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Evaluation of the Minimum Effective Concentration of Foam Sclerosant in an Ex-vivo Study

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WHAT THIS PAPER ADDS?

- This is the first preliminary ex-vivo study evaluating histopathological changes in the vein wall achieved by the use of different concentrations of foam sclerosants. Foam sclerotherapy exerts a certain amount of pathological damage even at the lowest concentration (0.5%). Therefore it seems to be a concentration-independent procedure in 5–10 mm caliber ex-vivo veins, although a 1% concentration was found to be near significantly the most injurious to the vein wall. Direct extrapolation of these findings to clinical settings needs further validation with in-vivo clinical studies.

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ABSTRACT

Background: Foam sclerosants are widely used in sclerotherapy and have been accepted as more effective than the liquid form; however, there is no consensus about the most applicable and effective concentration.
Objective: The aim of this study was to investigate the histopathological changes caused by various widely used concentrations of foam sclerosant.
Methods: Fifty-six varicose vein segments of 5–10 mm diameter were gently resected and exposed to various concentrations of foam sclerosant (0.5%, 1%, 2%, 3%) for 5 min, and were then prepared for routine histopathological examination. A total damage scoring system, including the presence of endothelial swelling, intimal thickening, cellular vacuolization in the muscle layer, edema in the tunica media and extent of necrosis, was established.
Results: The total damage score of the foam sclerosant groups was significantly higher than that of the control group (median 2.75 vs 1, $p = 0.007$). The highest damage score was achieved by 1% and 2% foam sclerosants (3.5 and 2.5). No significant difference was found among the different concentrations of sclerosant, although the 1% group caused more severe damage at a near significant level ($p = 0.074$).
Conclusion: Significant pathological damage can be caused by even the lowest doses of foam sclerosant. The most injurious concentrations were found to be 1% and 2%, morphologically. A working concentration of 1% could thus be preferable to 0.5%, especially in larger veins. Further in-vivo studies are needed in order to validate these findings.

Introduction

Sclerotherapy has been used for the treatment of varicose veins since Linser[1] first applied a liquid sclerosant, but the outcome of this method was not satisfactory. In 1944, Orbach[2,3] published the air block method in varicose vein sclerotherapy. He developed the foam form of sclerosant agent by shaking sodium tetradecyl sulfate.[3] Recently, foam sclerosants were proved to be more effective than the liquid form.[3–6] Of particular interest, in the comparative study of Hamel-Desnos et al.,[5] foam sclerosant applied to the greater saphenous vein achieved an 84% (38 of 45) success rate compared to 40% (17 of 43) from using liquid sclerosant.

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Morphologically, subendothelial edema and necrosis were observed in an in-vivo study after 1–2 ml of 3% sodium tetradecyl sulfate foam was injected into the great saphenous vein.[7] However, data about morphological changes caused by different concentrations of foam sclerosant are lacking.

In 2003 and 2006, experts on sclerotherapy met to standardize treatment with foam sclerotherapy. In 2003, it was stated that lower concentrations of foam sclerosant could be used for the treatment of varicose veins, instead of liquid sclerosant.[2] Then, in 2006, after the experts agreed that both the efficacy and tolerability of foam sclerotherapy depend on the concentration and volume injected, they published a consensus suggesting the applicable concentrations of foam sclerosant to varicose veins of different diameters. However, reliable clinical data allowing the selection of appropriate concentrations were still scarce.[8] In a randomized double-blind trial in which 148 patients with great saphenous vein reflux (saphenous trunk diameter 4–8 mm) were randomized to undergo ultrasound-guided foam sclerotherapy using either 1% or 3% polidocanol foam in a single session, clinical success rates after 3 weeks were not statistically different.[9]

We conducted a preliminary ex-vivo study investigating the degree of histopathological changes in the vein wall exerted by various widely used concentrations of foam sclerosant.

Material and Methods

Experimental design

Appropriate varicose vein segments of 5–10 mm diameter were gently resected through stab phlebectomy incisions and put into 0.9% NaCl solution. Each vein segment was divided into five equal pieces. In order to provide complete contact between foam sclerosant and the endothelium, each vein segment was cut along its full length. One of the pieces was placed in 10% formalin solution (control segment) and the rest were subjected to foam sclerosant at different concentrations. We used 0.5%, 1%, 2% and 3% concentrations of lauromacrogol 400 (Polydocanol, Kreussler & Co. GmbH). Foam was generated using the Tessari technique[10] with 2 ml of sclerosant and 5 ml of air.[11] Liquid sclerosant and air were mixed for 20 passages while the aperture in the three-way stop cock was narrowed to generate micro foam. Each piece of vessel was immersed into the selected concentration of foam sclerosant. The vein segments were soaked in sclerosant for 5 min. The specification of the duration was described elsewhere.[12] A fixed contact time of 5 min was employed because the foam was completely reverting to liquid by 287 s in 0.5%, 302 s in 1%, 362 s in 2% and 450 s in 3% concentration. After a 5-min exposure, the vein segments were washed out with isotonic saline solution and placed in 10% buffered formalin solution.

Pathological examination and grading

Fragments of varicose veins processed with different concentrations of sclerosant were fixed in 10% buffered formalin overnight and embedded in paraffin blocks separately. H&E staining was performed on the 5 micron thick cross-sections of the varicose veins. Slides were examined by a single pathologist (KK) who was blinded to the applied concentrations of sclerosant.

Microscopically, we evaluated the presence of endothelial swelling, intimal thickening, smooth muscle vacuolization, edema in tunica media and extent of necrosis. A total damage scoring system reflecting the damage to the vein wall was established: the absence of a pathological finding was scored as 0 while the presence was scored as 1. Exceptionally, necrosis was scored 0–2 depending on its extent, due to its relative importance in evaluation of tissue damage. Finally, a total damage score was calculated using the sum of scores gained from pathological findings (Table 1).

Statistics

Analyses were performed with SPSS (Statistical Package for Social Sciences) for Windows (Version 15.0.0; SPSS Inc). Using the Kolmogorov–Smirnov test, all parameters showed a non-normal distribution. Intergroup comparison of quantitative data was performed using the Kruskal–Wallis test and the parameter leading to difference was investigated by the Mann–Whitney *U*-test. $P < 0.05$ was regarded as significant. Chi square and Fisher's exact test were used to compare qualitative variables.

Results

Diameters of 56 varicose vein segments ranged between 5 and 10 mm. Control, 0.5%, 1% and 2% groups consisted of 12 vein segments, whereas 8 vein segments were available for the 3% group.

The distribution of histological findings among control and different concentration groups is summarized in Table 2. In the control group, the morphological examination of vessel sections was near normal (Fig. 1B). The sclerosant group (all concentration groups together) showed some of the investigated pathological changes, including endothelial swelling (Fig. 1A), necrosis (Fig. 2) and intimal thickening (Fig. 3) more frequently than the control group (Table 2). Vacuolization (Fig. 4) and edema did not differ significantly. When we compared the different concentration groups, there was no statistically significant difference regarding the presence of a single histological parameter (endothelial thickening, necrosis, vacuolization, edema, intimal thickening), except for the presence and extent of necrosis, which is more likely to be encountered with higher concentrations of foam sclerosant (Fig. 2).

Median damage score of the sclerosant group was significantly higher than that of the control group (2.75 vs 1, $p = 0.007$). The highest damage score was achieved by 1% and 2% sclerosants; however, divergent concentrations of sclerosant ranging between 0.5% and 3% did not alter morphology significantly (Table 3). It should also be mentioned that 1% foam sclerosant was the most effective concentration, exerting the most severe damage on the vein wall at a statistically near significant level ($p = 0.074$, compared to 0.5%).

Discussion

In this first ex-vivo study to compare the morphological effects of different foam sclerosant concentrations, we have found that

Table 1
Pathological grading system.

Pathological findings		Score
Endothelial swelling	Absent	0
	Present	1
Necrosis	Absent	0
	Focal	1
	Widespread	2
Edema of tunica media	Absent	0
	Present	1
Vacuolization of smooth muscle	Absent	0
	Present	1
Intimal thickening	Absent	0
	Present	1
Total damage score		**0–6**

Table 2
Distribution of histopathological findings.

Histological parameters		Total *n* (%)	Control *n* (%)	Sclerosant *n* (%)	*P*[a]	0.5% *n* (%)	1% *n* (%)	2% *n* (%)	3% *n* (%)	*P*[b]
Endothelial swelling	Absent	32 (57)	10 (83)	22 (50)	*.040*	8 (67)	4 (33)	6 (50)	4 (50)	*.136*
	Present	24 (43)	2 (17)	22 (50)		4 (33)	8 (67)	6 (50)	4 (50)	
Necrosis	Absent	25 (45)	9 (75)	16 (36)	*.013*	6 (50)	3 (25)	5 (42)	2 (25)	*.086*
	Focal	23 (41)	3 (25)	20 (46)		6 (50)	5 (42)	4 (33)	5 (63)	
	Widespread	8 (14)	0 (0)	8 (18)		0 (0)	4 (33)	3 (25)	1 (12)	
Vacuolization	Absent	34 (61)	10 (83)	24 (55)	*.073*	7 (58)	6 (50)	6 (50)	5 (63)	*.438*
	Present	22 (39)	2 (17)	20 (45)		5 (42)	6 (50)	6 (50)	3 (38)	
Edema	Absent	33 (59)	9 (75)	24 (55)	*.206*	6 (50)	4 (33)	8 (67)	6 (75)	*.194*
	Present	23 (41)	3 (25)	20 (45)		6 (50)	8 (67)	4 (33)	2 (25)	
Intimal thickening	Absent	36 (64)	11 (92)	25 (57)	*.045*	7 (58)	5 (42)	8 (67)	5 (63)	*.146*
	Present	20 (36)	1 (8)	19 (43)		5 (42)	7 (58)	4 (33)	3 (37)	

[a] Control vs sclerosants combined, Mann–Whitney *U*-test.
[b] Control vs different concentrations of sclerosant (0.5%, 1%, 2% and 3%), Kruskal–Wallis test.

significant pathological changes tend to occur independently from the concentration of foam sclerosant, starting from even the lowest concentration of 0.5%. At the Second European Consensus Meeting on Foam Sclerotherapy, experts came together to standardize the process of foam sclerotherapy.[8] According to participants' personal experiences, some suggestions were given for the two most commonly used sclerosant agents, polidocanol and tetradecyl sulfate. They pointed out that the diameter of the vein is an important determinant for the selection of the appropriate concentration of foam sclerosant. On the other hand, there are some controversial data about the importance of concentration. Hamel-Desnos et al.[9] reported in a functional in-vivo study that there was no statistically significant difference between the clinical success rates of 1% and 3% polidocanol foam after a 2-year follow-up. Similar results were also noted by Ikponmwosa et al.[12] Our data mainly agree with those of Ikponmwosa et al. and Hamel-Desnos et al. in indicating that the effectiveness of foam sclerotherapy is independent of the concentration used, as even the lowest concentration led to significant damage to the vein wall. It is also worth mentioning that our study demonstrated 1% foam sclerosant to be the most effective concentration in terms of the pathological damage it exerted, compared to the higher doses of 2% and 3%. These results suggest that the applied concentration of foam sclerosant can be adjusted as low as possible, regardless of the caliber of the varicose veins, since the concentration used did not significantly alter the severity of morphological damage and thus possibly the clinical outcome. Furthermore, using a low concentration of sclerosant may contribute to a decrease in complications and the total cost of the procedure. However, these results should be validated by clinical studies, since the usage of clinically ineffective concentrations may lead to re-treatment and drive the cost up.

Histomorphological changes caused by sclerotherapy were previously described by Orsini and Brotto.[7] In their in-vivo study, 3% sodium tetradecyl sulfate was injected into the proximal portion of the great saphenous vein. The saphenous tract was divided into three portions and removed after 2, 15 and 30 min for histopathological examination. Complete absence of endothelial cells and diffuse subendothelial edema were seen on vein walls even after 2 min. The saphena sections taken after 15 min showed larger areas of necrosis, also involving tunica media, and after 30 min early venous sclerosis, early parietal cover and shrinkage of the lumen were seen. However, these histological changes represent not only the effects of foam sclerosant but also the liquid form, since Ikponmwosa et al.[12] realized that foam sclerosant couldn't remain stable and completely reverted to liquid form within 300 s. Similarly, in our study, we realized that the foam completely reverted to liquid in a given amount of time for each concentration, and set the contact time of the experiment accordingly. In the study of Orsini and Brotto, histopathological changes seen at 15 and 30 min represented the cumulative effect of foam sclerosant and mostly the reverted liquid form without any foam left. In the current study, the pathological vascular changes were solely due to foam sclerosant or foam plus the reverted liquid form, but not to the liquid form without foam.

On histopathological examination, we realized that multiple findings were encountered in the same vessel wall, even in different sections. Therefore, we established a scoring system to sum up all histopathological data and quantify the

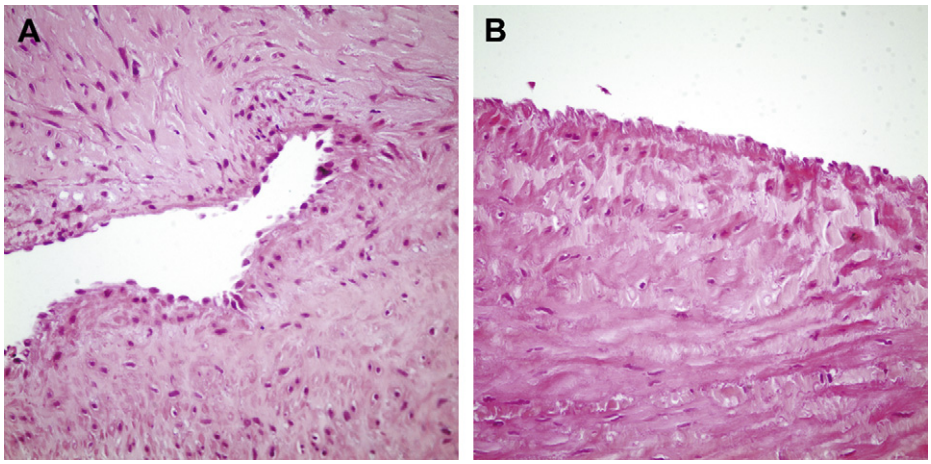

Figure 1. Note that endothelial nuclei of 2% sclerosant group (A) are hyperchromatic, enlarged and show hobnail-like extension toward lumen, compared to normal endothelial lining (B). H&E, 400×.

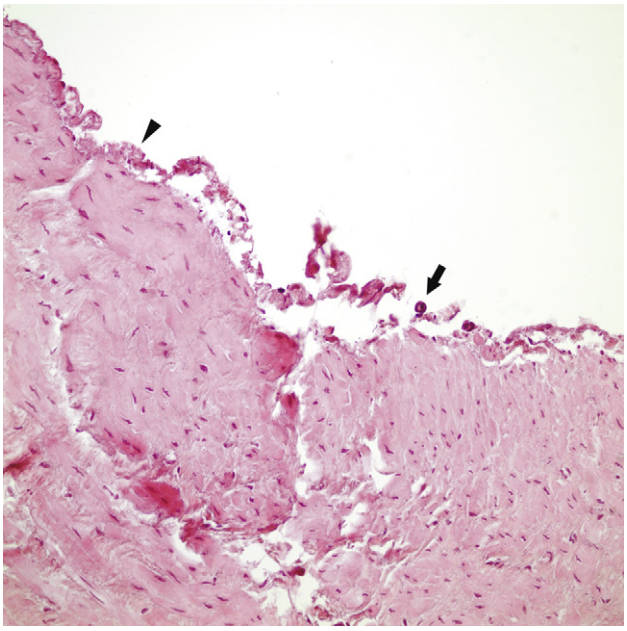

Figure 2. Prominent widespread necrosis is seen along the luminal border characterized by fibrin (arrowhead) and apoptotic endothelial cells (arrow). Necrosis was more likely to be encountered in vessels exposed to sclerosant. H&E, 200×.

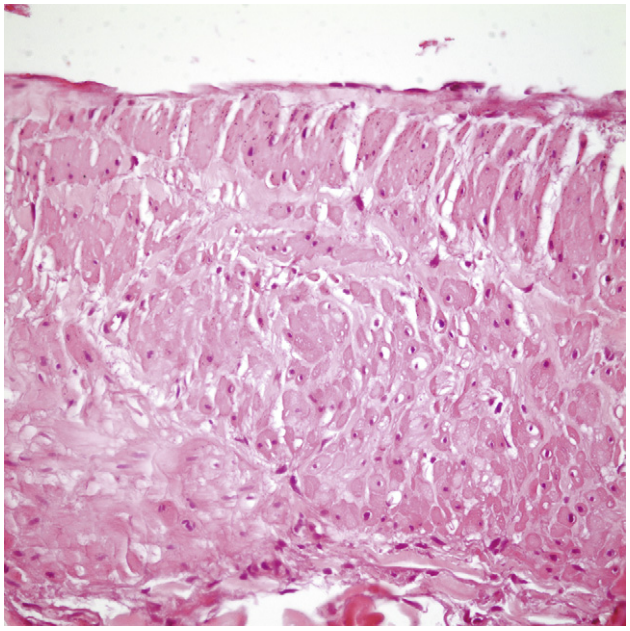

Figure 4. Vacuolization of smooth muscle cells. H&E, 400×.

histopathological outcomes of the control and foam sclerosant groups. Quantitatively, no statistical significance was determined among the foam sclerosant groups of different concentrations, although we obtained a significant difference between the control and foam sclerosant groups overall.

There are several limitations to our study. First of all, as soon as we removed the varicose veins for the application of sclerosant ex-vivo, we inevitably ignored the physiochemical effects of blood, which certainly affect the contact between foam sclerosant and vein wall, so we were unable to assess clinical outcomes directly. The conditions would be extremely different in the clinical situation, where blood is flowing through the vein and only the endothelium, instead of the whole vein wall, is subjected to foam sclerosant. Even so, we are also aware of the studies that revealed the insignificance of clinical outcome differences between foam concentrations.[9,12] In addition, at the consensus meeting in 2006,[6] experts listed other important determinants, such as access location, access material, preparation of foam, and especially foam volume per injection, that may directly affect the success of the process. Secondly, sclerosant was applied to the vein segment after its removal from the patient. Even though we tried to bring the vein segment and sclerosant together as soon as possible, there could

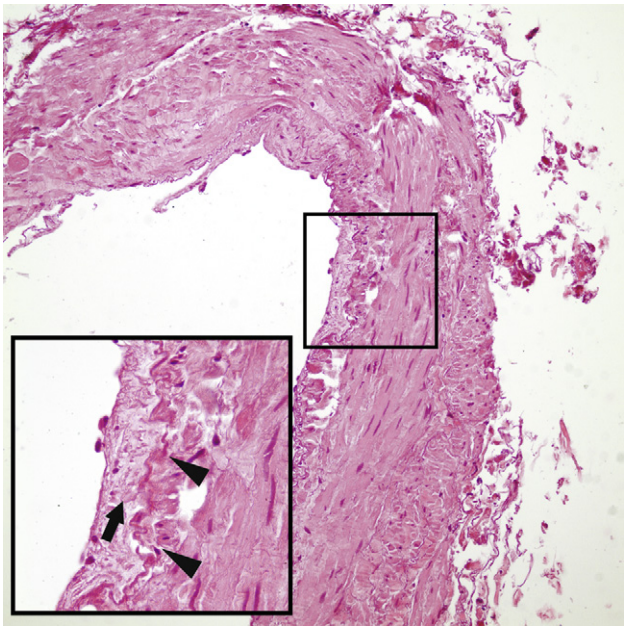

Figure 3. Intimal thickening (arrow) with edema is accompanied by prominent endothelial swelling and irregularity in elastic lamina (arrowheads). H&E, 200×; inlet H&E, 1000×.

Table 3
Comparison of total damage scores between control and all sclerosant concentrations.

	Total Damage Score			
	Mean ± SD	Median	[a]*P*	
Control	0.91 ± 0.90	1		*.007*
0.5%	2.16 ± 1.52	2	*.272*	
1%	3.50 ± 1.83	3.5		
2%	2.50 ± 1.56	3		
3%	2.37 ± 1.50	2.5		

Control − 0.5% [b]$P = 0.032$
Control − 1% [b]$P = 0.001$
Control − 2% [b]$P = 0.013$
Control − 3% [b]$P = 0.023$
0.5% − 1% [b]$P = 0.074$
0.5% − 2% [b]$P = 0.536$
0.5% − 3% [b]$P = 0.752$
1% − 2% [b]$P = 0.197$
1% − 3% [b]$P = 0.169$
2% − 3% [b]$P = 0.751$

[a] Kruskal–Wallis test.
[b] Mann–Whitney *U*-test.

still be unpredictable events, such as ischemic or hypoxic situations and lack of effective contact of sclerosant with vein wall, which could alter the results. Thirdly, as the details of the mechanism by which foam sclerosants act on vessel walls are not well known, minor differences between experimental models are inevitable. For example, we applied foam sclerosant for 5 min as described before.[12] However, it has been reported that the half time of polidocanol foam is around 100 s for 0.5% and 1% concentrations, suggesting that a shorter duration of foam application is required.[13] Wollmann[14] also pointed out that the stability, and thus the effectiveness and safety, of the foam depends on not only the foam half time, but also the drainage time and coalescence time, and therefore the duration of foam stability remains below 50 s under the optimum air-to-liquid ratio of foam sclerosant. Even syringe size may affect the properties of foam.[15] As a result, it would be interesting to see the morphological results of an in-vivo study, considering the variables mentioned above, summarized mainly from the studies of Rao and Goldman[13] and Wollmann.[14] Lastly, there are well-known limitations of histopathological examination studies. Nevertheless, this is the first study to compare the effects of different concentrations of foam sclerosant, and we believe that these results will be preliminary for future experiments.

Conclusion

In routine practice, a 0.5% or 1% concentration of foam sclerosant is preferred for vessels less than 5 mm in diameter, while 2% and 3% concentrations are used for vessels larger than 5 mm in diameter. In the current ex-vivo study, we were unable to demonstrate any statistically significant results among different foam concentrations on 5–10 mm diameter vessels in terms of pathological damage. However, due to the near significant difference between the outcomes of 0.5% and 1% foam sclerosants, the use of 1% foam sclerosant instead of 0.5% may be preferable. Again, 1% foam sclerosant may be preferred to 2% or 3% in larger vessels, as it exerts more severe damage on the vein wall. Further studies are necessary to validate our findings.

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Conflict of Interest/Funding

None.

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