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ORIGINAL ARTICLE

Histologic and sonographic features of holmium laser in the treatment of chronic venous disease

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ABSTRACT

BACKGROUND: A new holmium laser (HOL) has been introduced to the market. The device is able to reduce the great saphenous vein (GSV) caliber in a tumescence-free procedure, favoring an effective sclerotherapy of large vessels. Aim of the present investigation is to provide the first in vivo data about the effect of HOL on GSV histology.

METHODS: Six chronic venous disease (C_{2-5}, Ep, As, Pr) patients (M:F ratio 1:1; age: 57±8, BMI 24±2 kg/m^2) underwent HOL-assisted caliber reduction of the GSV, high-ligation and flush ligation of the incompetent tributaries. Three cm of proximal great saphenous vein not treated by laser and 3 cm of a contiguous segment that was just previously treated by HOL were harvested.

Histological assessments were performed. Patent GSV lumen caliber was assessed at the mid-thigh right before, and after the procedure. Periprocedural pain was graded by Visual Analogue Scale.

RESULTS: GSV samples after holmium laser therapy showed thickening of the vascular wall with a decreased, yet patent lumen. Immunostaining demonstrated intact endothelial lining in both the treated and not treated segments. Expansion of collagen fibers was observed in the laser-treated segments. Collagen appeared more homogeneous than in controls, with an amorphous appearance. Laser treated veins showed a reduction in elastic fibers with greater fragmentation. Smooth muscle cells appeared swollen. The caliber of the mid-thigh great saphenous vein lumen decreased from 8.1±0.8 mm to 3.9±0.2 mm (P<0.0001). The average periprocedural pain was 1±0.6.

CONCLUSIONS: HOL significantly reduces the caliber of the GSV. The endothelial lining is spared, while the remaining wall is thickened by a hyalinization-like process.

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Key words: Solid-state lasers - Holmium - Ablation techniques - Laser therapy - Sclerotherapy.

Endovenous thermal ablation has become a mainstay in the treatment of saphenous reflux [1] and is recommended as the best treatment option by international guidelines.[2, 3] So far, the main thermal sources for endovenous ablation are radiofrequency ablation (RFA) and endovenous laser ablation (EVLA).[4, 5] They are currently preferred over surgery, because they are safe and minimally invasive.[2, 3]

By introducing a fiber into the saphenous vein, thermal energy is delivered intra-luminally and absorbed by blood and vein wall. The aim of these techniques is to ablate the refluxing vein in a minimally invasive way. Nevertheless, tumescent anesthesia is required, leading to possible patient discomfort, occasional hematomas and to extremely rare but serious complications such as fiber fracture.[6, 7]

Recently, an innovative holmium laser (HOL) has been introduced to the market, offering the consistent advantage of not requiring tumescent anesthesia. This device is able to reduce the great saphenous vein (GSV) caliber, allowing immediate, subsequent foam sclerotherapy, even in the largest veins. The strategy is known by the acronym LAFOS (Laser Assisted FOam Sclerotherapy).[8] The specific effect of HOL is strictly related to the average lower temperature generated by the device in comparison to traditional RFA and EVLA: 42 °C *versus* more than 100 °C, respectively.[8-11]

Endovenous thermal ablation techniques will likely continue to play a persistent major role among modern and future phlebology therapeutic options. Nevertheless, their mechanistic features on the venous histologic layers and the related biological effects are described just by a limited number of papers.[12]

The aim of the present morphologic investigation is to provide a detailed description of the effect of HOL on the venous wall that can be used as a basis for future, deeper comprehension of the phenomena governing the action, and thus the performance of endovenous devices.

Materials and methods

Six chronic venous disease patients (M/F:1/1; Age:57±8; BMI:24±2) (C_2, 2 cases; C_3, 2 cases; C_4, 1 case; C_5, 1 case) ($C_{2\text{-}5}$, Ep, As, Pr) underwent a single procedure that included a HOL assisted caliber reduction of the GSV from the groin downward, a sapheno-femoral surgical high-ligation and a flush ligation of the incompetent saphenous tributaries along the leg. The HOL fiber (800 microns) was inserted directly into the GSV right above the knee by a 17-G needle. Neither a guide-wire nor tumescent anesthesia was used.

After positioning the device 3 cm below the sapheno-femoral junction, HOL was used in short pulse mode (350 µs), delivering 300 mJ per pulse at a frequency of 7 Hz for a total of 2.1 J/s. HOL was used in 3 repeated cycles of 2.5 s each, during which the fiber was kept in the same position, moving distally 1 cm after delivering 16 J/cm.

Subsequently, a high ligation was performed at the groin under local anesthesia. During the high ligation, 3 cm of proximal GSV not treated by HOL and 3 cm of the contiguous distal segment that was just previously treated by HOL were harvested.

All patients signed an informed consent prior to the procedure. Institutional Review Board approval was obtained.

For the histopathologic study, multiple sections of the samples were stained with standard ematoxylin-eosin, orcein staining (a specific histochemical staining used to identify elastic fibers) and Masson trichrome staining (a histochemical staining used to identify collagen fibers and muscle fibers).

Immunohistochemistry was also employed to verify the presence of factor VIII — which is normally expressed in endothelial cells — and of actin — which is normally expressed in smooth muscle cells.

Patent GSV lumen caliber was assessed at the mid-thigh right before and after the procedure in the supine position.

Periprocedural HOL-induced pain was graded by Visual Analogue Scale ranging from 0 (no pain) to 10 (unbearable pain).[15, 16]

Statistical analysis

InStat GraphPad (GraphPad Software, Inc., La Jolla, CA, USA) was used for statistical analysis. The data were expressed as mean ± standard deviation. The normal distribution of the data was verified by the Kolmogorov-Smirnov test. The differences between vein diameters pre- and post-procedure were performed using two-tailed paired Student's *t*-test. A P value of 0.05 or less was considered statistically significant.

Results

All 6 harvested samples were suitable for histological analysis. Gross examination of the GSV after HOL therapy showed a conspicuous thickening of the vascular wall with a decreased patent lumen (Figure 1).

On microscopic examination, the vascular wall showed sub-intimal fibrosis and hypertrophy of the smooth muscle coat in both HOL treated and not treated samples, because of the common chronic venous disease condition.

The endothelial lining remained intact in both groups, as demonstrated by factor VIII immunostaining (Figure 2). Various constituents of the vascular wall showed structural alterations. Compared to the contiguous GSV tract (Figure 3A) that was not previously shrunken, HOL samples demonstrated an expansion of the collagen fibers, with a more homogeneous hyalinization-like amorphous appearance and a loss of normal fibrillar characteristics (Figure 3B). In HOL treated samples, orcein staining demonstrated a significant reduction in elastic fibers with

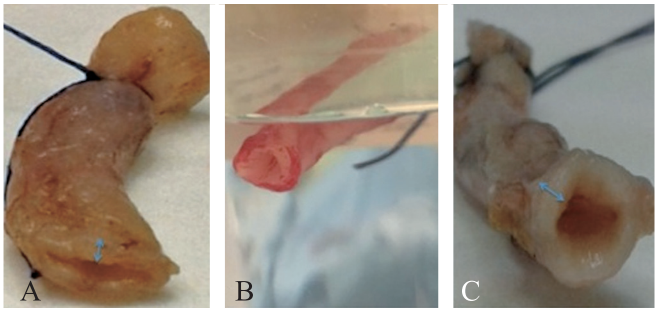

Figure 1.—Macroscopic comparative evaluation among GSV not treated by HL (A) and the GSV immediately below that was previously treated by HL (B, C). A clearly thickened wall is detected both at the harvesting (B) and after histological fixation (C).

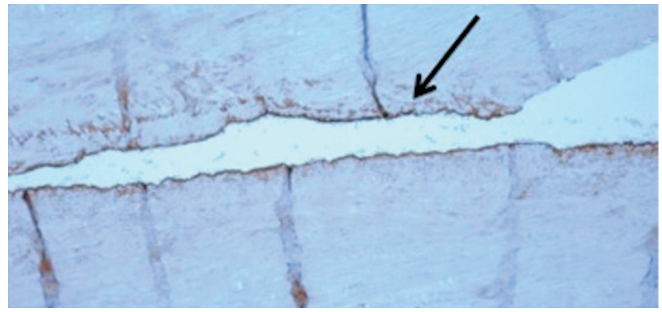

Figure 2.—Brown staining (black arrow) of endothelial cells with factor VIII immunostaining after HL shrinkage of the GSV (4X).

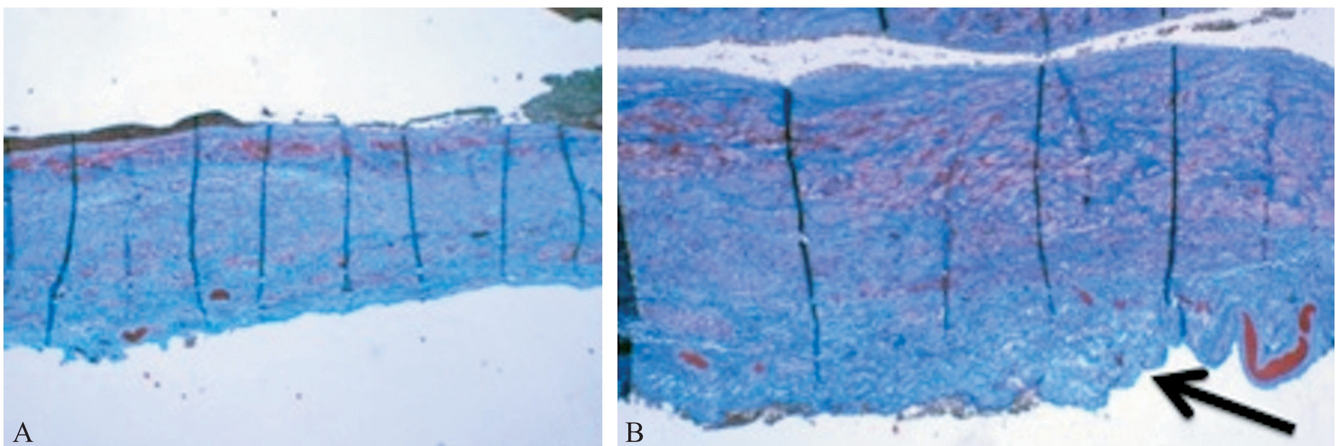

Figure 3.—GSV tract not treated by HL (trichrome staining 2×) (3A) compared to the contiguous GSV segment treated by HL (trichrome staining 2X)(3B). An expansion of the collagen fibers is clearly evident, in particular near the adventitia after the HL shrinkage (arrow, blue staining).

great fragmentation. Loss of the normal weave was clearly appreciated just in the HOL treated samples and not in the contiguous venous segment (Figure 4).

Actin immunostaining demonstrated significant swelling in the HOL samples that was not detected in the contiguous GSV segments (Figure 5).

After HOL shrinkage, thickening of the media was immediately detected by intraoperative sonographic scanning, together with a hyperechoic line at the border of the media and adventitia (Figure 6A). Even if shrunken, the venous lumen remained patent and, before the high-ligation, inhabited by a thoracic pump activated flow (Figure 6B). Caliber of the patent mid-thigh GSV lumen decreased from 8.1±0.8 mm to 3.9±0.2 mm ($P<0.0001$) (Figure 6). The average periprocedural pain was 1±0.6.

Neither minor nor major complications were reported.

Discussion

HOL is a device that has been on the market for more than a decade. Its main application has been in urology and orthopedics. Nowadays however, it is used in several other specialties including general surgery, neurosurgery, dentistry and phlebology.[8, 13, 14]

It is a pulsed Ho:YAG laser that uses a wavelength of 2100 nm, thus highly absorbed by water.[15]

In the phlebology setting, a HOL pulse lasts 350 µs and delivers from 100 to 500 mJ in cycles of 7 Hz, creating a peak temperature of 76 °C that is responsible for the shrinkage.

The average temperature never rises above 42 °C.

Until now, a limited number of histopathological evaluations have been performed on venous walls following EVLA and RFA treatments.[16-19]

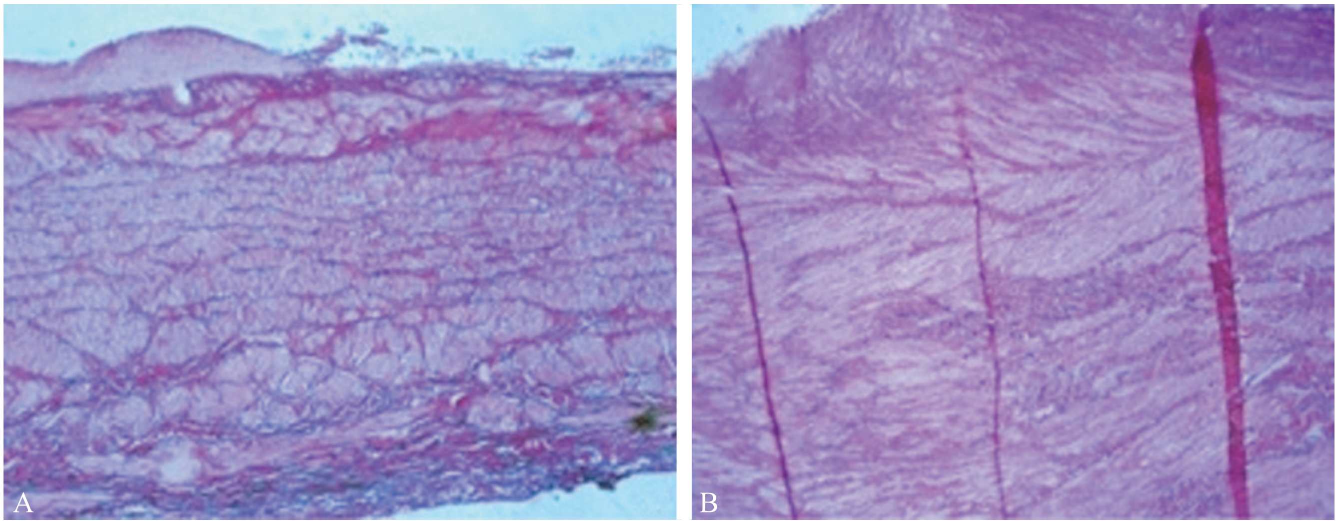

Figure 4.—A) Normal representation of elastic fibers (orcein staining 4×) in the segment not treated by HL. B) Significant reduction in elastic fibers with fragmentation and loss of normal weave after HL use in contiguous GSV segment in the same patient (orcein staining 4×). A) GSV segment not treated by HL compared to the contiguous GSV tract that was previously treated by HL. B) Significant swelling of smooth muscle cells is demonstrated after HL use by actin immunostaining (4×).

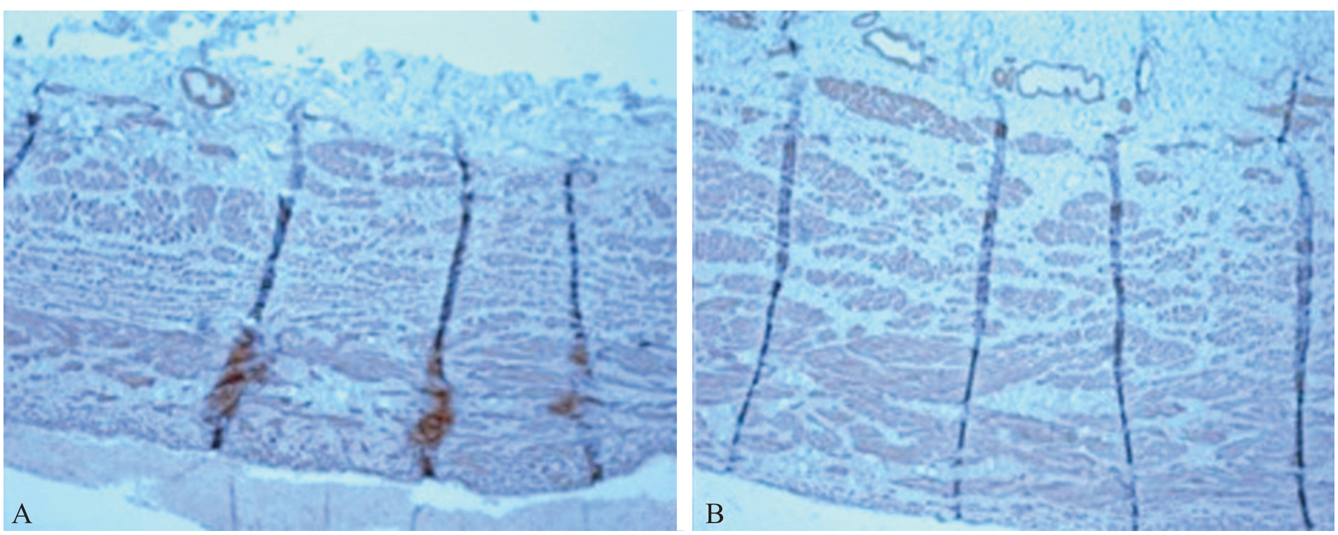

Figure 5.—A) GSV segment not treated by HL compared to the contiguous GSV tract that was previously treated by HL. B) Significant swelling of smooth muscle cells is demonstrated after HL use by Actin immunostaining (4X).

The high temperature produced by EVLA and RFA is associated with macroscopic induration of the vessel. Microscopic assessments have demonstrated endothelial and media disintegration, smooth muscle cell swelling and transmural thermal lesions.[16, 17, 20, 21]

Previous investigations have also reported a risk of venous perforation that was higher in continuous rather than pulsed lasers: a phenomenon that was linked to the carbonization and significant heating of the fiber tip.[20, 22]

In contrast to RFA and EVLA, the current investigation demonstrates that the lower temperature of HOL spares the endothelium.[20, 21]

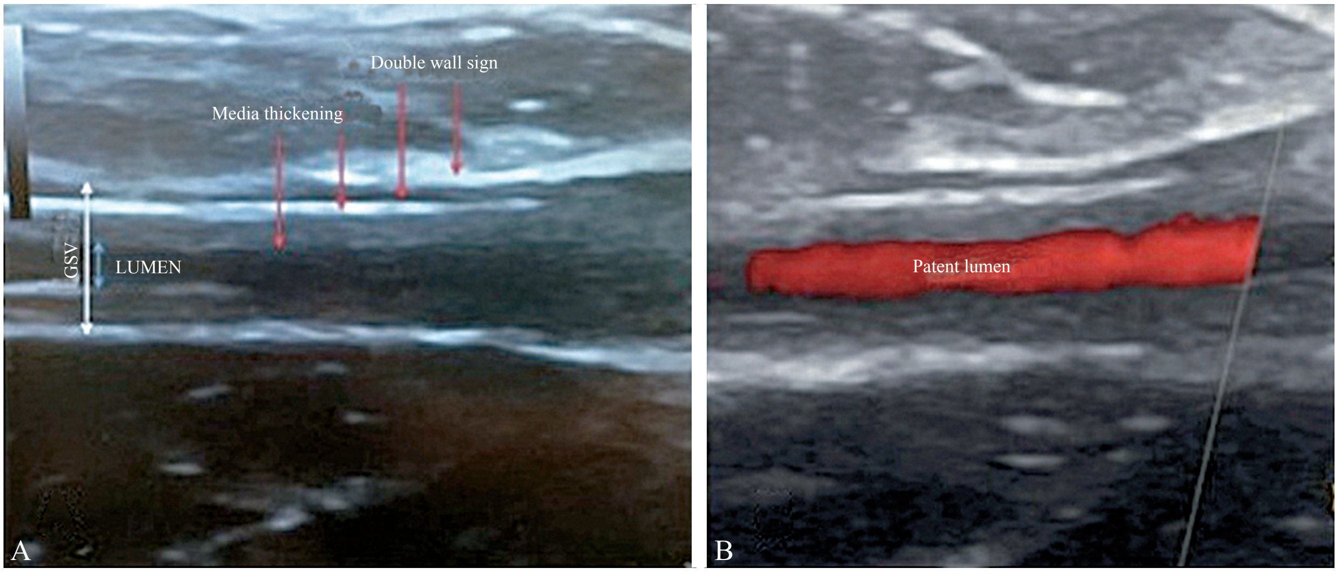

Figure 6.—A Venous wall thickening after HL shrinkage. A "double wall sign" is created by the appearance of the adventitia and the hyperechogenic line that is produced after HL use. A narrower but still patent lumen is clearly evident by color-Doppler investigation (B).

Collagen denatures between 50 °C and 60 °C.[23]

The circulating blood represents a fundamental factor in the process of wall cooling.

In fact, during the pulsed HL switched off phase, the endothelium is cooled by the thermal convection that is created by venous flow. Conversely, the media layer can only cool by conduction, a slower phenomenon that requires more time than the same HL switched off phase.

For this reason, the media layer progressively reaches temperatures above 60 °C, leading to wall damage despite the endothelium sparing.[8, 20, 24]

The present investigation demonstrates how, despite mild thermal damage, the microscopic features of the media are similar to those detected after effective high temperature RFA and EVLA: collagen denaturation, elastic fiber fragmentation, and smooth muscle cell swelling. At the same time, neither splits and gaps, nor wall perforations were reported in HOL samples.

Like traditional endovenous thermal devices, HOL-induced wall damage leads to a chronic rather than transitory process.

In the present investigation, a consequent lumen reduction was detected by intraoperative Doppler scanning. The efficacy of foam sclerotherapy is limited by GSV caliber.[25, 26]

The HOL-induced vessel caliber reduction paves the way for more effective sclerotherapy, decreasing the risks linked to high sclerotherapy drug concentrations.[27, 28]

Further investigations should assess the histopathological evolution of this brand new type of shrinkage over time, together with the eventual biological consequences of an intact endothelium. The HOL-induced low temperature also brings the great advantage of a tumescent free and painless office-based procedure. Another benefit comes from patient feedback in the absence of any anesthesia, so providing a potential further reduction of the neurological and thermal complications that are already diminished by the significantly lower energy delivered by HOL.[29]

The saphenous-sparing strategies' satisfying recurrence rate [30, 31] together with the mini-invasiveness of the modern endovenous techniques [32] have progressively stimulated pioneering investigations regarding GSV competence restoration by means of endovenous devices.[33-35]

In this environment, HOL offers the chance to switch from an ablative tool, as in LAFOS strategy, to a potential means of GSV flow restoration without the need of any subsequent foam sclerotherapy. Further hemodynamics investigations on this topic are currently ongoing and known as LAFE (Laser-Assisted Flow Enhancement).

Despite significant investigations in endovenous thermal techniques, there is still a lack of agreement regarding their principles of action and consequent therapeutic strategies.[36]

HOL adds a new histopathological scenario to the thermal induced venous wall evaluations, thus providing not only a potentially effective tumescent free therapeutic option, but also a deeper understanding of pathophysiology.

Conclusions

HOL offers a new option for tumescence-free treatment of varicose veins. The device reduces saphenous caliber, however investigations on the topic are lacking. This investigation provides the first *in vivo* evidences of the effect of HOL on the endothelium and on the remaining vessel wall, as well as the immediate reduction of vein caliber. The data offer the basis for further study on the histologic effects durability together with investigations addressed to increasing the efficacy of present and future endovenous thermal devices.

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Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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