Chapter 8

Histological observations one-year after mechanochemical endovenous ablation of the great saphenous vein

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Abstract
Mechanochemical endovenous ablation (MOCA), using the ClariVein® device, is a novel endovenous treatment modality of saphenous vein insufficiency. Results of MOCA on the cellular level are unknown and are essential to optimize treatment. Histological analysis was performed of a great saphenous vein one year after MOCA and compared with a healthy vein. Microscopic evaluation of the vein showed a circumferential disappearance of the endothelial layer and fibrosis of the vein. The media was considerably damaged with changes in collagen structure. These observations support the therapeutic effect of MOCA.
Introduction

Minimal invasive endovenous therapies are widely used for the treatment of great saphenous vein (GSV) incompetence and almost replaced traditional surgery. Mechanochemical endovenous ablation (MOCA) with the ClariVein® device is amongst the newest modalities, and supposedly combines mechanical damage using a rotating wire to the endothelial layer with the infusion of a liquid sclerosant. The aim of the mechanical damage is fourfold: (1) promoting the coagulation activation by damaging the endothelium, (2) inducing a vasospasm reducing the vein diameter, (3) increasing the action of sclerosant, and (4) ensuring an even distribution of the sclerosant. The liquid sclerosant then produces irreversible damage to the cellular membranes of the endothelium, resulting in fibrosis of the vein. However, evidence supporting these theories has not been published so far, while early data of anatomical and clinical success are promising\textsuperscript{1-3}. The aim of this study was to evaluate histological changes of a vein, one year after treatment with MOCA.

Case

Patient

A 59-year-old patient was treated with MOCA for bilateral GSV incompetence. Her medical history reported a high ligation of the right saphenofemoral junction (SFJ) in 2001. There were no comorbidities, and the patient did not use medication. The body mass index was 25.7 kg/m\textsuperscript{2}. Both legs were classified as C\textsubscript{3} E\textsubscript{r} A\textsubscript{1} P\textsubscript{r}. Duplex ultrasound examination of the right leg showed a recurrent GSV developing from the femoral vein with a diameter of 5.2 cm. At the left leg, an incompetent GSV was observed with a diameter of 5.0 cm. After informed consent, she was included in a prospective observational study\textsuperscript{4}. No complications after treatment were observed.

MOCA procedure

MOCA was performed using the ClariVein\textsuperscript{®} catheter (Vascular Insights LLC, Madison, CT, US), as previously described\textsuperscript{1}. Briefly, using a Seldinger technique a 4-F introducer sheath was introduced into the GSV and the ClariVein\textsuperscript{®} catheter was positioned with the tip of the dispersion wire 1.5 cm distal of the SFJ under ultrasound guidance. After the proper
positioning of the tip, the wire was activated for a few seconds to induce spasm of the proximal vein. Then, the activated catheter with rotating tip was steadily withdrawn at 1 cm every 7 seconds, dispersing liquid polidocanol (Aethoxysklerol®; Kreussler Pharma, Wiesbaden, Germany) to the damaged vein wall simultaneously. The right GSV was treated with 2 mL polidocanol 2% for the proximal segment, and 4 mL polidocanol 1.5% for the remaining segment over a total length of 43 cm. Since the maximum daily dose of polidocanol had been applied, the left GSV was treated on another day, using 2 mL of polidocanol 2% and 5 mL of polidocanol 1.5% to treat 42 cm of vein. After the procedure, the patient was discharged with a compression stocking (30-40 mmHg) continuously for the first 24 hours and during daytime for the next 2 weeks. No low molecular weight heparin was given. No complications were observed after treatment.

**Follow-up**

After 1-year follow-up, the patient had recurrent edema of the right leg, presenting in the evening. Duplex ultrasound imaging showed recurrent insufficiency of the SFJ and was suspect for recanalization. The patient was reoperated, and the deep femoral vein, including the SFJ, was explored. The GSV was completely obliterated, but an insufficient anterolateral branch, deriving from the SFJ, was found and removed. The proximal part of the GSV was excised and sent for histological processing.

**Histological analysis**

The histological effects of MOCA on the vein were compared to a normal, untreated GSV harvested from a patient who underwent a venous femoropopliteal bypass. Segments from both the normal and MOCA-treated GSV were fixed in 10% neutral buffered formalin and subsequently embedded in paraffin. Four micrometer sections were cut and stained with hematoxylin & eosin, elastica van Gieson, and Masson. Immunohistochemical staining was performed using antibodies to alpha-smooth muscle actin (α-SMA, clone 1A4; Cell Marque, Rocklin, CA, US), factor VIII–related antigen (factor VIII-R Ag, rabbit polyclonal; Cell Marque), and Ki-67 (clone 30-9; Ventana Medical Systems, Inc., Tucson, AZ, US). Stainings were performed using a Ventana Benchmark Ultra automated IHC/ISH slide staining system (Ventana Medical Systems, Inc.). The a-SMA stain was used to detect vascular smooth muscle cells (VSMCs) and myofibroblasts, whereas factor VIII-R Ag was used as a marker for endothelial cells. Ki-67 antigen is a large nuclear protein that is expressed during all phases of the cell cycle.
except G0 and was used for the detection of proliferating cells. Stained sections were scanned with a Hamamatsu NanoZoomer digital slide scanner (Hamamatsu Photonics, Almere, The Netherlands).

Results

Microscopic evaluation of the normal GSV (Figure 1, left two columns) revealed the presence of the three-layer structure of the vascular wall consisting of the tunica intima, tunica media and tunica adventitia (Figure 1A). The tunica intima contained α-SMA expressing VSMCs (Figure 1C) intermingled with thin elastin fibers (Figure 1B) and was covered with a monolayer of FVIII-expressing endothelial cells (Figure 1E). The tunica media in the normal GSV contained circular and longitudinal α-SMA expressing vascular smooth muscle cells (Figure 1B,C) with matrix (Figure 1D) and some microvessels (Figure 1E).

The MOCA-treated vein (Figure 1, right two columns) was characterized by complete luminal obstruction due to near complete neointima formation (Figure 1A). The original intimal area showed a circumferential disappearance of the endothelial layer and no remnant islands of endothelial cells were observed (Figure 1E). The neointima was characterized by excessive elastin deposition at the medial side (Figure 1B) and furthermore contained large numbers of α-SMA expressing myofibroblasts (Figure 1C) and collagenous matrix (Figure 1D). Venous and arteriolar structures as well as capillary microvessels were observed in the center of the neointima, embedded in extensive fibrosis (Figure 1E). In the Ki-67 staining, no mitotic activity was observed in the α-SMA expressing myofibroblasts and microvessel endothelial cells (not shown). The media was considerably damaged in an inhomogeneous pattern, whereas the width of the media varied widely in sections of the MOCA-treated vein compared to the proportional width in the normal GSV (Figure 1B,C). Damage to the media was generally seen in the inner third of the media at locations where the longitudinal VSMCs reside. Perforation of the vein wall or damage to the adventitia was not observed in the MOCA-treated vein sections.
Figure 1. Mechanochemical endovenous ablation (MOCA) results in complete obliteration of the great saphenous vein (GSV) with a myofibroblast and extracellular matrix–rich neointima (ni). Sections of a normal GSV and a MOCA-treated GSV (1 year after MOCA) were histochemically [A: hematoxylin & eosin (H&E), B: elastica van Gieson (EvG), and D: Masson] and immunohistochemically [C: α-smooth muscle actin (α-SMA) and E: factor VIII-R Ag (FVIII)] stained. Right column panels of both normal GSV and MOCA-treated GSV show higher-power magnifications of the framed areas in the respective low-power images on the left. Black dotted lines indicate the transition from media (m) to intima (in normal GSV); white dotted lines indicate the transition from media (m) to neointima (in MOCA-treated GSV). In B and C, open and closed black arrowheads indicate medial smooth muscle cells in, respectively, longitudinal and circular orientation. In C and E, white arrows indicate newly-formed neointimal microvessels. Asterisks indicate α-SMA–positive myofibroblasts (C) and matrix deposition (D) in the neointima. In these images, “a” indicates the adventitia, and “ec” is endothelial cells, “m” is the media, and “ni” is the neointima.
Discussion

Long-term effects of endovenous ablative techniques on the vein wall are scarcely described. This report shows a complete loss of the endothelial layer, damage to the medial layer, and fibrosis of the treated vein 1 year after MOCA of the GSV. To our knowledge, no other histological data are available 1 year after endovenous varicose vein treatment. For ethical reasons, it was impossible to find more patients for histological analysis. The GSV specimen was excised after a re-crossectomy due to a misinterpreted duplex ultrasound. Duplex ultrasound changes after endovenous laser ablation are described by Corcos et al. In their study, 44 limbs were followed with duplex ultrasound at 7 days to 1, 2, 6, and 12 months. The venous diameter of the GSV was decreased over time by fibrotic transformation, leading to definitive GSV atrophy after 12 months. At 12 months, 77% of the treated GSVs were no longer detectable by duplex ultrasound in the anatomical site. However, these findings were observed in patients treated with endovenous laser ablation, and data on duplex ultrasound changes after MOCA are insufficient. In the authors’ experience, the disappearance of the GSV with duplex ultrasound is also frequently seen after MOCA and could be an explanation for the misinterpreted duplex ultrasound in this case.

Several studies have evaluated the immediate effects of (foam) sclerotherapy on the vein wall. Injection of sodium tetradecyl sulphate 3% foam into the GSV results in complete damage of the endothelium within 2 minutes. After 15 and 30 minutes, edema of the subendothelial layer occurs with a progressive separation from the media. In an ex vivo study, endothelial cell loss was observed in 92.2%. However, all sections showed persistent islands of endothelial cells, and damage to the media was minimal, without disruption of the collagen structures. Complete denaturation of the endothelial layer is thought to be essential in achieving long-term occlusion. Though complete endothelial injury is often absent after endovenous laser ablation, the procedure is still associated with a high long-term success rate. Thermal damage to deeper layers of the vein wall, the media, and adventitia, probably contributes to the effect, causing a change in collagen structure, extensive fiber shortening, and vein contraction.

At the level of the original endothelium, no remnants of endothelial cells were observed in the MOCA-treated GSV specimen. However, some microvessels were located in the center of the neointima embedded in extensive fibrosis. Due to the small volume, these microvessels have no hemodynamic or clinical relevance in this case. The presence of
microvessels is also reported after endothermal treatment of varicose veins\(^5\). Histological analysis of endothermally-treated GSVs after 1 to 2 months showed large empty areas covered with endothelium in all 8 cases. Moreover, new microvessels were observed in organized thrombus. Hypothetically, the presence of microvessels may have the potential to evolve, leading to recanalization. This phenomenon is also seen in spontaneous recanalization after superficial or deep venous thrombosis\(^9\).

Recently, an ex-vivo experiment evaluated the histological effects of the mechanical part of the ClariVein\(^\text{®}\) catheter without using a sclerosant\(^10\). The mechanical part of the ClariVein\(^\text{®}\) catheter caused a subtle, incomplete destruction of the endothelium. No changes in the media or adventitia were seen. In the present study, we observed complete endothelial disappearance and considerable damage to the tunica media 1 year after MOCA, which is in contrast to foam sclerotherapy, where islands of endothelial cells usually persist\(^7\). Our observations thus support the hypothesis of an additional effect of mechanical damage using the rotating wire during MOCA. Additional studies, evaluating the immediate histological effects on the vein wall, are clearly indicated to further assess the effects of MOCA. These data may contribute to a better understanding of the technique and optimize the treatment strategy.
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References


