

Original Article

Effects of endovenous laser ablation on vascular tissue: molecular genetics approach

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Abstract: Background: Endovenous laser ablation (EVLA) is a treatment option for lower extremity varicose veins. In the present study, we investigate to the genetic changes and possibility of living tissue in the saphenous vein wall after the EVLA procedure. Methods: Eleven saphenous vein grafts were randomized in two groups: (1) 4 cm SVG segments of performed EVLA procedure in study group, (2) 4 cm segments of SVG none performed EVLA procedure in control group. SVG were taken from the remnants of distal saphenous vein grafts prepared for the bypass procedure but not used. SVG was approximately 8 cm in length and was divided into two parts 4 cm in length. One half was exposed to laser energy, while the other half of the same vein graft was untreated as a control. EVLA was performed on complete saphenous veins in the study group. Abnormal genetic changes of the SVG were observed with a Tri-Reagent method and quantified with a Nanodrop™ spectrophotometer. Results: Histopathological changes indicated that the intima including the endothelium was completely necrotized in the study group. It was observed that intimal thermal-energy-induced injury did not reach the media. Histopathological examination showed that homogenous eosinophilic discoloration and coagulation necrosis characterized the laser related thermal damage as well. Conclusions: In this preliminary study, we found that living tissue remained in the SVG wall after application of laser ablation, and we also detected abnormal genetic changes in the study group compared with the control group.

Keywords: Endovenous laser ablation, living tissue, apoptosis, genetic

Introduction

Treatment options for lower extremity varicose veins include surgical removal of the vena saphena magna (VSM) and superficial veins, ligation of the saphenofemoral junction, foam sclerotherapy, valvuloplasty, external banding, and radiofrequency or endovenous laser ablation (EVLA) [1]. The primary goal of EVLA procedures is to recognize transmural necrosis of the treated vein. Prevention of endoluminal thrombus formation may reduce pain, recanalization of the saphenous vein, and inflammation, and may also reduce the brownish pigmentation that develops secondary to hemosiderin accumulation due to hemorrhage (?). Thus, fibrosis develops in the vein wall and dead cells are cleared and complete atrophic scarring develops. Destruction of only the endothelium or inadequate injury in the vein wall may result in the media and the adventitia remaining viable

along with thrombotic occlusion of the venous lumen [2]. EVLA may result in complications, such as incomplete occlusion, skin burning, and postoperative pain [3].

Adequate intravascular warming is required for successful EVLA. While this warming leads to irreversible venous occlusion and subsequent fibrosis, this thermal energy should not harm the neighboring tissues of the involved vessel. Although it is essential to determine the thermal effects within and around the vessel, data regarding this issue are limited [4].

Cell proliferation is controlled by various mechanisms in the normal cell cycle, while cancer cells have abnormalities in levels of cell cycle regulators, such as cyclin (cyclin D1-CCND1) and cyclin-dependent kinase inhibitor (CDI) [5]. Apoptosis is a programmed cell death pathway, in which a number of genes, such as Bax and

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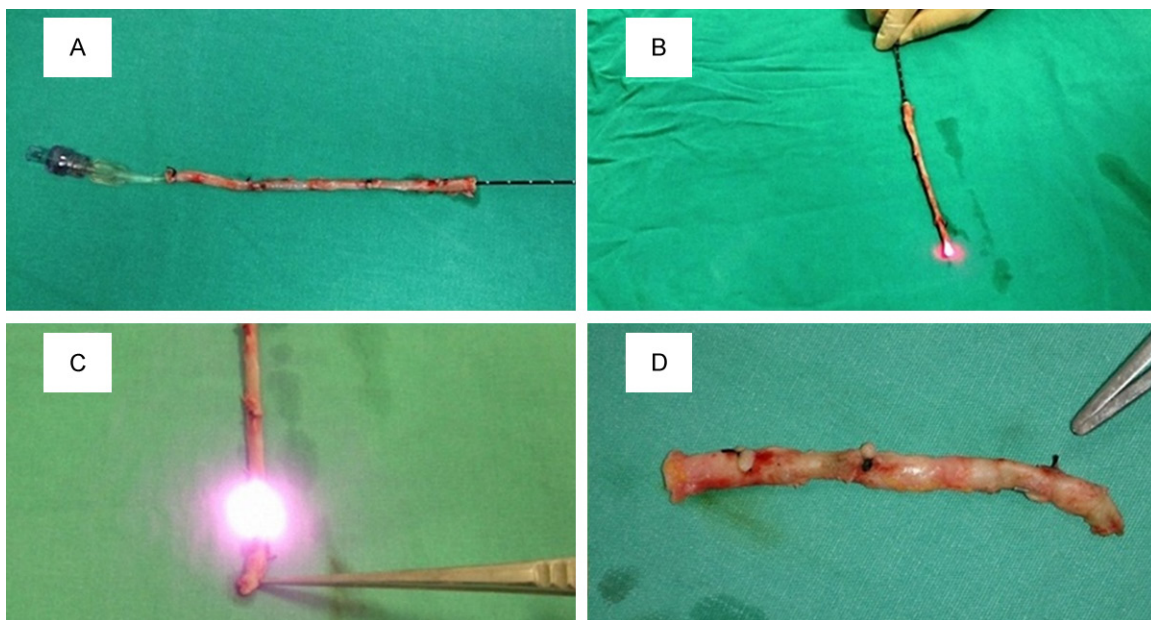
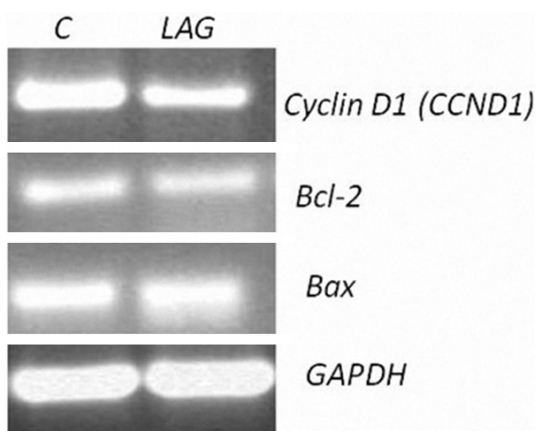


Figure 1. A: Saphenous graft. B: Laser fiber introduced into the saphenous vein graft (before the procedure). C: Application of laser ablation. D: After ablation.



C: Control, LAG: Laser Ablation Group

Figure 2. Changes in *CCND1*, *Bcl-2*, and *Bax* expression.

Bcl-2, play important roles. Apoptosis and the cell cycle work in close relation to each other, and it has recently been reported that imbalance between these two mechanisms can lead to disease. Apoptosis has been shown to be suppressed in some neurodegenerative diseases, and is thought to play a role in carcinogenesis [6].

During the EVLA procedure, the lumen of the saphenous vein is exposed to high thermal energy. Transmural necrosis is expected for

optimal results. We hypothesized that during the EVLA procedure, surviving vascular cells may remain in the saphenous vein wall; these cells may have experienced genetic changes due to laser irradiation. To examine this hypothesis, we designed and performed an experimental *ex vivo* study using normal human saphenous vein.

Materials and methods

The study was approved by the local Ethics Committee. Due to the study data is quantitative, statistical analysis not performed.

Surgical technique and tissue preparation

EVLA procedures applied human saphenous veins obtained from patients treated for coronary artery bypass grafting. Eleven human saphenous veins were taken from the remnants of distal saphenous vein grafts prepared for the bypass procedure but not used. Each saphenous vein graft was approximately 8 cm in length and was divided into two parts 4 cm in length. One half was exposed to laser energy, while the other half of the same vein graft was untreated as a control. EVLA was performed on complete saphenous veins in the study group. We placed laser fibers in the saphenous vein (**Figure 1A, 1B**) and adjusted the device (Fox

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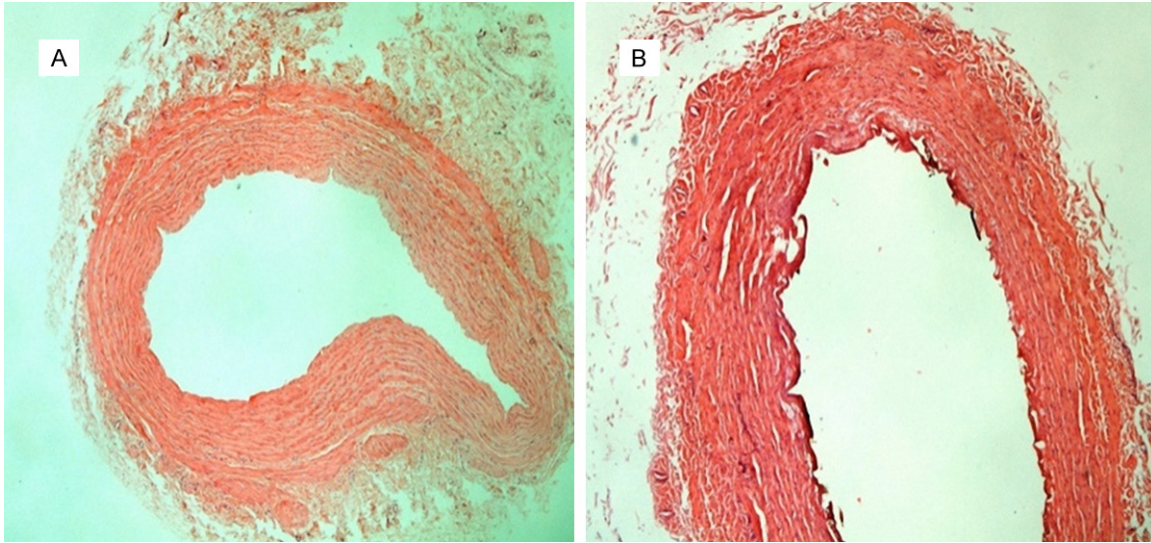


Figure 3. A: Without EVLA, all vessel layers were preserved in the control group (H&E, $\times 40$). B: With EVLA, in the study group necrosis was observed in the intima, but not in the media or adventitia (H&E, $\times 40$).

Diode laser 980 nm, ARC Medical laser) to a laser wavelength of 980 nm, 10 watts, 1-s pause, 10-s shooting time, and 100 J/cm LEED (**Figure 1C, 1D**).

Histopathology

Tissue samples of vessels from human saphenous vein grafts were fixed in 10% buffered formalin, placed in paraffin blocks, sectioned at 5 μm , and stained with hematoxylin and eosin for histological evaluation. Histopathological changes in saphenous veins were evaluated by a pathologist in a blinded manner.

RNA extraction and semi-quantitative reverse transcription-PCR

Laser ablated tissue ($n=11$) and control tissue ($n=11$) were cut into small pieces. Total RNA was isolated from tissue samples by the Tri-Reagent method and quantified with a NanodropTM spectrophotometer. Reverse transcriptase reaction was performed using a First-Strand cDNA Synthesis Kit according to the manufacturer's protocol (Roche Diagnostic, Germany). Appropriate cycles were chosen to ensure termination of PCR amplification before a stable stage was reached in each reaction. Gene expression is presented as the yield of PCR products from target sequences relative to that of the glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gene as a control. Semi-quantitative PCR products were analyzed by 2%

agarose gel electrophoresis and visualized by ethidium bromide staining.

Results

The effects of laser ablation on *CCND1* (cell cycle marker) and *Bcl-2* and *Bax* (apoptosis markers) gene expression are shown in **Figure 2**. Abnormalities in expression of these markers were detected in the laser ablation group compared with the control group (**Figure 2**).

Histopathological changes indicated that the intima including the endothelium was completely necrotized in the EVLA group examined. It was observed that the thermal-energy-induced injury in the intima did not reach deeper than the media and adventitia. Histopathological examination showed that homogeneous eosinophilic discoloration and coagulation necrosis characterized the laser related thermal damage as well. (**Figure 3A, 3B**).

The results of molecular genetic analysis indicated that some cells survived after complete saphenous vein laser ablation. Therefore, RNA was isolated from vascular tissue and assayed.

Discussion

In this experimental study, we proved out that laser energy during saphenous vein ablation procedure does not cause vein tissue necrosis in all of the cells. Besides, these remaining sur-

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vived live cells may show gene expression after exposed to laser energy. It is essential to have a good understanding of the EVLA mechanisms of action in order to prevent complications. Today, two mechanisms of action have been proposed for EVLA. The first of these mechanisms is the thrombotic occlusion of the vein by the effect of thermal injury caused by the heat energy spread from the fiber catheter and vaporization of the intravascular blood content and the second is the collapse and occlusion of the vein by direct heat [4, 7-9]. Although the temperature that may lead to permanent venous occlusion is not precisely known, a temperature of 70°C lasting for a few seconds within the vein is enough to cause endothelial injury. Venous contraction develops as a result of the denaturation of collagen fibers in the venous wall. While collagen contraction develops at 50°C, necrosis develops at 70-100°C. Corcos et al. [10] reported that the endothelium and intima are always destroyed in cases of permanent vein occlusion. As we mentioned at the beginning of the discussion, we think that these changes in the vessel wall occur after exposed to high temperatures.

Many studies have been conducted in order to clarify the mechanisms of action and the influences of heat energy on blood [11-13]. The blood clots at 70-80°C and adheres to the laser fiber. The volume of water in blood content expands 1600 fold at 100°C. At this stage, the heat of the vaporized clot reaches 200-300°C. Blood dissociates at this temperature and converts to carbon and gas. The molten clot adheres to the laser fiber and is carbonized with the effect of the heat. Carbonization is a process that occurs above 300°C. Subsequently the black carbon layer continues to absorb laser energy and begins to vaporize and converts to plasma at 1000-2000°C by spreading white light [14].

In a study evaluating the histopathological changes after EVLA, thermal damage of the intima and the internal part of the media was observed. Vascular perforation with subsequent perivascular bleeding was seen in 10% of the cases treated with 40 to 80 J/cm and in all cases treated with 110 to 200 J/cm. The saphenous nerve was not damaged [15].

In a report on laser energy for the treatment of the saphenous veins, histopathological exami-

nation of a specimen taken at 6 weeks after the procedure showed inconspicuous endothelial layer, subendothelial and intravascular fibrin deposition. And 6 months after the procedure, specimen showed a dilated vein with an interrupted muscularis and elastic tissue layer [16].

There are some potential considerations with the procedure, despite reported success of EVLA. Since the amount of energy delivered is correlated to the success of the procedure, insufficient energy levels (for instance, due to low energy settings or fast pullback of the laser fiber) are unlikely to damage the vessel wall or cause vessel occlusion; or may lead to early neovascularization [17]. On the contrary, very high energy levels can cause perforations in the vessel wall and blood extravasations may cause ecchymosed, pain and brownish stains [18]. During an EVLA procedure, it is important to deliver sufficient amount of energy which will cause transmural necrosis without causing vessel perforation. In the case of insufficient delivery of thermal energy to the vessel wall, several changes can be seen in the remaining cells. In this study, in order to investigate these changes, we applied laser energy to saphenous veins *ex vivo*. Comparing with the control group, we found an increased expression of bcl-2 gene expression.

There are several limitations of the present study. First, our study showed early changes after EVLA and gene expression may change in long term. Second, since we performed laser energy *ex vivo* using isolated saphenous veins, we did not obtain any data regarding surrounding tissues. However this limitation may even add more power to the study because we think that, if the same process was applied *in vivo*, thermal damage may be less than *ex vivo* environment due to the tissues surrounding the vessels and cooling-absorbing effect of tumescent solution. It is predictable that more cells would have survived if the EVLA procedure was applied *in vivo*. Another limitation of the study is that this model does not reflect the response of saphenous vein under chronic venous insufficiency but the response of normal saphenous vein. However saphenous vein with chronic venous insufficiency might have an alerted genetic profile and when subjected to EVLA energy might show further progression of this response. This should be studied by other studies. TUNEL or Annexin V staining were not per-

formed to show the alterations in antiapoptotic mRNAs which might add further data whether the mRNA alterations might have been transient or not.

Conclusion

According to the results of molecular genetic analysis, live tissue remains after application of laser ablation as well as abnormal changes in Bcl-2 and Bax gene expressions. These data may suggest that abnormal changes in the cells after laser ablation may neoplastic differentiate into cells in the future. In addition we think that the high temperature may also affect the adjacent perivascular tissues and also damage these cells. However, these results must be supported by further studies.

Disclosure of conflict of interest

None.

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