surrounding vascular proliferation in both treatment groups. Additional VEGF-cDNA application in the vicinity of TMLR channels does not improve long-term patency. Direct blood flow from the cavity into the myocardium is impossible due to the high endomyocardial pressure. This limitation might be overcome by implantation of endomyocardial stents.

Keywords: Myocardial tissue pressure; Chronic ischemia; Coronary artery disease; Growth factors; Transmyocardial laser revascularization

1. Introduction

Different hypotheses on the underlying mechanism of transmyocardial laser revascularization (TMLR) have been suggested: e.g. direct blood flow from the left ventricular (LV) cavity to the vasculature of the ischemic myocardium through laser-created channels in analogy to the physiology of the reptile heart [1], or induction of angiogenesis and expression of different growth factors [2].

The additional delivery of angiogenic factors in the form of protein or DNA to the ischemic target area may enhance this vessel development [3].

The two crucial points, pressure gradient and channel patency, are controversial. For years, the majority of investigators have demonstrated only transient patency [4]. Brilla and colleagues [5] found a higher patency rate of TMLR channels after the intramyocardial application of vascular endothelial growth factor (VEGF) in a porcine model.

Experimental studies investigating myocardial tissue pressure (MTP) revealed that MTP exceeds the LV pressure throughout the cardiac cycle in healthy hearts [6,7]. Thus, blood flow from the LV cavity into the myocardium should not be possible. In contrast, Baird et al. measured lower MTP values than left ventricular pressure (LVP) values using two different techniques [8], and, in an acute study, Krabatsch and co-workers [9] were able to demonstrate that under acute ischemic conditions MTP can fall below the intraventricular pressure. Blood flow into the ischemic myocardium could therefore be possible.

\textsuperscript{*}Corresponding author. Tel.: +49-431-597-4401; fax: +49-431-597-4402.
E-mail address: lutter@kielheart.uni-kiel.de (G. Lutter).
VEGF is a strong mitogen for vascular endothelial cells and therefore a major initiator of angiogenesis. It has been employed successfully in animal studies and clinical trials [10].

We therefore initiated this study to evaluate a possible indirect (non-coronary) revascularization in a model of chronic myocardial ischemia. We have investigated the effects of TMLR alone and TMLR combined with the intramyocardial application of naked VEGF\textsubscript{121} DNA, questioning firstly whether the transmural pressure state allows perfusion of laser channels, and secondly, whether additional application of VEGF in the vicinity of laser channels improves long-term patency.

Furthermore, we intended to examine the nature of the myocardial tissue pressure under healthy and under chronic ischemic conditions.

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). Pigs of the ‘German Landrace’ weighing 24–30 kg were pre-medicated and anesthetized, and monitored as described previously [11].

2.1. Experimental model

To mimic clinical coronary artery disease, we employed a model of chronic myocardial ischemia [11]. In the first operation, an operative stenosis of the left anterior descending artery (LAD) was created. One week later (second operation), the animals were studied by analyzing different parameters (see parameters below). Afterwards, pigs were designated to one of three groups. After 12 weeks (third operation), the animals were reanalyzed (same parameters as before) and sacrificed.

To define the area at risk the LAD was occluded for 10 s prior to TMLR treatment. The absence of a post occlusive hyperemic response provided physiological evidence for chronic ischemia. The pigs received therapy or were left untreated (see Section 2.2).

2.2. Experimental groups

The three experimental groups (Ischemic control \((n = 9)\), TMLR \((n = 8)\), TMLR + VEGF\textsubscript{121} \((n = 7)\)) were treated as described previously [11].

2.3. Parameters

2.3.1. Myocardial tissue pressure analysis

The regional MTPs were measured at three different myocardial levels (epi-, mid-, endomyocardium) in the free wall of the left ventricle (LAD and left circumflex (LCx) territory) using needle-tip pressure catheters (Millar Instruments, Inc., Houston, TX). The angle of insertion of the needle-probe was 90° to the epicardial surface (Fig. 1a).

We simultaneously recorded the MTP at one of the three myocardial levels and the LV pressure (Micro-Tip\textsuperscript{R} Millar Catheter). Myocardial penetration-depth was defined by a millimeter scale imprinted on the needle-tip catheter (epimyocardium: 2–4 mm; midmyocardium: 4–7 mm; endomyocardium 7–9 mm). Typical contours of the pressure curves could be demonstrated (Fig. 1b).

Data were recorded using Haemodyn\textsuperscript{R}-Software (Hugo Sachs Electronics, Hugstetten, Germany).

2.3.2. Immunohistology

Immunohistochemical double-staining of endothelial cells and smooth muscle cells were performed, as described elsewhere [11].

Channel patency and endothelialization was determined under a Zeiss light microscope.

2.4. Statistical analysis

Data were analyzed based upon Wilcoxon’s signed rank test to compare paired data and Mann–Whitney \(U\)-test to compare unpaired data for non-normally distributed data, as appropriate (SPSS-vers. 10.0). Results are expressed as mean \(\pm\) SD. A \(P\)-value less than 0.05 was considered statistically significant.

3. Results

The results are shown in Table 1 and in Figs. 1–3.

3.1. Procedural outcome

The entire study group included 35 animals. Shortly after the LAD stenosis had been established, four pigs died due to intractable ventricular fibrillation. Four additional animals were excluded due to no observed severe LAD stenosis (<90%) and another three with occlusion of the LAD artery as indicated in the angiography at the second operation. Between the second and third operation, two control and one pig of the TMLR + VEGF group died a sudden death. All other animals \((n = 21)\) survived until euthanasia without postoperative complications, had a high-grade LAD stenosis at the second and third operation (with no differences between experimental groups), and were used in analysis \((n = 7\) in the ischemic, \(n = 8\) in the TMLR, \(n = 6\) in the combined group).
Measurements of heart rate, left ventricular end diastolic pressure, mean arterial and left atrial pressure, electrocardiogramm (ECG) changes and all additional parameters revealed no statistically significant difference between the three study groups during the second operation ($P = \text{ns}$).

3.2. Myocardial tissue pressure (MTP)

Endo- and epimyocardial MTP in the LAD and LCx territories exceeded LVP continually through the cardiac cycle in all groups throughout the study ($P < 0.05$), whereas midmyocardial MTP was constantly below LVP during systole ($P < 0.05$).

There was sizeable heterogeneity in the end-diastolic MTP values observed. A wide range of midmyocardial diastolic pressure data emerged in particular, although end-diastolic MTP did not differ between groups and between the points of observation (Fig. 2, Table 1).

3.3. MTP in the LAD territory

Immediately after establishing a severe LAD stenosis (first operation), the MTP was not affected in any of the three layers during systole and diastole in the ischemic LAD territory compared to healthy control LAD territory and compared to remote myocardium (LCx territory).

After 1 week ischemia, no change was revealed either.

In contrast, shortly after therapy (second operation), the end-systolic endomyocardial pressure in the LAD territory was reduced in both treatment groups. In the TMLR group, systolic endo-MTP fell from $230.6 \pm 29.5$ mmHg (pre TMLR) to $187.3 \pm 13.8$ mmHg (post TMLR) ($P = 0.028$) and in the TMLR + VEGF group from $246.5 \pm 44.2$ mmHg (pre therapy) to $176.7 \pm 20.7$ mmHg (post therapy) ($P = 0.043$).

The end-systolic mid- and epimyocardial pressures were unaffected.

The end-diastolic MTP in all three layers was not changed by the TMLR- and TMLR + VEGF-treatment either.
Table 1
Endsystolic and end diastolic LVP and MTP in the LAD territory

<table>
<thead>
<tr>
<th></th>
<th>LVP (mmHg)</th>
<th>MTP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sys Dia</td>
<td>Epi sys Mid sys Endo sys</td>
</tr>
<tr>
<td>Ischemic control group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre stenosis</td>
<td>101.4 ± 15.6</td>
<td>6.5 ± 4.9</td>
</tr>
<tr>
<td>Post stenosis</td>
<td>103.9 ± 7.2</td>
<td>5.9 ± 4.9</td>
</tr>
<tr>
<td>1 week</td>
<td>88.6 ± 11.6</td>
<td>5.5 ± 2.3</td>
</tr>
<tr>
<td>3 months</td>
<td>92.4 ± 11.5</td>
<td>4.2 ± 1.3</td>
</tr>
<tr>
<td>TMLR group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre stenosis</td>
<td>102.0 ± 7.1</td>
<td>9.4 ± 5.0</td>
</tr>
<tr>
<td>Post stenosis</td>
<td>94.8 ± 16.8</td>
<td>11.4 ± 4.8</td>
</tr>
<tr>
<td>1 week</td>
<td>92.8 ± 15.7</td>
<td>9.2 ± 4.7</td>
</tr>
<tr>
<td>Post therapy</td>
<td>89.4 ± 5.3</td>
<td>5.9 ± 2.3</td>
</tr>
<tr>
<td>3 months</td>
<td>93.0 ± 8.3</td>
<td>8.8 ± 1.9</td>
</tr>
<tr>
<td>TMLR + VEGF group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre stenosis</td>
<td>101.5 ± 13.9</td>
<td>8.7 ± 5.9</td>
</tr>
<tr>
<td>Post stenosis</td>
<td>97.4 ± 19.6</td>
<td>8.3 ± 5.0</td>
</tr>
<tr>
<td>1 week</td>
<td>94.3 ± 11.8</td>
<td>7.7 ± 3.9</td>
</tr>
<tr>
<td>Post therapy</td>
<td>82.8 ± 7.8</td>
<td>9.0 ± 6.5</td>
</tr>
<tr>
<td>3 months</td>
<td>81.8 ± 5.3</td>
<td>8.8 ± 3.6</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD; LVP = left ventricular pressure, MTP = myocardial tissue pressure, dia = enddiastolic, sys = endsystolic, +P < 0.05 compared with 1 week value, *P < 0.05 compared with post therapy value.

After 3 months of ischemia (third operation), endo-MTP in the TMLR group increased to 230.9 ± 26.2 mmHg (P = 0.043 vs. post therapy) and in the TMLR + VEGF group to 225.6 ± 31.8 mmHg (P = 0.043 vs. post therapy), still being lower than after 1 week of ischemia (P = 0.043). Corresponding to the increase, there was no difference between the three groups in all three tissue layers.

3.4. MTP in the LCx territory

The tissue pressure values of the LCx territory of each myocardial level revealed no significant differences for all points of observation (first to third operation) during systole and diastole (P = ns).

3.5. Immunohistology

Laser channels were filled entirely with dense fibrinous tissue, leaving no central patent lumen (Fig. 3). The two TMLR groups did not differ concerning the occluded channels. Immunohistology provided no evidence of open endothelialized anastomosis between the left ventricular cavity and the lumina of any type of myocardial vessels. However, numerous small vessels abound in the channel remnant.

There was no macroscopic or microscopic evidence of angioma formation.

4. Discussion

This paper’s data were recorded together with data published recently [11]. Although these two papers resulted from one series of experiments, we primarily intended to present them separately due to the dissimilarity of their topics.

Our paper published recently [11] evaluates the combined therapy of TMLR and VEGF regarding regional and global contractility, regional myocardial blood flow, and angiogenesis (see below).

In this paper we highlight a physiologic and pathophysiologic topic: myocardial tissue pressure in healthy and in the chronically ischemic myocardium. We also scrutinize the effects of TMLR on MTP and the influence of VEGF-cDNA on laser channel patency.

Various studies report a pressure gradient from the endo- to the epicardium [8] which concurs with our results: endsystolic pressure in the endomyocardium exceeded epicardial pressure, both being higher than LV pressure. However, by advancing the needle-tip pressure transducer slowly from the epicardium towards the LV cavity, we were able to detect three particular levels, each revealing its characteristic pressure curve contour (Fig. 1b). Midmyocardial tissue pressure measurements revealed lower values than LV pressure measurements. Thus, a blood flow from the cavity into the midmyocardium is notionally conceivable, but made impossible by the high endomyocardial pressure state. Our observation that endo-MTP was
perpetually higher than LVP is in agreement with the results of several groups [7,12].

Concerning models of myocardial ischemia, one has to distinguish between infarction models and models with only reduced coronary flow. We worked with the latter.

The acute reduction in coronary perfusion pressure after setting the high-grade stenosis (first operation) as well as the chronically reduced perfusion after 1 week (second operation) did not influence MTP in any of the three myocardial layers. On the contrary, other research groups demonstrated a decrease in endystolic endo-MTP after the acute occlusion of a coronary artery [12]. It was also claimed that in the acute, severe ischemic myocardium, LVP can even exceed the MTP [9].

According to these findings, the MTP configuration and pressure values do not seem to change until a pronounced ischemia is induced.

The acute and chronic relative ischemia in our model did not alter the pressure state in the myocardium. Because TMLR is used in viable, chronically ischemic myocardium and not in infarcted myocardium, this finding is of great clinical importance. Long-term perfusion cannot occur directly from the LV cavity given the ischemic myocardial conditions under which the laser is used.

Krabatsch and co-workers found an increase in sub-endocardial pressure values after TMLR in acutely ischemic hearts. This increase was interpreted as indirect evidence of relief of the ischemia [9]. Contrarily, our treatment with TMLR or TMLR + VEGF after 1 week ischemia resulted in a reduction in the endo-MTP, whereas epi- and mid-MTP as well as the relation to LVP remained stable. Other groups’ efforts support our observation of MTP reduction after TMLR [13].

The drop of endo-MTP after both kinds of therapy can be explained by the manipulation of the heart. This fact is supported by the non-significant drop of LVP.

To our knowledge, this is the first report to show long-term results of MTP-measurements under chronic ischemia and TMLR. However, the 3 month-MTP values indicate no major difference from the values in healthy (pre stenosis) or short-term partially ischemic (1 week) myocardium regarding the basic message: endo-MTP exceeds LVP.

All laser channel remnants found in our immunohistologic examination were filled with scar-like tissue. In particular, the potential endomyocardial influx was obstructed. No endothelialization could be observed. Although some authors, mainly in early TMLR studies, reported patent channels, the idea that laser channels do not remain patent finds increasing acceptance [14]. The additional application of VEGF-cDNA in the vicinity of laser channels had no histological effect on channel morphology, so we cannot support the hypothesis of Brilla et al. [5] that growth factors enhance channel patency.

We administered 100 mg acetyl salicylic acid per day, the common dose for coronary patients in Europe. Unfortunately, the effect of different anticoagulation or platelet aggregation inhibition strategies on channel patency was not assessed.

But even if the channels were patent and if LVP exceeded MTP at some point during the cardiac cycle and clotting was absent, where would the blood be drained? What type of vessels would provide adequate run-off? The heart is not a giant sponge, after all [15].
As a matter of course myocardial perfusion can be enhanced after the combined therapy with TMLR and growth factors [3,11] even if it is not accomplished by blood flow from the LV cavity through laser channels. Endogenous collateralization, angio- and arteriogenesis are responsible for the positive effects of this combined revascularization method. Associated with these positive effects MTP can also increase.

The millar needle-tip pressure catheter has a beveled measuring sensor, which is sensitive to orientation towards or away from the cavity. Keeping this in mind, we changed the orientation of the millar needle in the myocardium several times between various measurements. Using this method we could not detect different pressure values.

It is desirable to measure the exact MTP, which is the pressure between two myocytes in the extracellular space. No damage to any myocyte should occur by introducing the pressure transducer into the myocardium. The needle-tip pressure catheter’s diameter is unfortunately too large (three French) to meet these ideal conditions.

We had five points of observation, but no data were acquired in the 11-week period between the second and third operations. It would have been very interesting to have had higher temporal resolution, making it possible to observe MTP and the evolution of channel closure. Furthermore, a higher local resolution would be desirable. We missed to record temporal or regional differences in tissue and cavitary pressure. So, we cannot say if at different points of time and in different regions of the ventricular wall pressure conditions make a blood flow into the myocardium possible.

Considerably high standard deviations appear to be intrinsic in MTP measurements and were also noted by others [8,13,16]. Hence, one cannot rule out the possibility that we have missed significant differences in pressure gradients. Nevertheless, data of the particular myocardial levels are consistent.

Theoretically, the endomyocardium could be passed by stents placed into the endomyocardial part of the laser channel to reach the midmyocardium with its lower pressure compared to LVP. The feasibility of this idea needs to be corroborated.

4.1. Conclusion

The present study was initiated to evaluate a possible indirect (non-coronary) revascularization in a model of chronic myocardial ischemia. We have investigated the effects of TMLR alone and TMLR combined with the intramyocardial application of naked VEGF (DNA), questioning firstly, whether the transmural pressure state allows perfusion of laser channels, and secondly, whether additional application of VEGF in the vicinity of laser channels improves long-term patency.

Considering our experimental MTP and histological results, the propitious clinical effects of TMLR cannot be attributed to direct blood flow through laser channels into the myocardium. Additional VEGF application in the vicinity of the laser channels could not improve long-term patency.

Acknowledgements

Dr Lutter is supported by the German Research Foundation (grant BE 931/7-1, grant BE 931/7-2, and grant LU 663/3-1), Bonn, Germany, and the Clinical Cardiovascular Research Center II and III at the Medical Center (grant B3 and 11-00-III), Albert-Ludwigs-University of Freiburg, Germany. We express our gratitude to Tina Brehmer and Kerstin Schlegel for their histochemical and immunological analysis, and Manfred Olschewski, Division of Medical Biometrics, Albert-Ludwigs-University, for his statistical analysis.

References


