SCIENTIFIC LETTER

Angiogenesis in chronically ischaemic human heart following percutaneous myocardial revascularisation

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Heart 2002;87:281–283

Patients with intractable angina and severe diffuse coronary artery disease not amenable to conventional revascularisation therapy have relatively few treatment options. A number of studies suggest myocardial laser revascularisation is of clinical benefit in such patients.^{1,2} Percutaneous myocardial revascularisation (PMR) involves the use of an intravascular catheter, positioned within the left ventricular cavity under fluoroscopic guidance, to deliver controlled bursts of holmium:YAG laser energy. PMR results in the formation of small channels (~1.75 mm diameter) that extend from the endocardial surface partly into the myocardial wall. Many uncontrolled studies suggest that PMR provides symptomatic relief, although the first randomised controlled trial demonstrated no benefit over a sham procedure.³ It has been suggested that PMR induces angiogenesis, although many other mechanisms of action have been suggested.

To determine whether PMR has any effects on angiogenesis in the human ischaemic myocardium we have undertaken a detailed histological and immunohistochemical examination of the hearts of two patients who died eight weeks and 52 weeks after apparently symptomatically successful PMR therapy. In this first detailed study of human myocardium subjected to percutaneous myocardial laser revascularisation, we report evidence of sustained myocardial neovascularisation in treated areas and of the presence of vascular endothelial growth factor (VEGF). Unexpectedly, most of the neovessels are abnormal and immature, lacking a smooth muscle

coat. Furthermore, neovessels are largely confined to scar tissue. Both the above factors are likely to limit the extent to which angiogenesis following PMR could improve perfusion. In a broader context, our findings that, once formed, immature and abnormal neovessels are sustained long term in human myocardium, may be relevant to the general design of strategies for therapeutic angiogenesis in patients—for example, the direct application of angiogenic factors (or genes).

METHODS

Hearts were recovered postmortem from two patients who died eight and 52 weeks following PMR (of non-PMR related causes). Specimens were fixed in 4% formalin, embedded in paraffin, and 4 µm serial sections stained with haematoxylin. Sections were immunostained with monoclonal antibodies against α-smooth muscle actin (0.25 µg/ml), and polyclonal antibodies against von Willebrand factor (vWF)/factor VIII related antigen (1/200), and pan VEGF (2 µg/ml). Samples were also immunostained for CD45 and CD68 to assess the presence of lymphocytes and macrophages, respectively.

Abbreviations: PMR, percutaneous myocardial revascularisation; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor

Figure 1 Comparison of vessels in control and PMR treated myocardium. Sections of non-treated left ventricular anterior wall from eight week patient (A); non-treated area of left ventricular lateral wall from 52 week patient (B); PMR treated at eight weeks (C) and 52 weeks (D) immunostained for von Willebrand factor. Negative control without primary antibody is shown in D. Examples of established microvessels and equivalent sized PMR induced vessels are indicated by arrows and large diameter vessels by arrowheads. Asterisks indicate possible vessel fusions. Bar = 200 µm.

Figure 2 Smooth muscle investment of vessels, presence of inflammatory cells and VEGF in control and PMR treated myocardium. Sections from non-treated areas (A) and PMR treated areas of myocardium at eight weeks (B) and 52 weeks (C) immunostained for von Willebrand factor (brown) and α -smooth muscle actin (red). VEGF (D, E). Negative control, primary antibody blocked with VEGF antigen peptide shown in inset (E). In panels A–C arrows indicate smooth muscle poor vessels and arrowheads indicate smooth muscle positive vessels. Bar = 100 μ m.

Primary antibodies were detected with biotin-conjugated secondary antibodies and the ABC system (DAKO Ltd, Cambridge, UK). Antibodies were from DAKO except for the pan specific VEGF antibody that was from Santa Cruz Biotechnology (Santa Cruz, California, USA).

RESULTS

Both patients responded positively to PMR with apparent symptomatic improvement. Areas of fibrotic scarring were evident at eight and 52 weeks following PMR, that were not present in sections of the non-treated regions of left ventricle. These fibrotic zones contained a high density of microvessels as evidenced by positive staining for vWF (fig 1). A prominent feature in these areas was the presence of large apparently thin walled vessels. These were evident not only at eight weeks, but were particularly notable at 52 weeks post-PMR. Neovessels, including the abnormal large vessels, in PMR treated regions at week 8 and 52 weeks contained blood cells (fig 1), suggesting that they were perfused.

To investigate microvessel maturity, sections were probed for the smooth muscle cell marker, α -smooth muscle actin (fig 2A–C). Microvessels in non-treated left ventricular myocardium had prominent smooth muscle cell coverage (fig 2A), as did microvessels outside the areas of laser scarring in PMR treated ventricles (fig 2B,C). In contrast, many of the microvessels within the PMR induced fibrotic areas had little or no smooth muscle cell cover, either at eight or 52 weeks post-treatment (Fig 2B,C). There was no evidence of differences in lymphocyte infiltration associated with any areas of the PMR treated ventricles. Immunostaining with anti-CD68 antibodies demonstrated that macrophages were not present in either PMR treated or control sections. Using an antibody which recognises VEGF isoforms 121, 165, and 189, this angiogenic factor was detected in untreated areas of the heart as well as PMR treated regions (fig 2). In control regions of left ventricle, VEGF was detected in cardiac myocytes and smooth muscle cells surrounding microvessels. Areas of fibrosis lacked VEGF, and although smooth muscle cells surrounding microvessels within these regions stained positively for the growth factor, vessels lacking this investment were VEGF negative.

DISCUSSION

This is the first report of sustained neovascularisation within the myocardium of human patients following PMR therapy. Clear evidence of increased numbers of neovessels was found not only shortly after PMR (eight weeks) but also longer term post-treatment (52 weeks). Notably the neovessels were abnormal. Specifically, many of these neovessels were smooth muscle cell negative and were large, dilated, thin walled vessels. Vessels lacking the regulatory influences on size and

structure normally exerted by smooth muscle cells could remodel and expand to produce the abnormal vasculature observed. Restriction of the neovessels to fibrotic tissue, as well as their immature nature, probably limits the impact these vessels have on myocardial perfusion.

Blood vessels form initially as endothelial tubes, which then become invested with supporting smooth muscle cells during vessel maturation. Before acquisition of supporting cells, newly formed microvessels are unstable and, in the absence of survival factors such as angiogenic growth factors, undergo regression.⁴ The continued existence of apparently immature neovessels within PMR treated ventricles even at 52 weeks would indicate the presence of such angiogenic factors. A likely candidate for maintenance of the immature neovessels is VEGF, which was expressed in myocardial cells of both PMR treated and untreated areas of ventricles. Levels of this growth factor in the heart may be sufficient to support the immature vessels and allow them to remodel into the dilated, thin walled structures observed here, but not enough to initiate angiogenesis. It is a strong possibility, therefore, that other strategies to initiate neovascularisation in a similar myocardial environment in patients with chronic coronary disease, such as application of growth factors,⁵ may also result in formation and persistence of abnormal vessels. The present findings emphasise the need for a better understanding of mechanisms controlling formation and maturation of neovessels in the human heart in order to optimise revascularisation strategies, be they PMR based or involving direct delivery of angiogenic factors.

ACKNOWLEDGEMENTS

JMC is a British Heart Foundation (BHF) Junior Research Fellow. AMS holds the BHF Chair of Cardiology at King's College, London. We are grateful to Professors JD Swales (deceased) and H Thurston for helpful discussions. We thank the Special Pathology Unit at Leicester Royal Infirmary for helpful advice.

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Accepted 8 November 2001

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IMAGES IN CARDIOLOGY

Microscopic evidence of effective ablation of calcium and metal from coronary arteries treated with directional coronary atherectomy using the Flexicut device

The presence of angiographically detected calcium in the atherosclerotic lesions that cause significant coronary stenosis has been assumed as a limit to the use of directional coronary atherectomy (DCA) because of the inability of the device to cut and remove the calcified plaque. The new Flexicut DCA device is smaller and more flexible, and the titanium cutter allows more extensive and effective ablation of tissue. Such technical improvements offer potential advantages that can make DCA also feasible in calcified lesions. As to its effectiveness for the treatment of in-stent restenotic lesions, experience is limited to small series performed with the former DCA device.

We present the histologic findings of a de-novo, calcified plaque (below, first left) and the macro- and microscopic aspects of in-stent restenotic material successfully retrieved with DCA (below, centre and right).

The images demonstrate that the new DCA Flexicut device has the potential for effective ablation of solid components of the coronary artery. On the one hand such capability could enlarge the applications of DCA in more complex lesions; on the other hand, the aggressive profile of the new cutter should be kept in mind when performing debulking of the soft tissue located within restenotic stents to avoid procedural complications. The ultrasound vascular assessment of the actual stent expansion may be useful to detect underexpanded stents and select the diameter of the DCA device, or to decide for a repeated balloon dilatation.

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Haematoxylin and eosin microscopy of a "de-novo" plaque with calcifications. The thin arrow indicates fine, superficial "sand-like" calcium. The thick arrow indicates a large calcified core deep in a fibrotic cellular plaque that caused unstable angina. The two aspects may represent different stages of the process of coronary calcification. Red blood cells identify the luminal side of the plaque (arrowheads).

Macroscopic view of in-stent restenotic material embedded in paraffin. The arrows indicate the presence of metallic fragments of the stent struts retrieved with DCA.

Radiographic image of the same stent struts embedded in the paraffin.

Haematoxylin and eosin microscopy of the in-stent restenotic plaque. The arrow shows a small metallic fragment retrieved with the DCA cutter. Close to the metal there is an inflammatory infiltrate. Deep in the plaque the neointima is composed mainly of smooth muscle cells and fibrosis.