Transmyocardial laser revascularisation. Clinical experience in patients with refractory angina pectoris

van der Sloot, J.A.P.

Citation for published version (APA):
Angiogenesis three months after clinical transmyocardial laser revascularisation

Jos AP van der Sloot
Menno Huikeshoven *
Allard C van der Wal †
Raymond Tukkie ‡
Martin JC van Gemert *
Johan F Beek *

*Laser Centre and Departments of †Cardio-Vascular Pathology and ‡Cardiology,
Academic Medical Centre, University of Amsterdam

Lasers in Surgery and Medicine 2001;29:369-73
Chapter 3

Abstract

We present for the first time histological findings 3 months after clinical TMLR using a XeCl excimer laser.

In the treated myocardium, no patent channels were found but scars were seen with a linear distribution and in continuity with circumscribed small fibrotic endocardial and epicardial scars. The scars were highly vascularised by new vessels, ranging from small capillaries to large thin-walled, and sometimes branching ectatic vessels. Sprouting of vessels into the adjacent myocardium was also observed.

These results suggest that angiogenesis might play a role in the clinical improvement after TMLR.
Angiogenesis three months after TMLR

Introduction

Transmyocardial laser revascularisation is an experimental technique used to treat patients with severe angina pectoris refractory to conventional therapies such as coronary angioplasty or bypass surgery. In ischaemic but viable myocardium transmyocardial channels with a 1 mm diameter are created using laser light. Three different types of lasers are clinically used: A high power CO\textsubscript{2} laser, a Ho:YAG laser and a XeCl excimer laser. Although the treatment has been under investigation for almost two decades, significant clinical improvement has only recently been shown in randomised clinical trials [72-76]. However, to date uncertainty and discussion prevails about the underlying working mechanism of this improvement. Post-mortem histology could contribute to gaining insight in this mechanism. So far, only post-mortem histology of human myocardium treated with a CO\textsubscript{2} laser has been reported. Our aims are (a) to present here the first case of human myocardium treated with a XeCl excimer laser, obtained from a patient who died 3 months after the intervention, and (b) to review the available clinical histology and compare it with our findings.

Patient history

The patient was an 81-year-old man whose cardiac history included a CABG in 1973 and 1984, followed by seven PTCA procedures, both in native vessels and in grafts, between 1984 and 1993. All these treatments were without long lasting improvement of anginal symptoms. In 1993 he suffered an anterior wall infarction after which he was admitted to the hospital several times for unstable angina. At referral to our hospital he presented with angina pectoris functional class IV/IV (NYHA classification) under maximal tolerable medication, caused by severe three-vessel disease considered untreatable with re-CABG or re-PTCA. The LVEF was 45% and myocardial perfusion scintigraphy showed reversible ischaemia in the anteroseptal and anterolateral region with corresponding hypokinesia on stress-echocardiography. With these findings, the patient was considered eligible for stand-alone TMLR and he was included in a TMLR pilot study in our hospital.

Under general anaesthesia, a left lateral thoracotomy was performed and 50 transmyocardial laser channels were created in the ischaemic anterolateral region of the left ventricular wall. Approximately one transmyocardial channel per cm\textsuperscript{2} was created with a XeCl excimer laser.
Chapter 3

*(Medolas MAX-20, Munich, Germany)* at 308 nm. A catheter was used with a tip diameter of 1 mm. An ECG-trigger device, developed at our hospital, was used in conjunction with the laser. This device operates a shutter that is positioned in the laser beam, just before the beam is coupled into the fibre. The laser was operated at 40 Hz and 40 mJ/pulse (pulse duration 110 ns). In combination with the trigger device on average 4-5 pulses were fired during each cardiac cycle. The fibre tip was advanced into the myocardium until perforation was confirmed by transoesophageal echocardiography. Using these settings, the myocardium was perforated in an average of three to four triggered cardiac cycles.

Peri-operatively the patient was haemodynamically stable. There was a slight CK-MB elevation up to 71.1 µg/l without signs of a localised myocardial infarction on ECG or echocardiography. He was discharged from the hospital 8 days after surgery but readmitted 4 days later with a severe pneumonia. Following a complicated post-operative period he died 88 days after TMLR due to respiratory insufficiency. During this period there had been no primary cardiac complications. Permission was obtained for a full autopsy.

Pathology

The heart was hypertrophied (heart weight 620 g) and showed moderate dilatation of both ventricles. Several pericardial adhesions were seen at the anterior left ventricular wall, which was also the site of TMLR. The remaining epicardium of the heart was remarkably free of adhesions. The TMLR area was marked with pins, the heart chambers were flushed with 4% formalin and fixed for a period of one week. Both ventricles were cross sectioned in 1 cm segments perpendicular to the long axis of the heart, which revealed patchy areas of fibrous scarring. From the area enclosed by the pins 18 transmural tissue blocks were taken for histology. Of all tissue blocks 5 µm serial sections were cut and at 100 µm intervals two sections were stained with haematoxylin & eosin (H&E) and elastin Van Gieson respectively. Adjacent serial sections of interesting H&E sections were mounted for immunohistochemistry and for a Perl stain to detect iron deposits. In a Streptavidin Biotin Complex method antibodies were applied for recognising endothelial cells (anti-Von Willebrand factor, anti-VWF, DAKO, dilution 1:500), macrophages (anti cd68, DAKO, dilution 1:400), T-lymphocytes (anti cd3, Becton & Dickinson, dilution 1:100), B-lymphocytes (anti cd79a, dilution 1:50), smooth muscle cells (SMA-1,
Angiogenesis three months after TMLR

DAKO, dilution 1:400) and the proliferation associated nuclear antigen Ki67 (MIB-1, Dianova, dilution 1:100).

On low power view, in all sections areas of fibrosis were noticed amidst hypertrophic myocardium. These areas were characterised by cellular connective tissue containing fibroblasts and foci of mononuclear cells (majority of cd68+ macrophages, cd3+ T-cells and a few clusters of cd79a+ B-cells). Moreover, areas with deposition of iron pigment were seen in macrophages and the interstitium, indicating previous haemorrhage. The connective tissue was highly vascularised by vessel structures, ranging from small capillaries to large thin-walled and sometimes branching ectatic vessels lined with flat endothelium (anti-VWF+) and a media consisting of 1-3 layers of smooth muscle cells (alpha-actin+) (figure 1). These ectatic vessels contained red blood cells and often extended into the adjacent hypertrophic myocardium. In between, we found scars with a linear distribution and in continuity with small, circumscribed fibrotic scars of the epicardium, which was otherwise composed of fat tissue. Circumscribed scars of similar size were also noticed at the endocardial surface. Interpreting these epicardial and endocardial scars as highly suggestive for respectively the site of entrance and exit of laser fibre (see figure 2), nine channel scars were identified. Additionally, at three sites linear areas of vascularised connective tissue without obvious continuity with an epicardial or endocardial scar were found. These sites were also considered to be channel remnants. Patent channels were not observed, neither were connections present between large ectatic vessels and the endocardium. Ki67 staining was positive in a number of T-cells and macrophages, but was always negative in endothelial cells or smooth muscle cells of vessels. In addition to the cellular scar tissue described above, a few areas included dense sclerotic hypocellular connective tissue.

Discussion

To our best knowledge, we present in this paper for the first time post-mortem histology of a patient treated with XeCl excimer TMLR. We did not see any patent channels and our findings indicate that vessel growth may not only occur inside the remains of the channels but also extends into the surrounding myocardium. Until now, the clinical post-mortem literature on TMLR has only reported on CO₂ laser findings. These papers mainly discussed channel patency and few papers also reported on neovascularisation. No human post-mortem histology has been reported
Figure 1. High power view of large ectatic vessels (arrows, not all vessels indicated) in an area with scarring fibrosis and remnants of myocardium. Anti-alpha actin stain reactive with vascular smooth muscle cells. Original magnification x 125.

Figure 2. Example of a linear scar in continuity with the epicardium, showing large ectatic vessels in loosely arranged connective tissue, with sprouting of vessels (arrow) to adjacent myocardium. Anti-VWF antibody staining reactive with endothelial cells. Original magnification x 80.
Angiogenesis three months after TMLR. However, experimental results of this (and other) laser(s) have been described in detail by Whittaker in a review on the fate of myocardial channels [98].

The primary goal of post-mortem histological analysis should be to gain insight into the working mechanism underlying clinical improvement after TMLR. The most discussed and investigated hypotheses have been direct blood flow from the left ventricle into the myocardium through patent channels [67], denervation of the myocardium resulting in a reduced perception of anginal pain [99] and induction of angiogenesis leading to improved perfusion in the ischaemic area [100]. Other hypotheses include improved cardiac compliance [91], improved oxygenation of surviving myocardium by a redistribution of blood flow [92], or placebo. Given the findings in our patient and the fact that we could not assess denervation, we will focus on channel patency and neovascularisation.

Of 50 created channels, only nine (18%) could be identified at post-mortem investigation. None of these remnants showed any form of lumen or central passage, which could have contributed to direct perfusion from the ventricular cavity into the myocardium. This finding is in accordance with clinical CO₂ laser reports showing non-patent channels [101-104]. In contrast, other clinical CO₂ laser reports claim patent channels in patients at a time span ranging from 2 hours to 4 years after TMLR [105-108]. Results of human post-mortem histology have been described in 18 patients (not including the one described here). In 78% (14 patients) non-patent channels were reported after TMLR and in 22% (4 patients) patent channels were seen. Recently, in five published randomised trials clinical improvement (defined as a decrease after one year of at least 2 angina classes) was found in 56% of a total of 331 patients that received stand-alone TMLR. If channel patency is the mechanism underlying the clinical improvement, the percentage of patients having patent channels at post-mortem investigation is expected to be similar or higher than the percentage of patients showing clinical improvement. Since the percentage of patients with patent channels is much lower (22%), it is unlikely that the clinical improvement is caused by (blood flow through) patent channels. Only 1 of the 18 patients died at the same time as the patient described in this paper. In that patient the investigators found multiple patent channels containing red blood cells and concluded that the channels were not only open but also functional. We do not think that this difference with our findings should be attributed to the different laser-myocardium interaction
Chapter 3

of the CO₂ and the excimer laser, given the fact that other CO₂ publications reported closed channels at both shorter and longer time spans after TMLR.

The hypothesis of enhanced angiogenesis has gained interest during the last years. Neovascularisation in the zone surrounding the channels would improve perfusion in ischaemic myocardium and is thought to be induced by an increase in local growth factor production following the laser irradiation. This improvement can either be achieved through connections with native vessels of the surrounding ‘healthy’ myocardium or by way of redistributing the blood flow within the ischaemic area. The latter mechanism could be effective if the ischaemia is not completely transmural but predominantly epi- or endocardial. In the post-mortem investigation of the patient presented here, angiogenesis was seen in the fibrotic channel remnants and in the adjacent myocardium. It is likely that the original channels were completely filled with clots and that reorganisation of these clots as well as the thermally damaged tissue adjacent to the original channel resulted in scar formation and angiogenesis. Not all reports on human post-mortem histology have described the presence (or absence) of neovascularisation in the treated area. In one report on a patient 4 years after TMLR, “an increase in the number of vessels in the laser area” was described without specifying the nature of these ‘vessels’ [105]. In this patient, patent channels were claimed to be found. In four papers that reported on non-patent channels, (neo)vascularisation was investigated [101-104]. Of the 14 patients described in these papers, 10 died 2-15 days after TMLR and, as might be expected after such a short time, no neovascularisation was seen. Two of the other 4 patients died at respectively 20 and 24 days after TMLR and the presence of mostly thin-walled capillaries was described in the channel remnants. However, in these 2 patients these capillaries were not found outside the fibrotic channel remnants [101,102]. In hearts of the remaining 2 patients (1 patient died 5 months after TMLR and the other received a heart transplant 9 months after TMLR), formation of new vessels was found both inside and outside the fibrotic channel remnants. In the patient that died 5 months after TMLR “occasionally a link between the capillary network in the laser created channels and the original small myocardial vessels” was found [103]. In the native heart that was explanted 9 months after TMLR “multiple vessels within the channel remnant and adjacent to the channel” were observed, with a positive cd31 and anti-VWF staining, demonstrating the presence of endothelial linings. Furthermore, capillary vascular density analysis on randomly chosen lasered areas showed a mean vessel count that
Angiogenesis three months after TMLR

was 2.5 times higher than in non-lased areas of the left and right ventricle [104]. Although we used an excimer laser and not a CO₂ laser, our result of sprouting of vessels to the myocardium adjacent to the channel remnant 88 days after TMLR, is in accordance with the findings in these last 2 patients. A pattern of abundant vascularisation including large ectatic vessels is also observed in the healing phase of 'normal' myocardial infarction. It is likely that the multiple small infarctions made during TMLR and 'normal' myocardial infarctions follow the same cascade of events leading to sprouting of new vessels. Consequently, the angiogenic response found after TMLR is likely just part of a normal healing process following myocardial injury. However, in TMLR the injury has a predefined pattern, is controlled and smaller compared to a myocardial infarction. Thus, although injury is inflicted, the advantage of increased vascular density may be larger than the disadvantage of the myocardial destruction.

In conclusion, the pathologic characteristics found in this patient indicate that clinical TMLR using an excimer laser causes damage that resembles the majority of post-mortem histology following clinical TMLR using a CO₂ laser: Scarring of the myocardium without any evidence of channel patency. The scars are highly vascularised with sprouting of vessels into surrounding myocardium, indicating induced angiogenesis. Perfusion through these new vessels might be the explanation for the excellent results 5 years after TMLR that have recently been reported [109].