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SHORT REPORT

Histological and Immunofluorescent Analysis of a Large Tributary of the Great Saphenous Vein Treated with a 1920 nm Endovenous Laser: Preliminary Findings☆

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Objectives: To analyse the biological effects of a 1920 nm endovenous laser (EVL) on extra-fascial great saphenous vein (GSV) *in vitro*.
Methods: A 10 cm length of a large tributary bypassing a hypoplastic segment of the GSV (sometimes called an "extra-fascial GSV") was obtained during routine varicose vein surgery. The length was treated in five sections with different LEEDs (0 (control), 20, 40, 60, and 80 J/cm) with a 1920 nm EVL at 4W power, in a novel *in vitro* treatment model. The biological effects were assessed by histological staining of the samples for haematoxylin and eosin (HE) and Martius Scarlet Blue (MSB), and by immunofluorescent detection of p-p53 and VCAM-1.
Results: Histological analysis showed significant structural damage at LEEDs above 60 J/cm, especially in the intima and media, with the treatment at 80 J/cm causing perforation of the vein wall. In addition, there was a significant increase in p-p53 expression in treated tissue at 60 and 80 J/cm.
Conclusions: Using this *ex vivo* model, the results indicate that *in vitro* treatment with a 1920 nm EVL, at or above an LEED of 60 J/cm and 4 W power, causes significant vein wall cell death reaching deep into the media by a combination of direct thermal damage and apoptosis. A wavelength of 1920 nm appears to be effective for the endovenous ablation of truncal veins.

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OBJECTIVES

Over the last two decades varicose vein surgery has progressed from invasive open surgical techniques to minimally invasive endovenous surgery performed under duplex ultrasound guidance. Radiofrequency ablation (RFA) was the first successful percutaneous endovenous thermal ablation technique to be administered for truncal venous reflux. More recently, endovenous lasers (EVL) have been used in EVL ablation (EVLA), targeting haemoglobin and more recently water as the chromophore for the laser energy.

There have been many clinical studies looking at the success rates of EVLA relative to open surgery. Research has negatively favoured the latter, with reports of revascularisation, neovascularisation, and, consequently, high rates of varicose vein recurrence.[1] By comparison, the post-operative pain, scarring, bruising, and overall recovery time has been reported to be significantly lower with RFA and EVLA.[2] However, a recent meta-analysis looking at the results of treatment of the great saphenous vein (GSV) 5 years after open surgery, EVLA, and ultrasound foam sclerotherapy, has shown that these advantages of EVLA over open surgery appear to be early, and outcomes at 5 years appear to be similar.[3] Thus, endovenous thermal ablation has been the recommended treatment for truncal venous reflux by the National Institute of Health and Care Excellence[4] since 2013 and by the European Society for Vascular Surgery guidelines in 2015.[5]

Although the exact biological mechanism of thermal in induced venous occlusion has not yet been demonstrated, it has been proposed that transmural vein wall cell death is essential for fibrotic occlusion after treatment. Following recent findings regarding sclerotherapy, apoptosis has been proposed as a cell death mechanism after thermal ablation.[6] The objective of this study was to analyse the biological effects of a 1920 nm EVL on an *ex vivo* large tributary bypassing a hypoplastic segment of the GSV, often called an

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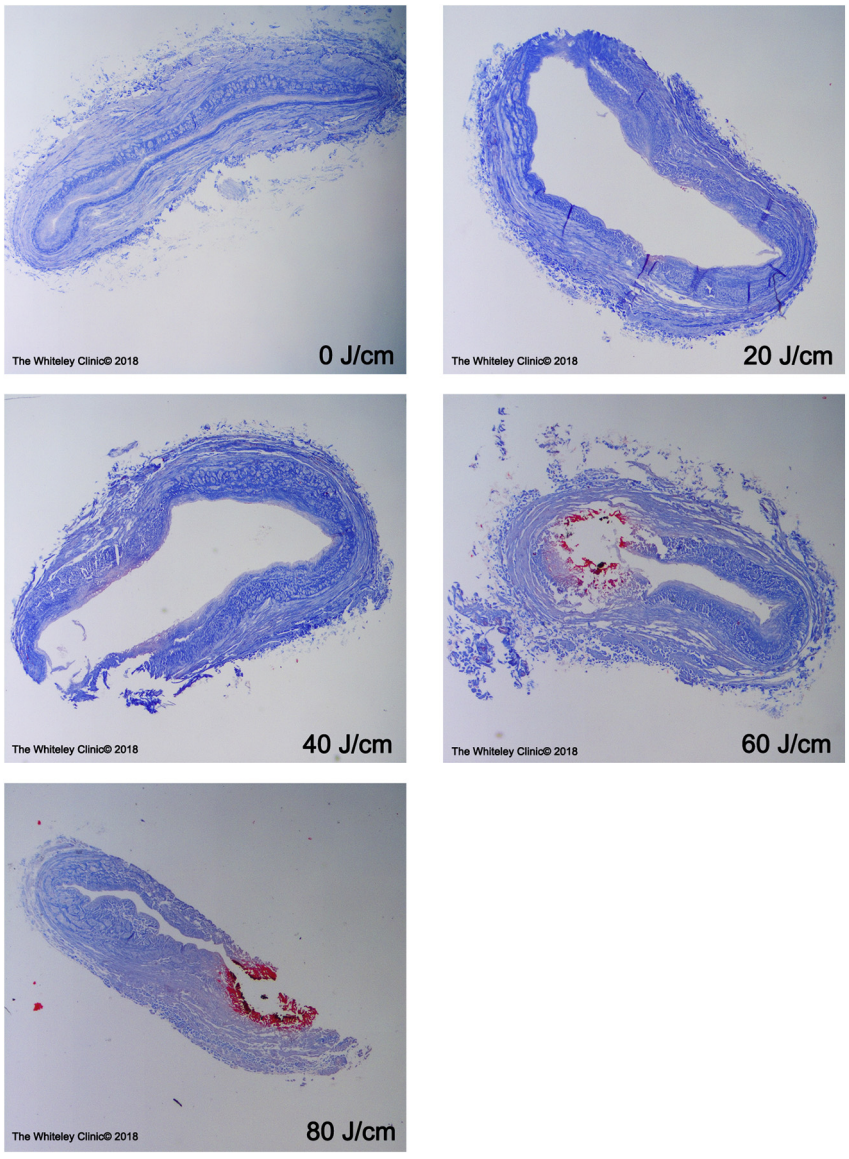

Figure 1. Histological analysis of a large extra-fascial tributary bypassing a hypoplastic segment of the great saphenous vein (GSV) treated with varying LEEDs by a 1920 nm endovenous laser. Stained with Martius Scarlet Blue. Connective tissue appears blue and fibrin red. Pictures taken at ×40 magnification.

"extra-fascial GSV," treated *in vitro*, by histology and immunofluorescence.

MATERIALS AND METHODS

A 10 cm length of a large tributary of the GSV was obtained from a patient undergoing routine varicose vein surgery. After extraction, the length was immediately placed in a Petri dish containing phosphate buffered saline (PBS). The vein length was split into five equal sections. A jacket tipped laser fibre (NeverTouch Direct, AngioDynamics, Albany, NY, USA), attached to a 1920 nm EVL (AngioDynamics, Albany, NY, USA) was inserted into the open end of each section, and passed up the length of vein. One section was untreated, to act as a control, and the other four were treated with the 1920 nm EVL at 4W, with four different pull back speeds; 5, 10, 15, and 20 s/cm. Thus, the five sections taken from the length of vein were treated with five different linear endovenous energy densities (LEEDs): 0, 20, 40, 60, and 80 J/cm. After treatment, the sections were immediately halved.

One half was immediately immersed in 10% buffered formalin and fixed for 24 hours. Following fixation, the tissue samples were placed into an automatic tissue processor (Shandon Citadel, Thermo Electron Corporation, Runcorn, UK) and processed routinely. The samples were then embedded into paraffin wax blocks using an embedding station (Raymond Lamb Blockmaster II, Thermo Fisher Scientific, Loughborough, UK). A microtome (Reichert 2040 Microtome, Leica Biosystems, Milton Keynes, UK) was then used to cut 5 µm sections and these were placed onto Superfrost slides. The slides were stained with haematoxylin and eosin (H&E) and Martius Scarlet Blue (MSB). The staining was performed using an Autostainer (Sakura Tissue-Tek DRS 2000, Sakura, Alphen aan den Rijn, Netherlands), to