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Histologic findings after mechanochemical ablation in a caprine model with use of ClariVein

Michael G. Tal, MD, MBA,[a] Scott J. Dos Santos,[b,c] John P. Marano, MS, PhD,[d] and Mark S. Whiteley, MS, FRCS(Gen),[b,c] *New Haven and Madison, Conn; and Guildford, Surrey, United Kingdom*

Objective: **The use of foam and liquid sclerotherapy for the treatment of varicose veins and underlying venous reflux is widespread. A novel device, the ClariVein Occlusion Catheter (Vascular Insights LLC, Madison, Conn), has been the subject of several clinical trials in humans. We report the initial histologic results obtained with use of the device in a caprine vein model.**
Methods: **A total of 11 male goats (12 veins) underwent minimally invasive procedures. Unilateral mechanochemical ablation of the lateral saphenous vein by the ClariVein Occlusion Catheter with an E-140° tip was performed under fluoroscopic guidance in five veins with 5 mL of 1.5% sodium tetradecyl sulfate (STS) and in one vein with 5 mL of 0.9% saline. The remaining six received injection sclerotherapy with 5 mL of 1.5% STS or 0.9% saline. All subjects were assessed with ultrasound before the procedure and intermittently afterward during a period of 12 weeks. Subsequent termination was immediately followed by necropsy and histologic examination of the treated veins.**
Results: **Complete occlusion of the lateral saphenous vein was observed in all subjects treated with ClariVein and STS, whereas complete patency was noted in all other treatment modalities. Histologic staining with hematoxylin and eosin and Masson trichrome stain revealed total fibrotic sealing with extensive collagen production in all ClariVein/STS veins. A statistical significance was observed in the difference in the number of occluded veins between subjects treated with ClariVein/STS and those treated by injection sclerotherapy (Fisher exact test, $P < .01$).**
Conclusions: **The ClariVein Occlusion Catheter with 1.5% STS can be used to achieve complete mechanochemical ablation of the lateral saphenous vein in a caprine model. The evidence in this report can be used to justify the device's use for the treatment of the great saphenous vein in subsequent human clinical trials. (J Vasc Surg: Venous and Lym Dis 2014;■:1-5.)**

Clinical Relevance: **This animal study shows that the combination of mechanical and chemical ablation performed by the ClariVein device results in occlusion of the normal caprine lateral saphenous vein, whereas mechanical trauma or sclerosant alone does not. This study along with the histology is essential in helping us understand how this technique works in human incompetent saphenous veins.**

The introduction of minimally invasive endovascular techniques for the treatment of chronic venous reflux during the last 15 years has completely revolutionized the field of vascular surgery. Since the introduction of the first catheter-based radiofrequency ablation (RFA) techniques involving tumescent anesthesia in the new millennium,[1] treatment methods have evolved to the point where patients are no longer hospitalized and experience significantly less if any postoperative pain.[2,3] Some procedures aimed at eliminating reflux in the great and small saphenous veins and their tributaries boast long-term success rates of more than 90%.[4]

From the Yale University School of Medicine, New Haven[a]; the Whiteley Clinic, Guildford[b]; the Faculty of Health and Medical Sciences, University of Surrey, Guildford[c]; and Vascular Insights LLC, Madison.[d]

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Reprint requests: Mark S. Whiteley, MS, FRCS(Gen), The Whiteley Clinic, Stirling House, Stirling Road, Guildford, Surrey GU2 7RF, United Kingdom (e-mail: mark@thewhiteleyclinic.co.uk).

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At roughly the same time as the emergence of these novel methods and devices, sclerotherapy experienced a renewed interest because of increased safety and efficiency made possible by duplex ultrasonography[5] and was reintroduced as a treatment for varicose veins. The transition from the use of liquid sclerosant to foamed sclerosants, pioneered by the likes of Cabrera[6] and Tessari,[7] was proved to be more efficient, with some studies reporting double the number of successful treatments with sclerosing foam.[8,9] Sclerosants such as sodium tetradecyl sulfate (STS) and polidocanol are commonly employed to fibrose the vein, preventing the venous reflux. Phlebologists routinely perform sclerotherapy worldwide, and its use is now recommended above stripping by the National Institute for Health and Care Excellence for varicose vein treatment.[10]

Despite the excellent clinical outcomes of these two techniques, both are accompanied by some significant drawbacks and various side effects, ranging from fairly minor to potentially fatal. Although patients report less pain with RFA or endovenous laser ablation (EVLA) compared with stripping,[2,3] postprocedure complications can include deep venous thrombosis,[11] paresthesia,[3] and thermal skin injury.[12] In addition, the multiple injections of anesthesia required for these interventions can be a painful stage in the procedure[13] and, understandably, may deter patients from treatment. Similarly, sclerotherapy is not free from side effects; skin necrosis,[14] skin

staining,[15] thrombophlebitis,[16] and deep venous thrombosis[17] have all been reported.

In light of these drawbacks, it was decided that a less painful technique capable of achieving the same outstanding success rates as RFA, EVLA, and foam sclerotherapy was required. The ClariVein Occlusion Catheter (Vascular Insights LLC, Madison, Conn) somewhat amalgamates the two procedures, using mechanochemical ablation (MOCA) to occlude a variety of vessels, primarily the great and small saphenous veins, without the possibility of thermal skin injury or nerve damage.[18]

Previous research including clinical trials[13] and safety evaluations[19] has been performed despite no prior published evidence to support the device's suitability for human use. To justify the use of ClariVein in human clinical trials and indeed any future comparative investigations or randomized clinical trials, we present the initial results of the use of the device in a caprine model and accompanying histologic evidence.

METHODS

Initially 18 male goats received from a farm in Upperco, Maryland, all of whom were negative for caprine arthritic encephalitis and Johne disease, were studied with different versions of this device. This report includes 11 of the animals, randomly assigned to one of the following four treatment groups:

- Group 1: ClariVein and 1.5% STS (five veins)
- Group 2: ClariVein and 0.9% saline (one vein)
- Group 3: 5-mL injection of 1.5% STS (five veins)
- Group 4: 5-mL injection of 0.9% saline (one vein)

Group 2 was a control vein for group 1, checking that any effect was not merely the result of the physical effects of the ClariVein device. Similarly, group 4 was a control for group 3, checking that any effect seen at all was not a result of intravenous injection alone. The vein to be treated, left or right, was also randomly determined. All caprine subjects were acclimated to the testing facility for 7 days before any treatment. Animal housing was arranged in appropriately sized pens and compatible groups as required by the *Guide for the Care and Use of Laboratory Animals* with a 12-hour light/dark cycle.

Duplex ultrasound examination was performed before surgery and on days 14, 28, 56, and 84 (±2 days) to assess the state of the lateral saphenous vein and (postprocedurally) the extent of occlusion, if any. Scans were performed while subjects were conscious, standing and gently restrained.

Procedures were performed under a general anesthetic. A premedication of acepromazine maleate 0.05 mg/kg intramuscularly and buprenorphine 0.005 mg/kg intramuscularly was given, followed by induction of general anesthesia with an intravenous injection of diazepam 0.22 mg/kg and ketamine 10 mg/kg. Each goat was intubated, and general anesthesia was maintained with isoflurane 0.5% to 2% delivered in oxygen through a rebreathing system. Percutaneous access to the lateral saphenous vein of the hind leg was achieved with a 16-gauge intravenous cannula. The vessel to be treated was evaluated at the time of surgery with fluoroscopy, after the injection of contrast media. The ClariVein Occlusion Catheter is fitted with a rotating tip that is at 140 degrees to the longitudinal access of the catheter. This was inserted and advanced up the vessel under fluoroscopic guidance to within approximately 2 cm proximal to the saphenofemoral junction. After the loading of a 5-mL syringe onto the device, treatment was initiated at a speed of 3500 rpm and pullback rate of around 1 to 2 mm/s, terminating at the insertion point. Syringes were filled with 5 mL of 1.5% STS (group 1) or 5 mL of 0.9% saline (group 2). Graduation marks on the catheter shaft were observed by the physician during the procedure to ensure a correct pullback rate and to aid in the optimum distribution of sclerosing liquid. After the conclusion of the procedure, the treated hind leg was wrapped along the entire treated area in a Vetrap compression bandage (3M, St Paul, Minn). A total of 12 veins in 11 goats were treated.

The injection of liquid sclerosant or saline was carried out in a manner identical to that of liquid sclerotherapy in humans; 5 mL of 1.5% STS (group 3) or 5 mL of 0.9% saline (group 4) was injected through a short peripheral catheter at the access site. After sclerotherapy, identical compression bandaging as described before was applied to the treated hind leg.

At day 84, all animals were weighed, euthanized with an overdose of sodium pentobarbital, and immediately subjected to a general gross necropsy. The lateral saphenous veins were examined and gently extracted before being placed in a formalin cup and immersed in neutral buffered formalin for examination. All veins were segmented into three equal pieces (proximal, medial, and distal), processed into standard paraffin blocks, and subject to staining with hematoxylin and eosin and Masson trichrome stain for microscopic evaluation and assessment of fibrosis.

Histologic evaluation of each vein segment was approached by analyzing the lumen and then the layers of the vein wall: the subintima, media, and adventitia.

For the lumen, the assessment was made as to whether the lumen was occluded, was patent, or showed subintimal

Table I. Grading system used for severity of fibrosis found in each layer of the vein wall and how much of each layer was affected

Grade of fibrosis	
1	Minimal
2	Mild
3	Moderate
4	Severe
Where appropriate, grades were assigned according to the following percentage of affected area:	
Grade 1	<10%
Grade 2	11%-49%
Grade 3	50%-74%
Grade 4	>75%

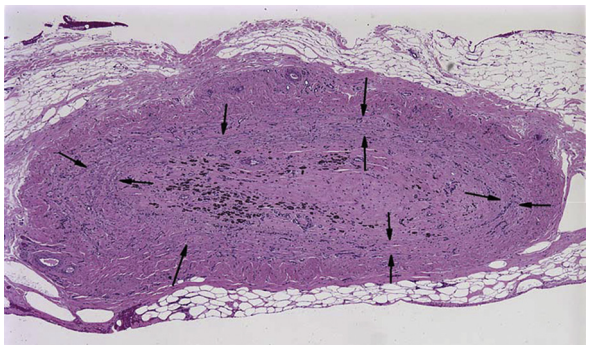

Fig 1. Histologic section of a caprine lateral saphenous vein 84 days after treatment with ClariVein and sodium tetradecyl sulfate (STS). The vein is occluded with attenuated and fibrotic media (*arrows*).

impingement reducing the vein lumen. Subintimal impingement was based on the presence of subintimal proliferation of fibrosis and whether this change resulted in the subintima's being raised and projecting into the lumen to a level that could be considered sufficient to have impinged on blood flow and the total luminal diameter.

As far as the vein wall was considered, fibrosis in each layer (subintima, media, and adventitia) was graded on a scale of 1 to 4 if present (Table I). When present, the fibrosis noted was then also classified into what percentage of area of that layer of the vein wall was affected to that level (Table I).

Histologic analysis took place at Experimental Pathology Laboratories Inc (Sterling, Va) in accordance with their standard operating procedures. Statistical analysis was outsourced to ENVIRON International (Washington, DC) and carried out on SAS v9.1.

RESULTS

All 11 goats underwent the procedure with no immediate complications; however, six presented mild swelling in one or both legs in the days after surgery. This required no intervention and resolved within 1 or 2 days, with no analgesic intervention required. Despite the swelling, no subject displayed evidence of discomfort throughout the entire study.

Duplex ultrasonography showed complete or almost complete occlusion of the lateral saphenous vein in all five goats treated with ClariVein and STS during each of the ultrasound assessments, up to 84 days. Veins treated with the other three treatment modalities showed complete patency during all of the four postprocedure ultrasound scans. The difference in the number of occluded vessels between group 1 and group 3 was found to be statistically significant at every examination (Fisher exact test, $P < .01$).

Group 1 experienced the most severe luminal and subintimal changes. Thickening of the vein wall along with endothelial denudation could be observed from the histology samples. In all five of these veins, complete fibrotic sealing and extensive new collagen production could be seen in at least one segment (Fig 1). Of the 15 vein segments from this group, 10 were found to be completely occluded compared with 0 of 15 in group 3 (Table II). Despite an equal number of diagnoses of subintimal impingement, its mean severity was markedly higher in group 1 (3.3) than in group 3 (1.8), indicating a greater reduction in lumen size. This was attributed to the increased occlusion rate in group 1 and the fact that subintimal impingement was diagnosed only in patent veins. Medial and adventitial fibrosis were also significantly higher; adventitial fibrosis and medial fibrosis were observed in 14 of 15 and 13 of 15 segments in group 1 compared with only 2 of 15 and 0 of 15 in group 3, respectively.

In comparing the two saline control groups, despite there only being one vein in each group, group 2 (ClariVein and saline) (Fig 2) not surprisingly showed more changes than group 4 (saline alone). Regardless of complete patency in all six vein segments, subintimal impingement and medial fibrosis in vein segments were both higher in group 2 than in group 4. Fibrosis of the adventitia was not observed in either saline group.

Group 2 (ClariVein and saline), the saline control of group 1, showed some subintimal impingement into the lumen and some subintimal fibrosis but considerably less that that found in group 1 (ClariVein and 1.5% STS), showing the physical effects of the rotating wire tip of ClariVein alone. Group 2 showed results similar to those of group 3, injection of STS alone. Group 4, being the control of saline injection alone, not surprisingly showed no subintimal impingements or fibrosis at all.

The overall results of all groups are shown in Tables II and III.

DISCUSSION

The venous pathologic change observed during histologic examination was notably more abundant in group 1

Table II. Occlusions in treated veins and treated vein segments

Group	*No. of veins*	*DUS-identified occluded veins*				*No. of vein segments*	*No. of occluded segments, n (%)*
		Day 14, No. (%)	*Day 28, No. (%)*	*Day 56, No. (%)*	*Day 84, No. (%)*		
1	5	5 (100)	5 (100)	5 (100)	5 (100)	15	10 (67)
2	1	0	0	0	0	3	0
3	5	0	0	0	0	15	0
4	1	0	0	0	0	3	0

DUS, Duplex ultrasonography.

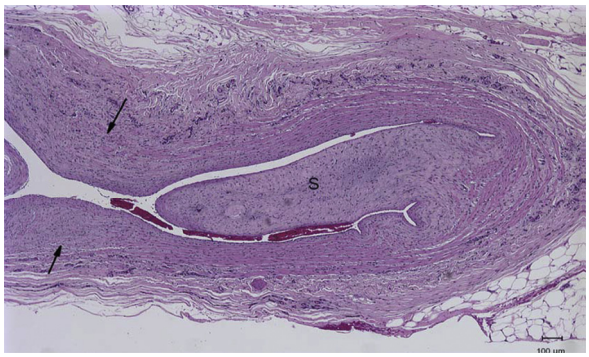

Fig 2. Histologic section of a caprine lateral saphenous vein 84 days after treatment with ClariVein and normal saline. The vein is open and shows a subintimal proliferative tag (*S*) but minimal damage in the media (*arrows*).

than in any other group. The vast differences in fibrosis and occlusion between group 1 and group 3 illustrate just how much of an effect MOCA can have as opposed to the sole use of liquid sclerosant. We are currently investigating the reason that the combination of the physical ClariVein procedure enhances the depth of effect of the STS alone, with fibrosis found out to the adventitial layer. It might be an increased inflammation in the wall or an effect of the physical damage to the vein wall, allowing deeper penetration of the sclerosant.

Similarly, virtually the same differences between group 2 and group 3 show that the mechanical properties of the ClariVein's rotating tip alone only has a similar effect as liquid sclerosant. This shows that both ClariVein and sclerosant are needed to obtain the transmural fibrosis of the vein wall and occlusion of the vein.

It was expected that saline on its own would have absolutely no effect on the vessel, and this is indeed shown by our data. In addition to this, it transpired that the mechanical action of ClariVein in conjunction with saline produced no lasting ablation whatsoever.

A comparison of two hematoxylin and eosin–stained vessel samples from groups 1 and 2 shows the superiority of MOCA over mechanical ablation alone. Fig 1 shows a completely occluded proximal segment of a lateral saphenous vein from group 1. Its lumen is effaced by fibrous tissue with newly formed vessels and hemosiderin-laden macrophages. The media is attenuated and fibrotic with increased vascular prominence. This vessel is in stark contrast with Fig 2, a group 2 vein. A subintimal proliferative tag composed of fibrous tissue and very little neovascularization can be seen. The smooth muscle of the media is more delineated to the right of the vessel, and its myofibers are more attenuated and difficult to discern.

With regard to the actual performance of the device during surgery and its ease of use, no issues such as fluid leaks or mechanical malfunctions were observed. The catheter was easily inserted and positioned in the vein smoothly; any venous obstructions encountered were successfully navigated by a slight clockwise-anticlockwise rocking motion of the device while maintaining axial distal motion. None of the procedures were affected by abnormalities or postprocedure reactions by the animals (eg, rashes or staining), and the ultrasound examinations presented little problem.

As the treatments in this study were performed under anesthesia, we are unable to comment as to pain in the goats during the procedure itself, although experience from use in humans without anesthetic is discussed later. In view of the treatment's being nonthermal and directed from the vein lumen outward, and as the fibrosis extends only into the adventitial layer and not beyond, there is no suggestion that nerve damage might be a problem with this procedure, although this cannot be proved in this study. However, subsequent use in humans has not shown this to be a problem.

Although it was not possible for us to obtain information about pain during the procedure, patients from the first clinical trial in humans reported absolutely no pain. Aside from the 1 mL of anesthetic required for the cannulation of the great saphenous vein, no further medication was requested. One patient treated with the device described the sensation during the procedure as a "vibration." As stated earlier, the unique mechanochemical action of ClariVein means that the delivery of tumescent anesthesia, deemed to be a painful or uncomfortable part of RFA or EVLA procedures, is not required. It is this aspect of MOCA that presents it as an alternative to thermal ablation for patients anxious about the pain.

One study investigating the safety of ClariVein reported that the device was safe to use and that MOCA resulted in an occlusion rate of 86%.[19] In a letter to the editor of the *Journal of Endovascular Therapy*, Lurie[20] agreed with the original authors that the device was safe but

Table III. Histologic changes in segments of treated veins

		Histologic changes in segments of vein (3 segments per saphenous vein)			
Group	*No. of vein segments, No.*	*Subintimal impingement, No. (mean)*	*Subintimal fibrosis, No. (mean)*	*Medial fibrosis, No. (mean)*	*Adventitial fibrosis, No. (mean)*
1	15	4 (3.8)	14 (4)	14 (2.9)	13 (2.0)
2	3	1 (2.0)	2 (2.5)	1 (1.0)	0
3	15	4 (1.7)	9 (1.7)	2 (1.5)	0
4	3	0	0	0	0

argued that success rates of up to 10% higher could be obtained with RFA or EVLA at the expense of increased pain to the patient. This argument is invalidated by the results of the clinical trial, which obtained an occlusion rate of 96.7%,[13] showing that MOCA can achieve the same outcome as the other techniques as well as having none of the associated immediate complications.

CONCLUSIONS

We have reported the initial histologic evidence for the use of a novel device, ClariVein, in MOCA of the lateral saphenous vein in a caprine model. Our study shows that the physical effect of ClariVein needs to be combined with a sclerosant. Compared with liquid sclerotherapy with use of STS and mechanical ablation, MOCA resulted in 100% occlusion of all veins, which remained occluded at 12 weeks, as proven by duplex ultrasonography. Further supporting research and randomized controlled trials must now be undertaken to determine if MOCA has any advantages as a treatment option over the more well established thermoablation techniques.

AUTHOR CONTRIBUTIONS

Conception and design: MT, JM
Analysis and interpretation: MT, SDS, JM
Data collection: MT, JM
Writing the article: SDS, MW
Critical revision of the article: MT, KJ, JM, MW
Final approval of the article: MT, KJ, JM, MW
Statistical analysis: MT, JM
Obtained funding: MT, JM
Overall responsibility: MT

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