Comparative Stability of Sodium Tetradecyl Sulphate (STD) and Polidocanol Foam: Impact on Vein Damage in an In-vitro Model

B. McAree, A. Ikponmwosa, K. Brockbank, C. Abbott, S. Homer-Vanniasinkam, M.J. Gough

Leeds Vascular Institute, The General Infirmary at Leeds, Great George Street, Leeds LS1 3EX, UK
Institute of Pharmaceutical Innovation, University of Bradford, UK

WHAT THIS PAPER ADDS

This study provides histological evidence in an in vivo model to support the clinical impression that sodium tetradecyl sulphate is more effective than polidocanol for the sclerotherapy of varicose veins. Further it shows that these sclerosants, particularly polidocanol cause incomplete endothelial cell loss and minimal injury to the media thus providing a rational explanation for venous recannalisation and treatment failure.

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ABSTRACT

Objectives: To compare the half-life of STD and Polidocanol air-based foams and the damage they inflict upon human great saphenous vein in an in-vitro model.

Methods: The time for the volume of 3% STD and polidocanol foams to reduce by 10% (T90) and 50% (T50) was recorded in an incubator at 37°C. Segments of proximal GSV harvested during varicose vein surgery were filled with foam for 5 or 15 min. Histological analysis determined percentage endothelial cell loss and depth of media injury.

Results: Median (±IQR) T90 and T50 for polidocanol were 123.3 s (111.7–165.6) and 266.3 s (245.6–383.1) versus 102.03 s (91.1–112) and 213.13 s (201–231.6) for STD (T90 p = 0.008, T50 p = 0.004). Median endothelial loss with polidocanol was; 63.5% (62.2–82.8) and 85.9% (83.8–92.5) versus 86.3% (84.8–93.7) and 97.64% (97.3–97.8) for STD after 5 and 15 min (p = 0.076 and p = 0.009). The median depth and % media thickness injured were 0 (0) and 0% for both assessments with polidocanol versus 37.4 μm (35.3–45.8 and 43.4 μm (42.1–46.7) and 3.5% (3.1–3.6) and 5.3% (3.7–6.0) after 5 and 15 min for STD (p < 0.01 for all comparisons).

Conclusion: Although polidocanol foam shows greater stability than STD foam perhaps remaining in the vein for longer, endothelial cell loss and damage to the media were significantly greater with STD.

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guided foam sclerotherapy (UGFS) has become common-place in the management of varicose veins.\textsuperscript{12}

Foam prepared using the Tessari technique displaces blood from the vein and increases both the duration and surface area of contact between the sclerosant and the vein wall.\textsuperscript{6,13} Effective sclerosis (fibrosis) of a vein requires endothelial destruction and exposure of sub-endothelial collagen fibres to the sclerosant. This initiates the intrinsic pathway of blood coagulation by activating factor XII. Nevertheless the aim of sclerotherapy is not to merely achieve vein thrombosis, which may be amenable to recanalisation, but to achieve transformation into a fibrinous cord.\textsuperscript{14}

The latter is more likely to occur where the damage inflicted upon the vein wall involves both the endothelium and the sub-endothelial medial layer. Whilst this has been demonstrated with other minimally invasive treatments (endovenous laser ablation [ELVA] and radiofrequency ablation [RFA]) that are associated with high ablation rates, little is known about the ability of either STD or polidocanol to achieve this.\textsuperscript{5,16} Further, foam sclerotherapy has early recanalisation rates of up to 32\% as well as poor medium to long-term occlusion rates compared to these other minimally invasive treatments.\textsuperscript{17,18} This suggests that the principle mode of action of these agents is to promote thrombotic occlusion rather than permanent vein wall injury. Although there is much work describing the in-vitro effects of sclerosants on coagulation and cultured cell lines using normal arterial or super-ficial venous tissue there is minimal data on their effect on the superficial veins of patients with varicose veins.\textsuperscript{19–22} Further there is no literature comparing the effects of STD and polidocanol on human veins.

The aim of this study was to compare both the stability and degree of injury inflicted by STD and polidocanol in an in-vitro model using incompetent GSV.

Materials/Methods

**In-vitro half-life of 3\% STD and 3\% polidocanol foams**

Foam was prepared using Tessari’s technique (1:3 ratio of sclerosant:air) with 20 passes through a double syringe system and immediately transferred to a preheated (37 °C) 15 ml graduated polyester tube. The initial foam volume was recorded and subsequent readings were taken every 30 s until the volume of the foam fell below 2 mls. Experiments were conducted at 37 °C (Stuart Incubator S16D, Bibby Scientific Ltd, Staffordshire, UK). 10 runs were performed for both 3\% STD and 3\% polidocanol.

Previous research has shown that the rate of destabilisation of foam is non-linear with periods of apparent stability before sudden decreases in the volume of foam.\textsuperscript{23} Further, the initial volume of the foam varied slightly between experiments. Thus, rate of change in foam volume cannot be used as a measure of foam stability. Instead the experimental data was converted to percentage of foam varied slightly between experiments. Thus, rate of change in foam volume cannot be used as a measure of foam stability.

**In-vitro human great saphenous vein wall treated with STD and polidocanol**

3–5 cm segments of proximal GSV were harvested prior to stripping from patients undergoing surgery for primary varicose veins with documented evidence of sapheno-femoral junction (SFJ) incompetence and STD reflux on ultrasound. A clip was placed on one end of the test vein that was then filled with either 3\% STD or polidocanol foam. Each sclerosant was tested blindly (coded A or B). A second clip was then used to occlude the other end of the segment. Control samples of the same vein were filled with the patient's blood (heparinised) to account for potential mechanical or pressure effects of fluid in the lumen of the vein. Test and control sections were simultaneously placed in 2 mls of patient’s heparinised blood and left in-situ for either 5 or 15 min. Five veins were tested with each sclerosant for both durations. After the prescribed time the veins were flushed with heparinised blood and the mid-portion of each vein (avoiding the clipped ends) was immediately fixed in 10% buffered formal saline and subsequently sectioned and stained with haemotoxylin and eosin. Each sample was allocated a random number (random number generator) to ensure blinding during histological analysis.

Histological analysis was performed by a consultant cardiovascular pathologist who was blinded in respect of whether samples were controls or experimental, the duration of the experiment and the type of sclerosant. 20 × magnification sections were analysed using an Aperio ImageScope v.10.2.2.2319 (Aperio Technologies, Inc. Vista, CA, USA) to determine the percentage luminal endothelial loss and the depth of injury to the media.

To determine the extent of endothelial cell loss the luminal circumference and the length of endothelial cells remaining were measured using the Aperio "Pen Tool." Endothelial cell loss was calculated as a percentage of the luminal circumference without an endothelial layer.

The depth of medial injury (μm) and the total depth of the media were assessed at the “12 points” of a clock-face in each section and both the absolute depth of injury (the depth over which sub-endothelial vacuolation extended from the intima) and the % depth of injury were determined. Sub-endothelial vacuolation was characterised by swollen, pale smooth muscle cells, with unraveling of the nucleus. Two sections from each vein (providing 24 measurements for each parameter) were examined and the median (±IQR) depth and % depth of injury determined.

**Statistical Analysis**

Analysis was performed using PASW (SPSS, Statistical Package for Social Sciences Inc, Chicago, Illinois, USA) version 18.0. The Mann–Whitney U Test was used to compare data. A p value of <0.05 was considered significant.

**Results**

- **In-vitro half-life of STD and polidocanol foams**

  $T_{90}$ and $T_{50}$ values are shown in the Table 1 and Fig. 1. The median $T_{90}$ and $T_{50}$ for polidocanol were significantly longer than those for STD ($p = 0.008$ and $p = 0.004$ respectively). Thus 3\% polidocanol shows greater stability than 3\% STD foam.

- **In-vitro GSV injury following exposure to STD and polidocanol**

  Tables 2 and 3 summarise the results for STD and polidocanol at 5 and 15 min.

  Mean endothelial loss (Fig. 2) after exposure to polidocanol was less than that for STD ($p = 0.076$ and $p = 0.009$ respectively). By comparison median endothelial cell loss in controls was negligible (5.7% and 7.26%).

<table>
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<th>Table 1</th>
<th>Median $T_{50}$ and $T_{90}$ Values for STD and polidocanol 3% foams (10 measurements).</th>
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<tr>
<td>STD</td>
<td>Polidocanol</td>
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<tr>
<td>$T_{50}$ (sec)</td>
<td>231.1 s (201–231.6)</td>
</tr>
<tr>
<td>$T_{90}$ (sec)</td>
<td>123.3 s (111.7–165.6)</td>
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\[p = 0.004\]
Similarly the median depth of media injury (and % of media thickness injured) was greater with STD compared to polidocanol (median depth of injury at 5 and 15 min: p < 0.01 for both; % median thickness injury at 5 and 15 min: p < 0.01 for both). No medial injury occurred in controls. These data are shown in Figs. 3 and 4.

Fig. 5 shows a histology slide of GSV treated for 15 min with 3% STD foam confirming injury to the superficial part of the media whilst Fig. 6 (3% polidocanol for 15 min) shows intact endothelium with no discernible media injury. This slide is typical of those for polidocanol.

**Discussion**

Despite 150 years of unregulated human experimentation encompassing a range of more or less toxic agents, the “perfect sclerosant,” free from complications and causing permanent vein occlusion has not been found. Thus, currently available sclerosants represent a compromise between efficacy and toxicity.1

The desired functions of sclerosing agents are the destruction of venous endothelial cells and exposure of sub-endothelial collagen fibres, and ultimately fibrotic occlusion of the treated vein. Polidocanol and STD are detergent agents containing a hydrophilic and a hydrophobic pole. They act by altering the surface tension around endothelial cells. The hydrophobic pole binds to the cell surface, whereas the hydrophilic portion attracts water into the cell, resulting in a rapid and intense cell hydration.2

Endothelial damage is enhanced by greater concentrations of sclerosant and this would be consistent with a similar clinical efficacy for both sclerosants despite the greater vein wall injury inflicted by STD in this model. One potential mechanism for enhanced in vivo injury might be vein wall hypoxia following thrombosis.

There are few studies detailing the pathological changes induced by sclerotherapy on varicose veins. Orsini et al.32 described changes in the GSV vein wall treated with 3% STD (in-vivo), reporting complete loss of endothelium after 2 min with sub-endothelial oedema developing after 15 min exposure to STD. Ikponmwosa et al.22 found almost complete endothelial cell loss after exposure to 1% and 3% STD after 2 min but with minimal sub-endothelial damage and no collagen disruption. These findings for STD would seem consistent with those of this study and at the very least a sclerosant which causes maximal endothelial destruction is likely to have a longer lasting therapeutic effect. Nevertheless even

<table>
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<td><strong>Vein wall injury data for STD and polidocanol at 5 min.</strong></td>
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<td>Median endothelial cell loss (%)</td>
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<tr>
<td>Median depth of injury (µm)</td>
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<td>Median depth of injury as % of media thickness (%)</td>
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**Table 3**

| Median endothelial cell loss (%) | 97.6 (97.3–97.9) | 85.9 (83.8–92.5) | 0.009 |
| Median depth of injury (µm) | 43.4 (42.1–46.7) | 0 (0–0) | 0.005 |
| Median depth of injury as % of media thickness (%) | 5.3 (3.7–6.0) | 0 (0–0) | 0.007 |

and EVLA. These induce heat-dependant changes in collagen in the vein wall with loss of periodicity, dissolution and fusion of fibres, coagulation of collagen bundles and shrinking of smooth muscle, resulting in vessel contraction.25–27

Duplex ultrasound follow-up after truncal vein foam sclerotherapy demonstrates recanalisation and recurrent reflux in 20–32% of limbs at 1–3 years, compared to a rate of 1–16% following RFA and EVLA.28–30 It is likely that the more favourable early and mid-term occlusion rates offered by these techniques are the result of a greater damage inflicted upon the vessel wall. It therefore appears that denaturing collagen is necessary for sustainable vessel closure.31

Early treatment failure following sclerotherapy is associated with recanalisation of the treated vein. On the basis of this work it seems likely that the initial success of sclerotherapy is associated with simple thrombotic occlusion and that recanalisation is promoted by persisting islands of endothelial cells and the absence of significant fibrosis due to the failure to inflict a significant injury to the media.

Although this hypothesis seems logical this study only assesses the immediate impact of sclerotherapy in an in vitro model. It is possible that sclerotherapy may cause greater tissue damage in vivo and this would be consistent with a similar clinical efficacy for both sclerosants despite the greater vein wall injury inflicted by STD in this model. One potential mechanism for enhanced in vivo injury might be vein wall hypoxia following thrombosis.

![Figure 1. T90 and T50 (sec) for 3% STD and 3% polidocanol foams.](image1)

![Figure 2. Percentage endothelial loss with 3% STD, 3% polidocanol and heparinised blood (controls).](image2)
when complete endothelial loss is achieved re-endothelialisation may still occur via circulating endothelial progenitor cells.\(^{33,34}\)

Our results also show that 3% polidocanol foam lasts longer than 3% STD foam with a median difference of 21.3 s and 53.2 s for \(T_{90}\) and \(T_{50}\) respectively. Similar differences between the longevity of the respective foams have been found by others using different strengths of these sclerosants.\(^{35}\) This may be related to the larger molecular size of polidocanol thus enhancing its surfactant and foaming properties. This ought to be beneficial given that foam sclerotherapy is considered more efficacious than liquid sclerotherapy because of a longer contact time between the sclerosant and the endothelial cells.\(^{13}\) However, Parsi et al. found that the therapeutic effect of a sclerosant appears to occur in the first few seconds after injection.\(^{19}\) Thus it is possible that as long as the active drug is distributed to the target area, longer exposure may not significantly increase its efficacy. Certainly our results suggest that the longevity of the foam matters less than the active ingredient as evidenced by the difference in sub-endothelial damage between the two agents.

The mean depth of injury and the percentage of media injured were significantly higher for STD compared to polidocanol. Although there are no other histological studies with which to compare these findings they would support the clinical view that STD is a more potent sclerosant than polidocanol with higher concentrations of the latter necessary to produce the same effect.\(^{3,5,35,36}\) Nevertheless the EASI study showed that polidocanol 0.5% and 1% were as effective as STD in the treatment of patients with reticular and spider veins, and that more patients were satisfied with the effect of polidocanol because of more adverse events with STD.\(^{14}\) Similarly, Goldman treated patients with varicose and telangiectatic veins (none with truncal vein incompetence) with either polidocanol (0.5%, 1%) or STD (0.25%, 0.5%) and observed that polidocanol and STD were equally effective although tissue necrosis and swelling was less common in the polidocanol group.\(^{4}\) Rao et al., in the only study using foam also found that the two sclerosants had similar efficacy, tolerability and patient satisfaction.\(^{5}\)

Although the findings of this study seem robust it has a number of shortcomings. These include the relatively small number of veins tested and its in-vitro design. Ethical considerations make in-vivo humans studies impossible. Whilst animal models could be used for in-vivo research expense would preclude large experimental numbers. In addition there is no established animal model with SFJ and GSV incompetence.

One advantage of the model used in this study is that it can easily be used in the initial assessment of longer lasting or novel sclerosants.
Conclusions

Although polidocanol foam shows greater stability than STD foam and may thus remain in the treated segment of vein for longer (in vivo), damage to the media is significantly greater with STD.

Conflict of Interest

None declared.

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References