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The Lytic Effects of Detergent Sclerosants on Erythrocytes, Platelets, Endothelial Cells and Microparticles are Attenuated by Albumin and other Plasma Components *in Vitro*

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Abstract *Objective:* To investigate the lytic effects of sodium tetradecyl sulphate (STS) and polidocanol (POL) on erythrocytes, platelets, endothelial cells and platelet-derived microparticle (PDMP) formation *in vitro* and the potential protective effects of serum albumin and agents such as procaine.
Materials and methods: The effects of sclerosants were studied in blood samples obtained from normal individuals. Absorbance densitometry was used to assess the lytic effects of sclerosants on blood cells and cultured human microvascular endothelial cells (HMEC) in plasma and in saline. PDMP were quantified by flow cytometry.
Results: Haemolysis occurred in whole blood at sclerosant concentrations greater than 0.25% for STS and above 0.45% for POL. Similar concentrations of both agents caused platelet and endothelial cell lysis. Both sclerosants released PDMP at low concentrations but destroyed PDMP at higher concentrations. Albumin significantly reduced the lytic effect of both sclerosants on all cells but had a greater inhibitory effect on POL. Protamine at 0.01% had a neutralising effect on STS, whereas procaine and lignocaine showed no such activity.
Conclusions: Sclerosants at therapeutic concentrations lyse blood cells and endothelial cells *in vitro*. This effect is strongly reduced by serum albumin possibly contributing towards the low incidence of thromboembolic complications of sclerotherapy.

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Introduction

Detergent sclerosants such as sodium tetradecyl sulphate (STS) and polidocanol (POL) are widely known to have lytic effects on endothelial cells.[1] The destruction of the endothelial lining of the vessel wall leads to exposure of the sub-endothelial collagen and a cascade of events that can either lead to endovascular sclerofibrosis, sclerothrombosis or even thrombophlebitis.[1]

Despite the acknowledged lytic effects that these drugs have on endothelial cells, little is known about their effects on circulating blood cells and platelets. The related literature remains contradictory with some authors reporting these sclerosants to be non-haemolytic *in vitro*[2] while others have reported them to cause haematuria[3] or haemoglobinuria[2,4] when used clinically in high volumes. In this study, we investigated the haemolytic effects of these agents *in vitro*.

We have previously reported on the effects of detergent sclerosants on clotting times, clotting factors, platelets and formation of platelet-derived microparticles (PDMP).[5] At low concentrations, both drugs shorten phospholipid sensitive clotting times and demonstrate procoagulant properties *in vitro*. This is achieved by damage to platelet membranes and the release of procoagulant PDMP. At high concentrations, STS destroys platelets and PDMP, prolongs the clotting times and demonstrates anticoagulant properties *in vitro*. By contrast, high concentration POL does not prolong the clotting times to the same extent.[5]

Clinically, the sclerosing effects of these drugs are thought to be limited to a short distance and usually more than a single injection is required to sclerose a vein segment which is more than a few centimetres long. The lack of distal sclerosing power has been presumed to be due to dilution of these agents in blood.[1,6] Similarly, the low thrombotic complication rate of sclerotherapy and the rarity of distal complications has been presumed to be due to dilution effects. Here, we investigated the possibility of a neutralising interaction between detergent sclerosants and albumin and human plasma.

Another rare but important complication of sclerotherapy is tissue necrosis and skin ulceration.[1,7] The local anaesthetic Procaine has been thought to bind and neutralise STS and is used clinically if impending STS induced tissue necrosis is suspected.[8] We also investigated the presumed protective benefit of Procaine and similar agents such as lignocaine hydrochloride and protamine sulphate in an *in vitro* setting.

Materials and Methods

Sample collection

Blood from normal healthy volunteer donors was obtained by clean venepuncture and collected in Vacutainer tubes (Becton Dickinson, USA) containing 0.109 M trisodium citrate.

Sclerosants and other agents

STS was obtained as FIBRO-VEIN 3% (Australian Medical and Scientific Limited, Chatswood, NSW, Australia) and POL as AETHOXYSKLEROL 3% (Chemische Fabrik Kreussler & Co, GMBH, Wiesbaden, Germany). Bovine serum albumin (BSA) was obtained from Bovogen (Melbourne, Vic, Australia). 20 g of BSA was dissolved in 100 ml of saline to produce a stock solution. A 4% physiologic concentration of albumin was used in these experiments. Procaine hydrochloride 2% was obtained as PROCAINE HYDROCHLORIDE INJECTION (DBL) from Mayne Pharma International (Melbourne, Vic, Australia). Lignocaine hydrochloride 2% was obtained as XYLOCAINE AMPOULES (PLAIN) INJECTION from AstraZeneca Pty Ltd (North Ryde, NSW, Australia). Protamine sulphate 1% was obtained as PROTAMINE SULPHATE INJECTION BP from Aventis Pharma Pty Limited Sanofi-Aventis Group (Macquarie Park, NSW, Australia).

Sample preparation

Preparation of Platelet Rich and Platelet Poor Plasma. Platelet rich plasma (PRP) was prepared by centrifugation of whole blood samples at 150 g for 10 minutes. Platelet poor plasma (PPP) was obtained by centrifugation of citrated blood at 1500 g for 20 minutes. Platelet counts were carried out with a Cell-Dyn 4000 (Abbott Diagnostics Division, Santa Clara, CA, USA).

Preparation of Washed Red Cells. Washed red cells were obtained by centrifugation of citrated whole blood. PPP was removed and the remaining red cells were mixed with a large volume of saline followed by recentrifugation at 2000 g for 15 minutes. The supernatant was then discarded and the washed red cells were resuspended in a volume of saline equal to the packed cell volume of 50%.

Preparation of Washed Platelets. PRP was centrifuged for 15 minutes at 3000 g to sediment platelets which were then pooled and washed in 20 ml total volume of 0.15 M NaCl, 0.01 M HEPES, 0.001 M EDTA pH 7.4 solution (diluting buffer). After centrifuging down again at 3000 g for 15 minutes and discarding the supernatant, the washed platelets were resuspended in 4.5 ml of HEPES-buffered saline. This was described as ''10×'' washed platelet suspension because it would have contained most of the original platelets in 1/10th the volume of the blood (45 ml).

Haemolysis studies

A) Detection of Haemoglobin Released From Lysed Red Blood Cells. The haemolytic effect of sclerosants was assessed by the addition of plasma containing various concentrations of sclerosants to 1/10th its volume of sedimented red blood cells that had been centrifuged down in microwells. Red blood cells were sedimented in microwells by centrifugation. The supernatant was removed and the pellet resuspended in plasma containing various concentrations of sclerosants. The sclerosants were dispersed into the plasma component before resuspending the red cells to avoid excessive localised lytic effects of high concentration sclerosants. After a ten minute incubation, the microplate was again centrifuged. The supernatant was liberated and transferred to another microplate. Free haemoglobin was then measured using a microplate reader (Titertek Multiskan MCC, Finland) at an absorbance of 520 nm.[9] Absorbances were converted to percent

haemolysis by interpolating onto a standard curve constructed from dilutions of red cells that had been fully haemolysed with excess STS.

B) Detection of Protective Effects of Albumin on Haemolysis. To examine the potentially protective effects of albumin, mixtures of sclerosants and bovine serum albumin (BSA) in saline were first prepared in round bottomed microwells. Next, small volumes of washed red cells were added and the mixtures were centrifuged at 200 g for 10 minutes. Unlysed red cells sedimented to form a compact spot in the centre of the wells. Absorbance measurements were then carried out at 405 nm.

Platelet lysis studies

Platelet lysis was assessed by means of changes in turbidity as previously described.[5] The inhibition of platelet lysis by the addition of bovine serum albumin (BSA) was assessed in mixtures of washed platelet suspensions and sclerosants. BSA was diluted to a concentration of 16% using diluting buffer. Serial dilutions were carried out down 2 columns of flat bottomed wells in a microplate with the bottom well containing 0% BSA. To each well, small volumes of the 10× washed platelet suspension were dispensed and absorbances were measured at 414 nm in a microplate reader (Titertek Multiskan MCC, Finland). The instrument was blanked with a column of buffer-containing wells before each reading. Small volumes of STS and POL were then added to the respective columns for a final sclerosant concentration of 0.015%. Absorbances were measured and then additional volumes of sclerosants were added stepwise to achieve a final concentration of 0.6%.

Effect of plasma on the release of platelet-derived microparticles

To examine the protective effects of plasma components, PDMP formation in response to STS or POL was assessed in both washed platelet samples and PRP. Samples (0.08 ml) were incubated with saline (0.02 ml) containing varying concentrations of sclerosant for 30 minutes. 0.05 ml of this mixture was then diluted using 0.2 ml of 0.02 M HEPES pH 7.0 buffered 0.15 M sodium chloride solution. 0.01 ml of this mixture was then added to CD41a-PerCP-Cy5.5 antibody (0.005 ml) and Annexin V-APC (0.002 ml) with the volume made up to 0.05 ml using HEPES-bufferred saline solution containing 2.5 mM $CaCl_2$. This was incubated for 30 minutes before the addition of 1 ml HEPES buffer containing 2.5 mM $CaCl_2$. Counting was performed using TRU-Count tubes. Platelet-derived microparticles (PDMP) were defined as events, less than 1.09 μm in diameter that bound Annexin V and CD41a-PerCP-Cy5.5.

Neutralisation of haemolytic effects of sclerosants by procaine, lignocaine and protamine

We investigated the potential protective benefits of these drugs in comparison with albumin. Microwells were filled with increasing concentrations of sclerosants in saline and then 1% washed red cells were added. After mixing and centrifuging, absorbances at 405 nm were determined in a microplate reader. These experiments were done with washed red cells in saline to avoid interference from plasma proteins.

Endothelial cell lysis

A human microvascular endothelial cell line (HMEC-1)[10] was cultured to confluence in endothelial growth medium: MCDB131 (JRH Biosciences, Lenexa, KS, USA) containing 10% Fetal Bovine Serum, Penicillin-Streptomycin and L-Glutamine (Gibco Invitrogen, Carlsbad, CA, USA) in 96 well microplates pre-coated with human fibronectin (Gibco Invitrogen). Mixtures of sclerosants and BSA or plasma dilutions were prepared in a separate microplate and added to the microwells after a single wash with saline. Following a 10 minute incubation at 20 °C, the solutions were gently removed, the residual cells washed twice with saline, dried and stained with Leishman's stain. Residual adherent material representing non-lysed cells was quantified by densitometry at 540 nm.

Results

Haemolysis studies

STS concentrations above 0.25% caused haemolysis in plasma whereas POL concentrations above 0.45% were necessary to induce haemolysis in this system (Fig. 1).

Fig. 2 shows the progressive inhibitory effects of albumin on the haemolytic activity of the sclerosants in saline. In the absence of albumin, much lower concentrations of both agents induced haemolysis. STS induced haemolysis at 0.02% and POL at 0.005%. Increasing concentrations

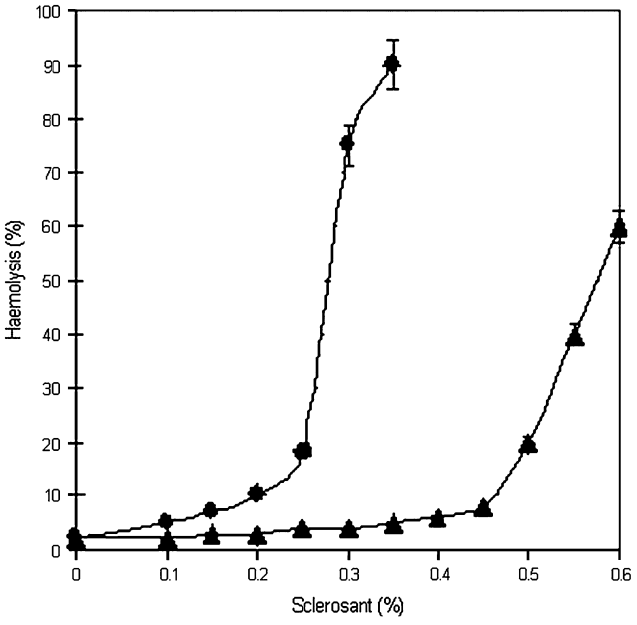

Figure 1 Haemolysis induced by sclerosants added to normal plasma containing 50% packed red cells. STS (●), POL (▲).

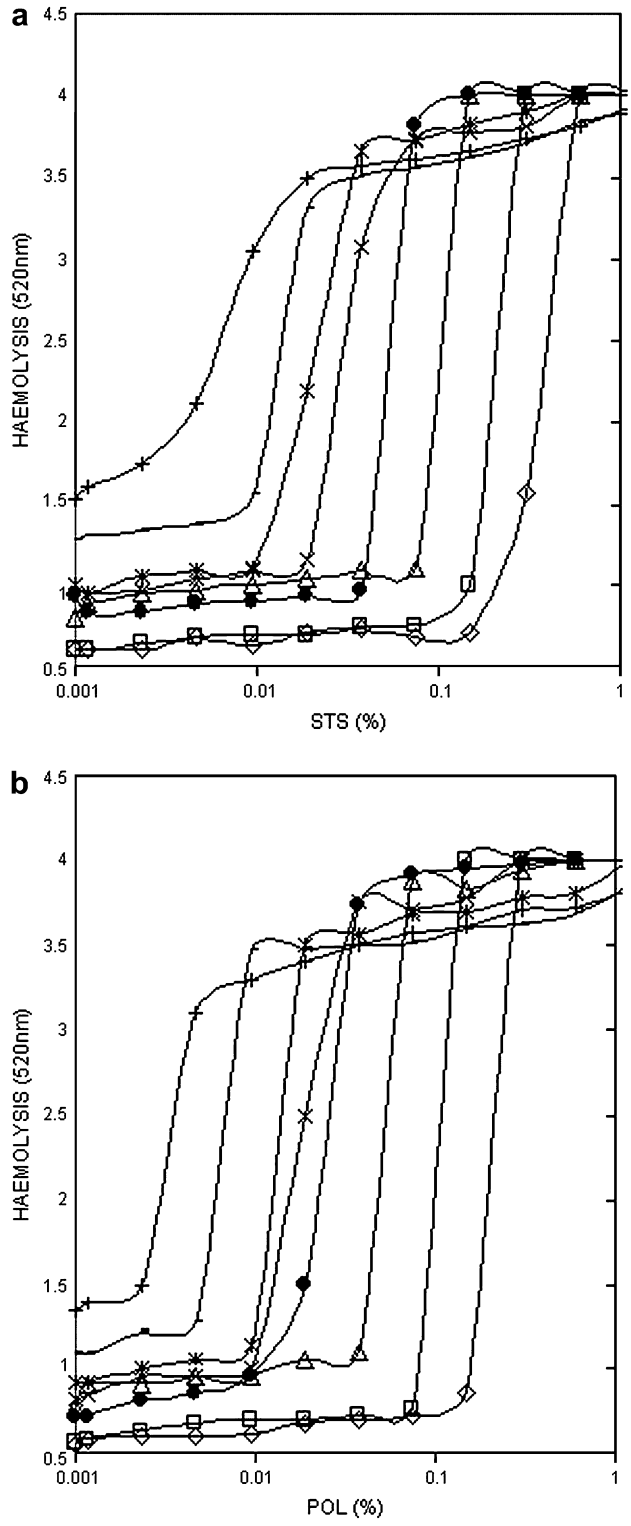

Figure 2 Haemolysis induced by sclerosants in the presence of varying concentrations of bovine serum albumin (BSA 8% ◇, 4% □, 2% △, 1% ●, 0.5% ×, 0.25% *, 0.125% −, 0% +). (a) STS, (b) POL.

of albumin in saline required higher concentrations of sclerosants to induce haemolysis. STS was 50 fold and POL was 163 fold less haemolytic in blood than in saline (Table 1).

Table 1 Effects of sclerosants on red blood cells and platelets in saline, with 4% BSA present, and in whole blood

	STS (%)	POL (%)
	Red cells	
In saline alone	0.006 (1×)	0.0035 (1×)
In saline + 4% BSA	0.2 (33.3×)	0.1 (28.6×)
In whole blood	0.3 (50×)	0.57 (163×)
	Platelets	
In saline alone	0.03 (1×)	0.03 (1×)
In saline + 4% BSA	0.3 (10×)	0.3 (10×)
In whole blood	See below*	0.3 (10×)

Results extracted from 50% lysis points in Figs. 2a & b and 3a & b and Ref. 5. A 4% BSA concentration was chosen as the physiologic concentration.

* Due to a precipitate of apolipoprotein B and fibrinogen the turbidity rises after an initial drop with STS in whole blood and hence measurement of 50% platelet lysis with this method cannot be achieved.

Platelet lysis studies

In the absence of BSA, platelets were lysed by STS and POL concentrations above 0.02% (Fig. 3a and 3b). However, increasing concentrations of BSA interfered with the lytic activity of the sclerosants. At BSA concentrations above 4%, sclerosant concentrations greater than 0.2% were required to lyse platelets. POL did not dissolve platelets as completely as STS.

Effect of plasma on the release of platelet-derived microparticles

We have previously shown that low concentrations of sclerosants release PDMP.[5] Here we investigated the potential inhibitory effects of plasma on the release of PDMP. Microparticle formation was induced by STS or POL concentrations of 0.01% in washed platelet samples, whereas higher concentrations (0.15%) were required to achieve the same in PRP samples. This suggests that plasma confers a degree of lytic protection to the platelets. Both agents at higher concentrations (>0.2%) in saline reduced the PDMP count (Fig. 4b).

Neutralisation of haemolytic effects of sclerosants by procaine, lignocaine and protamine

Results obtained are shown in Tables 2 and 3. It is apparent that the haemolytic activity of STS which emerged at a concentration of 0.25% was not impeded by 0.1% procaine or lignocaine. Indeed these drugs slightly increased the haemolytic activity of STS. The best neutralising agent for STS was BSA which at a low concentration of 0.5%, completely blocked haemolysis at STS concentrations of up to 0.05%. Protamine sulphate had a mild neutralising effect on STS. As noted earlier, POL was more haemolytic at lower concentrations when compared with STS in saline. The haemolytic activity of POL was inhibited only by BSA and not procaine, lignocaine or protamine at the concentrations examined.

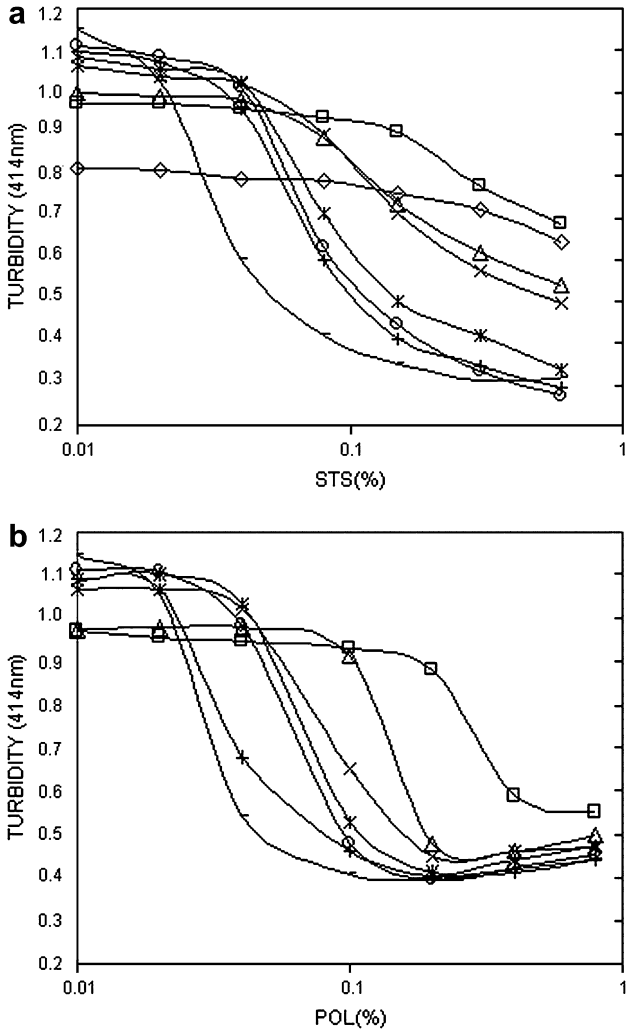

Figure 3 Turbidity studies performed to determine the effects of detergent sclerosants on washed platelets in the presence of varying concentrations of bovine serum albumin (BSA 4% □, 2% △, 1% ×, 0.5% *, 0.25% ○, 0.125 +, 0% −). Baseline zero STS or POL is shown as ''0.001%'' so as to be accommodated on a logarithmic scale. (a) STS, (b) POL.

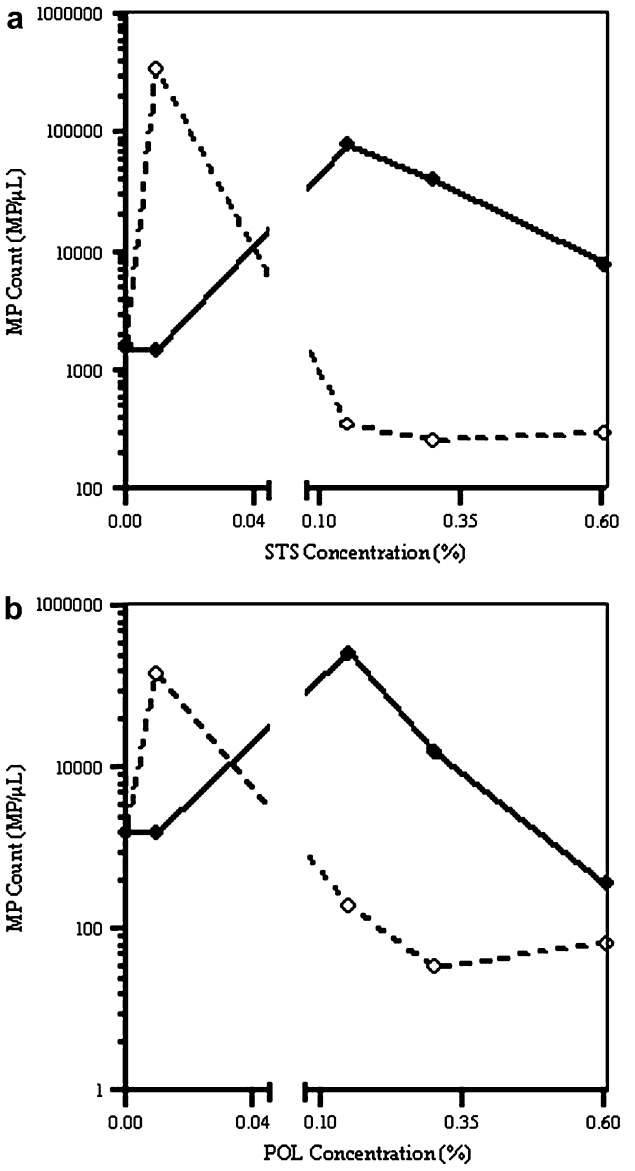

Figure 4 Effect of plasma proteins on sclerosant induced platele-derived microparticle (PDMP) formation. PDMP counts in platelet rich plasma (●) and a washed platelet suspension (○) incubated with varying concentrations of (a) STS and (b) POL.

Effect of sclerosants on endothelial cells

Results obtained are shown in Fig. 5a and b. STS reduced the amount of stainable material in the microwells in a concentration-dependent manner. POL had less lytic effects on endothelial cells (results not shown). Both plasma and BSA reduced the lytic effects of STS on the endothelial cells (Fig. 5a and b).

Discussion

It is well known that detergents including STS and POL disrupt lipid and phospholipid membranes.[1] Detergents are widely used to permeabilise cells allowing the ingress of various dyes and markers. For instance, sodium dodecyl sulphate (SDS), an anionic detergent with a similar chemical structure to STS, readily dissolves most cells even at relatively low concentrations.[11] Fegan noted that red cell lysis was caused by STS concentrations of 0.125% or greater in normal saline added to heparinised blood.[12]

In this study, both detergent sclerosants were shown to cause haemolysis, platelet lysis and endothelial cell lysis. Both drugs released PDMP in low concentrations but destroyed them at higher concentrations. Platelets appeared to be more resistant to lysis by sclerosants when compared with red blood cells (Table 1). This is possibly due to the differences in the composition of the cell membranes and the internal structure of these cells. Previous studies have reported platelet rich clots occluding femoral arterial eversion grafts to be much more resistant to lysis than erythrocyte rich whole blood clots.[13]

Table 2 Haemolysis of washed red cells in saline by STS and the effect of various therapeutic agent additives. No haemolysis is indicated by ''O'', partial haemolysis by ''+'', and complete haemolysis by ''++''. Haemolysis defined as lysis of more than 50% of red blood cells

STS (%)	No additive	Lignocaine 0.1%	Procaine 0.1%	Protamine 0.01%	BSA 0.5%
0	O	O	O	O	O
0.010	O	O	O	O	O
0.020	O	O	O	O	O
0.025	O	++	+	O	O
0.030	+	++	++	O	O
0.040	++	++	++	O	O
0.050	++	++	++	+	O

We demonstrated for the first time that plasma components appeared to neutralise the lytic effects of sclerosants in an *in vitro* setting. STS was 50 fold and POL was 163 fold less haemolytic in blood than in saline (Table 1). These findings highlight the importance of plasma components and in particular albumin in protecting against the lytic effects of detergent sclerosants. It can be concluded that lower concentrations of sclerosants can achieve the same lytic effects if blood is removed from the target vessels. In a clinical setting, this can be partially achieved by an empty vein technique or if blood is displaced by foam, diluted by an infusion of saline, or forced out by perivenous infiltration of saline or tumescent anaesthesia. The displacement of blood from the vessel lumen decreases the exposure of the sclerosant to plasma components and increases the exposure of the endothelial lining of the vessel lumen to the active agent. This concept may partly explain why foam sclerosants are found to be more effective when compared with the liquid counterparts.

Compared with the 3 other drugs tested (procaine, lignocaine and protamine), albumin proved to be the best neutralising drug with the potential to completely block sclerosant-dependent cell lysis. Albumin is a transport protein with the potential to bind to a number of drugs via two major and three minor binding sites.[14] The binding strength of a drug to serum albumin is the main factor that

Table 3 Haemolysis of washed red cells in saline by POL and the effect of various therapeutic agent additives. No haemolysis is indicated by ''O'', partial haemolysis by ''+'' and complete haemolysis by ''++''

POL (%)	No additive	Lignocaine 0.1%	Procaine 0.1%	Protamine 0.01%	BSA 0.5%
0	O	O	O	O	O
0.008	O	O	O	O	O
0.015	++	++	++	++	O
0.02	++	++	++	++	O
0.03	++	++	++	++	O
0.035	++	++	++	++	O
0.05	++	++	++	++	+

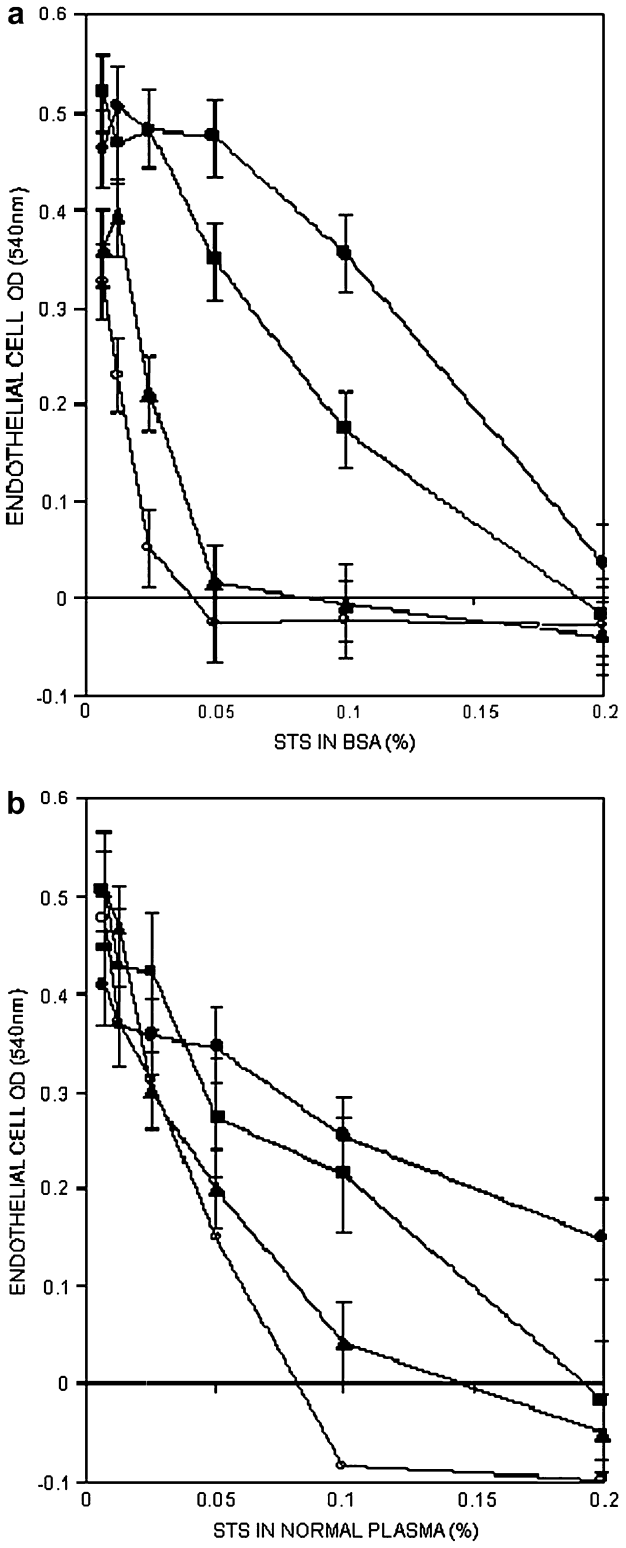

Figure 5 Effect of STS on cultured endothelial cells in the presence of varying concentrations of (a) bovine serum albumin (BSA 1.5% ●, 0.5% ■, 0.17% ▲, 0% ○) and (b) normal plasma (NP/2 ●, NP/6 ■, NP/18 ▲, NP/54 ○).

determines the amount of free agent in the circulation available to reach the target tissue. STS has been shown to bind to 10-11 sites on BSA with Kd (M) of 6.7×10^{-7}.[15]

Apart from albumin, other plasma proteins and possibly lipoproteins may also play a role in neutralisation of detergent sclerosants. We have previously isolated a precipitate induced by STS which was found to be mainly apolipoprotein B and fibrin(ogen).[5] STS can also form complexes with other proteins such as gammaglobulin.[16] Association of detergents with lipid-related proteins is probably not unexpected and we have also noticed an insoluble STS-red cell complex form when washed red cells are lysed by STS. It is also conceivable that POL is binding to plasma components other than albumin. We observed POL to be more active than STS in both saline and with 4% BSA present (Table 1). 0.0035% POL was required to achieve 50% haemolysis in saline, whereas 0.1% (28.6×) was required in the presence of 4% BSA and 0.57% (163×) was required in whole blood (Table 1). These findings imply that other plasma components play an important role in neutralisation of POL. These plasma components and their interaction with POL have not been characterised.

Sclerosants have been observed to enter the deep venous system during sclerotherapy. It is likely that the concentration of the active drug entering the deep veins is quite low due to neutralisation and dilution effects. However, we have previously demonstrated low concentrations of sclerosants to possess procoagulant properties[5] and deep vein thrombosis has been described in association with the clinical use of low concentration sclerosants.[17] Therefore, infusion of large volumes of detergent sclerosants from a single access point should be avoided as an initial high concentration will undergo a neutralisation and dilution process reaching the deep veins in a low concentration 'pro-coagulant window' which may lead to deep vein thrombosis (DVT). The extent of the neutralisation process depends on the volume of blood with which the sclerosant is mixed at the time of injection. Larger blood volumes will dilute and neutralise the sclerosants more significantly. Based on our present findings, 1 ml of blood will contain enough plasma protein to neutralise 0.2 ml of STS and 0.4 ml of POL at the therapeutic concentration of 3%. The quantity of the intravascular blood volume depends on the diameter of the target vessel and the length of the target segment. The sympathetic tone is responsible for the vessel diameter at any time. Anxiety, pain, cold temperature, vasoactive medications and other factors can induce vasoconstriction reducing the volume of the intravascular blood in the target segment. Leg elevation can help with emptying the vessel and reducing the intravascular blood. Needle penetration can also induce vasospasm and further reduce the vessel diameter. Furthermore, bubbles generated from foam sclerosants have been observed at distant sites such as the right atrium.[18,19] Sample collection from the right atrium is an invasive procedure and to our knowledge has not been attempted as yet. Given our present findings however, these bubbles are highly unlikely to have any sclerosing activity due to the neutralisation and dilution process.

We compared the protective effects of three other agents with albumin. Procaine is an anaesthetic agent and its role in preventing STS induced tissue necrosis is not well understood. Procaine has been presumed to bind and inactivate STS.[20,21] In this study, procaine and lignocaine slightly increased the haemolytic activity of STS and showed no neutralising properties. Veno-arteriolar axonal reflex has been previously proposed by one of the authors to be one of the pathogenic mechanisms of post-sclerotherapy ischemic ulcers.[7] Procaine used as a local anaesthetic possibly impedes this axonal reflex, thus exerting a protective effect. The clinical utility of Procaine in prevention of STS induced tissue necrosis requires further evaluation.

In this study, Protamine was found to mildly neutralize STS. A slight turbidity was apparent after this interaction. This is possibly because the positively charged protamine ionically binds to the negatively charged STS forming an insoluble complex.[22] Protamine is well known to neutralize other negatively charged polymeric molecules such as heparin.[22,23] POL is an anionic agent and predictably, Protamine did not inhibit the lytic effects of POL. None of the other agents, except for BSA, inhibited the lytic effects of POL.

In summary, both STS and POL at therapeutic concentrations have lytic effects on red cells, platelets, and endothelial cells and release procoagulant platelet-derived microparticles. STS causes significant haemolysis at concentrations higher than 0.3% in whole blood. Serum albumin neutralizes the lytic effects of both sclerosants. Lower sclerosant concentrations are required to achieve equivalent lytic effects in the absence of plasma components. Foam sclerosants are more effective than liquids in sclerosing blood vessels possibly due to displacement of blood and a reduced exposure to serum albumin and other plasma components. Neutralisation of sclerosants by albumin possibly plays a role in the low incidence of post-sclerotherapy deep vein thrombosis and low incidence of distal effects. Finally, procaine and lignocaine have no protective effect on the lytic properties of detergent sclerosants while protamine confers a mild degree of protection.

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References

1 Goldman MP, Bergan JJ, Guex JJ. *Sclerotherapy. Treatment of varicose and telangiectatic leg veins*. 4th ed. United States of America: Mosby Elsevier; 2007. p. 165—209.
2 Berenguer B, Burrows PE, Zurakowski D, Mulliken JB. Sclerotherapy of craniofacial venous malformations: complications and results. *Plast Reconstr Surg* 1999;**104**(1):1—11 [discussion 12—15].
3 Goldman MP. *Sclerotherapy. Treatment of varicose and telangiectatic leg veins*. 2nd ed. United States of America: Mosby Elsevier; 1995. p. 315—318.
4 Marrocco-Trischitta MM, Guerrini P, Abeni D, Stillo F. Reversible cardiac arrest after polidocanol sclerotherapy of peripheral venous malformation. *Dermatol Surg* 2002;**28**:153—5.
5 Parsi K, Exner T, Connor DE, Ma DD, Joseph JE. In vitro effects of detergent sclerosants on coagulation, platelets and microparticles. *Eur J Vasc Endovasc Surg* 2007;**34**:731—40.

6 Weiss RA, Feied CF, Weiss MA. *Vein diagnosis and treatment. A comprehensive approach*. United States of America: McGraw-Hill Medical Publishing Division; 2001. p. 167—174.

7 Tran D, Parsi K. Veno-arteriolar reflex vasospasm of small saphenous artery complicating sclerotherapy of the small saphenous vein. *Aust NZ J Phleb* 2007;**10**(1):29—32.

8 Goldman MP. *Sclerotherapy. Treatment of varicose and telangiectatic leg veins*. 2nd ed. United States of America: Mosby Elsevier; 1995. p. 64—68; 280—349.

9 Dacie J, Lewis S. *Practical haematology*. 5th ed. Churchill Livingstone Publication; 1975. p. 195.

10 Ades EW, Candal FJ, Swerlick RA, George VG, Summers S, Bosse DC, et al. HMEC-1: establishment of an immortalized human microvascular endothelial cell line. *J Invest Dermatol* 1992;**99**(6):683—90.

11 Singer MM, Tjeerdema RS. Fate and effects of the surfactant sodium dodecyl sulfate. *Rev Environ Contam Toxicol* 1993; **133**:95—149.

12 Fegan G. *Fegan's compression sclerotherapy for varicose veins*. London: Springer-Verlag; 2003. p. 56—57.

13 Jang IK, Gold HK, Ziskind AA, Fallon JT, Holt RE, Leinbach RC, et al. Differential sensitivity oferythrocyte-rich and platelet-rich arterial thrombi to lysis with recombinant tissue-type plasminogen activator. A possible explanation for resistance to coronary thrombolysis. *Circulation* 1989;**79**(4):920—8.

14 Zhao X, You T, Liu J, Sun X, Yan J, Yang X, et al. Drug-human serum albumin binding studied by capillary electrophoresis with electrochemiluminescence detection. *Electrophoresis* 2004;**25**(20):3422—6.

15 Reynolds JA, Herbert S, Polet H, Steinhardt J. The binding of diverse detergent anions to bovine serum albumin. *Biochemistry* 1967;**6**(3):937—47.

16 Jones MN. A theoretical approach to the binding of amphipathic molecules to globular proteins. *Biochem J* 1975;**151**:109—14.

17 Conrad P, Malouf GM, Stacey MC. The Australian polidocanol (aethoxysclerol) study. *Dermatol Surg* 1995;**21**(4):334—6.

18 Forlee MV, Grouden M, Moore DJ, Shanik G. Stroke after varicose vein foam injection sclerotherapy. *J Vasc Surg* 2006; **43**(1):162—4.

19 Morrison N, Neuhardt DL, Hansen K, Levin S, Salles-Cunha SX. Tracking foam to the heart and brain following ultrasound-guided sclerotherapy of lower extremity veins. *Aust NZ J Phleb* 2007;**10**(1):6—10.

20 Orbach J. A new look at sclerotherapy. *Folia Angiologica* 1977; **25**:181.

21 Fegan G. The complication of compression sclerotherapy. *Practitioner* 1971;**207**(242):797—9.

22 Mittermayr M, Margreiter J, Velik-Salchner C, Klingler A, Streif W, Fries D, et al. Effects of protamine and heparin can be detected and easily differentiated by modified thrombelastography (Rotem®): an *in vitro* study. *Br J Anaesth* 2005; **95**(3):310—6.

23 Kim SK, Lee DY, Kim CY, Moon HT, Byun Y. Prevention effect of orally active heparin derivative on deep vein thrombosis. *Thromb Haemost* 2006;**96**(2):149—53.