In Vitro Effects of Detergent Sclerosants on Clot Formation and Fibrinolysis

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Abstract  Objective: To investigate the in vitro effects of detergent sclerosants sodium tetradecyl sulphate (STS) and polidocanol (POL) on clot formation and lysis.

Materials and methods: clot kinetics were assessed in whole blood by thromboelastography (TEG®) and rotational thromboelastometry (ROTEM®). Fibrinogen was measured by the Clauss method in plasma and factor XIII (FXIII) by enzyme-linked immunosorbent assay (ELISA). Turbidity measurements were used to assess clot lysis in plasma, and fibrinolysis in non-cross-linked and cross-linked fibrin. D-dimer was measured by VIDAS®, STA® Liatest® and AxSYM® assays.

Results: Strong clots were formed at low sclerosant concentrations (0.075–0.1%). At midrange concentrations (0.15% STS, 0.15–0.3% POL), both agents inhibited the contribution of platelets to clot firmness and formed weak clots prone to lysis. At higher concentrations (STS ≥0.3% and POL ≥0.6%), clot formation was inhibited. STS destroyed FXIII at ≥0.15% and fibrinogen at ≥0.6%. Neither sclerosant had a significant effect on cross-linked fibrin, but STS had a lytic effect on non-cross-linked fibrin. STS caused an artefactual elevation of D-dimer in the VIDAS® assay when fibrinogen was present.

Conclusion: Detergent sclerosants demonstrated a trimodal effect on clot formation, initiating strong clots at low concentrations, weak clots at midrange concentrations and preventing clot formation at higher concentrations. Neither agent had fibrinolytic activity.

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Introduction

Detergent sclerosants, sodium tetradeyl sulphate (STS) and polidocanol (POL), function by lysing the intimal lining of blood vessels, exposing the underlying collagen with the ultimate aim of inducing endovascular fibrosis. These agents were historically assumed to achieve this by forming a clot within the target vessel, the organisation of which could lead to vessel fibrosis and the lysis of which would lead to vessel recanalisation and treatment failure. Our group has previously shown that these detergents are biologically active and interfere with the coagulation, anti-thrombotic and fibrinolytic mechanisms. We have also shown that these agents have a concentration-dependent effect on the coagulation system in vitro with STS exhibiting anticoagulant activity at >0.3%, while both agents demonstrate procoagulant activity at lower concentrations. Nonetheless, the direct effect of sclerosants on clot formation and lysis was not previously investigated and was the subject of the present study.

The ultimate step in the formation of a fibrin clot is the conversion of soluble fibrinogen to insoluble fibrin. This process is mediated by thrombin, which also activates Factor XIII (FXIII). Fibrin monomers are first assembled in a non-covalent fashion and then covalently cross-linked by activated FXIII (FXIIIa). Cross-linking enhances the mechanical strength of the fibrin polymer, and leads to increased clot stability, stiffness and resistance to fibrinolysis and deformation.

Fibrin is degraded by plasmin generated from plasminogen by the action of tissue plasminogen activator (t-PA) in blood and urokinase in tissues. The enzymatic degradation of fibrin leads to the formation of fibrin degradation products and, in particular, D-dimer. As shown by the authors, both sclerosants interfere with fibrinolytic enzymes and inhibitors and demonstrate antifibrinolytic activity. Here, we studied the direct lytic effects of sclerosants on FXIII, fibrinogen and fibrin and their potential for generation of D-dimer.

Materials and Methods

Materials

The following were used in this study: STS (FIBRO-VEIN 3%; Australian Medical and Scientific, NSW, Australia); POL (AETHOXYSKLEROL 3%; Chemische Fabrik Kreussler, Wiesbaden, Germany); TEG® reagents (Haemoscope, IL, USA); ROTEM® reagents (Pentapharm, Munich, Germany); Echis carinatus venom (ECV; RUDINtest, Haematex Research, NSW); urea, low-melting-point agarose (type VII), Agkistrodon rhodostoma venom (ARV) and hydrolysed gelatin (Sigma Chemical, MO, USA); human thrombin (Enzyme Research Laboratories, IN, USA); recombinant human t-PA (Haematologic Technologies, Vermont, USA) and bovine serum albumin (BSA; Bovogen, Victoria, Australia).

Sample collection for fresh-frozen plasma (FFP)

Normal plasma (NP) was collected as FFP derived from donor blood unsuitable for clinical indications and obtained from the Australian Red Cross Blood Transfusion Service, Sydney. Samples were collected in acid citrate dextrose (ACD) venous blood vacuum collection tubes.

Preparation of platelet-rich (PRP) and platelet-poor plasma (PPP)

Centrifugation of whole blood (WB) for 10 min at 150 g was performed to generate platelet-rich plasma (PRP) and for 30 min at 1700 g to generate platelet-poor plasma (PPP).

Preparation of freeze-dried plasma

Freeze-dried samples were prepared using NP (2.0 ml, 0.5% 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid (HEPES) dispensed into siliconised vials spiked with sclerosants to a final stated concentration (0%, 0.075%, 0.1%, 0.3%, 0.6% and 1%). The vials were frozen at −50 °C and vacuum dried for 2 days. Prior to testing, each vial was reconstituted with 2 ml of water.

Preparation of fibrin agarose gel

Fibrinogen was isolated from NP by precipitation with 20% ammonium sulphate. Fibrinogen solution and low-melting point agarose (2%w/v in 0.1 M sodium chloride (NaCl), 0.02 M HEPES pH 7.0, 37 °C) were mixed, 0.1 U ml−1 thrombin and 0.01 M calcium chloride (CaCl2) were added, and 0.1-ml volumes were dispensed into pre-warmed microwells. For non-cross-linked fibrin, thrombin was replaced with 0.0001% ARV (containing the thrombin-like enzyme Ancrod) without CaCl2. The agarose set on cooling and the fibrinogen within each well was slowly converted with time to non-cross-linked fibrin (with ARV) and to cross-linked fibrin (with thrombin that activates FXIII). Urea (6M), known to dissolve non-cross-linked fibrin but not cross-linked fibrin, was used as a control to confirm the cross-linking status of the two types of fibrin agarose gels.

Clot kinetics

Clot viscoelastic properties were assessed by thromboelastography (TEG®, Haemoscope) and rotational thromboelastometry (ROTEM®, Pentapharm). Both systems use a vertical pin within a cup that contains the WB sample. In TEG®, the cup oscillates and the pin is stationary whereas in ROTEM®, the cup is stationary while the pin oscillates. As a clot forms between the cup and the pin, the reduction in transmitted rotation from the cup to the pin (TEG®) or impedance to the oscillatory movement of the pin (ROTEM®) is detected and a trace is generated (Fig. 1(a)).

WB was collected from consenting healthy volunteers on no medications or supplements into vacutainer tubes containing 0.105 M buffered sodium citrate (BD Diagnostics, Basel, Switzerland) and spiked with increasing concentrations of sclerosants. Parameters measured are detailed in Table 1 and assays performed are summarised in Table 2.

For TEG®, 1 ml volumes of WB-spiked samples were pipetted into Kaolin vials. A total of 20 µl of 0.2 M CaCl2 was placed in each cup followed by 300 µl of the Kaolin-spiked samples.
### Sclerosants, Clot Formation and Fibrinolysis

![Diagram]

#### b

<table>
<thead>
<tr>
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<th>NATEM</th>
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<th>EXTEM</th>
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### FIBTEM

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<td>0.1%</td>
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<td>High Cn.</td>
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<tr>
<td>1.2%</td>
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</table>
Figure 1  (a) Diagrammatic representation of a typical TEG®/ROTEM® trace indicating the commonly reported variables. r, reaction time; CT, clotting time; k, coagulation time; CFT, clot formation time; MA, maximum amplitude; MCF, maximum clot formation. (b) Representative sclerosant clot kinetics as analysed by ROTEM®. Clot firmness <20 mm is marked with pink, and ≥20 mm with blue. Green line indicates no clot formation. Cn, concentration; Mid., midrange. (c) TEG®/ROTEM® parameters measured in various assays. In FIBTEM, CFT is indefinitely prolonged as by definition, no strong clots with amplitudes ≥20 mm (which requires a contribution from platelets) can be achieved. This is a normal finding for FIBTEM (NATEM, n = 5; other assays, n = 2; STS and POL).
In all ROTEM® assays (except ECATEM), 20 μl of the STARTEM reagent (0.2 M CaCl₂ in HEPES pH 7.4 buffer) was placed in each cup followed by 20 μl of the relevant reagent per assay (EXTEM, INTEM, APTEM or FIBTEM) or 20 μl of RUDINtest (ECATEM) or no other reagent (NATEM). A total of 300 μl volumes of WB-spiked samples were then added. All TEG® and ROTEM® assays were performed at 37 °C and run for 30 min except NATEM, which was run for 60 min due to the prolonged initial clotting time.

### Clot lysis time (CLT)
NP (FFP, 1 ml) was briefly mixed with small volumes of t-PA stock solution to a final concentration of 25 ng l⁻¹. Volumes (0.1 ml) were distributed into microwells and mixed with increasing concentrations of sclerosants. Volumes (0.1 ml) of thrombin (2U ml⁻¹) were then added and the mixtures containing approximately 50% diluted NP were allowed to clot. Turbidities were determined periodically at 414 nm.

### Interaction with exogenous t-PA
A total of 1.0 μg l⁻¹ of t-PA in 0.2% hydrolysed gelatine (protein-poor) and 8.0 μg l⁻¹ in NP (protein rich) was pre-incubated briefly with serial dilutions of sclerosants in NaCl pH 7.0 buffer. 0.02 ml volumes were applied to fibrin

<table>
<thead>
<tr>
<th>Table 1</th>
<th>TEG® and ROTEM® parameters measured in this study.</th>
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<tr>
<td>Parameters</td>
<td>Description</td>
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<tr>
<td>TEG® Reaction time (r)</td>
<td>Clotting Time (CT)</td>
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<tr>
<td>Coagulation time (k)</td>
<td>Clot Formation Time (CFT)</td>
</tr>
<tr>
<td>—</td>
<td>Clot Formation Rate (CFR)</td>
</tr>
<tr>
<td>Maximum Amplitude (MA)</td>
<td>Maximum Clot Firmness (MCF)</td>
</tr>
<tr>
<td>Coagulation Index (CI)</td>
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### In all ROTEM® assays (except ECATEM), 20 μl of the STARTEM reagent (0.2 M CaCl₂ in HEPES pH 7.4 buffer) was placed in each cup followed by 20 μl of the relevant reagent per assay (EXTEM, INTEM, APTEM or FIBTEM) or 20 μl of RUDINtest (ECATEM) or no other reagent (NATEM). A total of 300 μl volumes of WB-spiked samples were then added.

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### Table 2  TEG® and ROTEM® assays used in this study and reagents used in each assay.

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<tr>
<th>Assays</th>
<th>Reagent</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEG Calcium Chloride (CaCl₂)</td>
<td>Thromboelastography (TEG) was used to assess the global effects of sclerosants in the assay, the clotting cascade was activated with kaolin.</td>
<td></td>
</tr>
<tr>
<td>NATEM 'STARTEM' (CaCl₂)</td>
<td>Non-activated (NA) Thromboelastometry (TEM), NATEM, is sensitive towards any kind of coagulation activation or inhibition in the sample and provides a general overview of the haemostatic process.</td>
<td></td>
</tr>
<tr>
<td>INTEM CaCl₂ + Ellagic acid</td>
<td>Intrinsic pathway TEM, INTEM, is an assay that initiates a controlled activation of the contact system using ellagic acid and phospholipids to evaluate the intrinsic pathway.</td>
<td></td>
</tr>
<tr>
<td>+ Phospholipids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EXTEM CaCl₂ + Tissue factor (TF)</td>
<td>Extrinsic pathway TEM, EXTEM, uses a stabilised preparation of TF for a mild but consistent activation of coagulation to examine the extrinsic pathway.</td>
<td></td>
</tr>
<tr>
<td>ECATEM Echis Carinatus Venom (ECV)</td>
<td>Ecarin TEM, ECATEM, uses ECV to mediate the conversion of prothrombin to thrombin. ECATEM evaluates the final two steps of the coagulation cascade i.e. the conversion of prothrombin to thrombin and the subsequent generation of fibrin from fibrinogen. The trace generated is independent of phospholipids and other plasma clotting factors.</td>
<td></td>
</tr>
<tr>
<td>APTEM CaCl₂ + TF + Aprotinin</td>
<td>Aprotinin TEM, APTEM, incorporates a plasmin inhibitor (aprotinin) in the ap-TEM® reagent together with ex-TEM® activation. The trace generated by this assay is compared with EXTEM. In presence of fibrinolysis, aprotinin will inhibit fibrinolysis and the abnormal EXTEM trace will be corrected to a normal trace in APTEM.</td>
<td></td>
</tr>
<tr>
<td>FIBTEM CaCl₂ + TF + Cytochalasin D</td>
<td>Fibrinogen TEM, FIBTEM, incorporates Cytochalasin D, an inhibitor of platelet cytoskeletal re-organisation. The trace generated would represent the contribution of clotting factors and in particular fibrinogen to clot strength and is compared with EXTEM to assess the contribution of platelets to clot formation. The FIBTEM maximum clot firmness (MCF) normal range is much lower (9–25 mm) than the EXTEM (50–72 mm).</td>
<td></td>
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</table>
agarose wells and incubated at 37 °C overnight. Turbidities were determined periodically at 414 nm.

Fibrinogen

Fibrinogen levels were measured in freeze-dried plasma samples by the Clauss method, using a Thrombin reagent (Dade Behring, Marburg, Germany) on an STA-R analyzer (Diagnostica Stago, Asniere, France).

FXIII

Fresh donor WB, PRP and PPP samples containing serial dilutions of sclerosants were prepared on non-coated plates and tested following the standard procedure in the FXIII ELISA kit (EF 1013-1, Assaypro, MI, USA). In control experiments, serial dilutions of sclerosants in 5% BSA were spiked with 12 ng of FXIII and tested in the assay system (inter-assay coefficient of variation: 6.8%). All assays were done in duplicate.

Fibrinolytic activity

Serial dilutions of sclerosants and t-PA (0.02 ml, 25 ng ml⁻¹) in 0.1% BSA or hydrolysed gelatin buffer were applied to cross-linked and non-cross-linked fibrin agarose gels (0.1 ml) and incubated overnight at 37 °C

0.1 ml volumes of 2% cross-linked fibrin powder suspensions in HEPES-buffered saline were dispensed into microwells, mixed with 0.1 ml of sclerosant dilutions and incubated for 4 h at 37 °C.

For both methods, turbidities were measured periodically at 414 nm following the incubation.

D-dimer

D-dimer was measured in freeze-dried samples by the VIDAS® D-dimer Exclusion assay (bioMérieux®sa, Marcy-l’Étoile, France) on the Mini-VIDAS® instrument (bioMérieux Vitek, MI, USA), AxSYM® D-dimer (Abbott Laboratories, IL, USA) and STA®Liatest® D-dimer (Diagnostica Stago).

To investigate the observed elevation of D-dimer by STS (see Results 7), the D-dimer levels were re-measured in the following samples using the VIDAS® assay:

1. Original plasma samples following removal of a previously reported STS-induced precipitate;
2. Serial dilutions of STS in saline or in VIDAS® kit diluent;
3. 1% STS in heat-defibrinated pooled NP (PNP, 30 min, 56 °C) neat and after centrifugation (10 min at 16,100 g); and
4. Purified fibrinogen (Fibrinogen Fraction 1, type I Sigma Cat F3879) added to the VIDAS® kit diluent to a concentration of 2.3 g l⁻¹ and then spiked with STS.

Presentation of Sampling Fluctuations

Sampling fluctuations are reflected by the standard errors (standard deviation divided by the square root of the number of sample) for Figures 1, 5 and 6 and within the 10-15 % range for the remaining Figures.

Results

Clot kinetics

Low concentrations (0.075–0.1%)

Strong clots (amplitude >20 mm) were formed in TEG® and all ROTEM® assays (Fig. 1(b)). The reaction (r)/clotting time (CT) and coagulation time (k)/clot formation time (CFT) were reduced in TEG® and NATEM (Fig. 1(c)) while the clot formation rate (CFR) was increased (results not shown). TEG® Coagulation Index showed a hypercoagulable state (CI > +3.0).

Midrange concentrations (0.15% STS, 0.15–0.3% POL)

Weak clots (amplitude <20 mm) formed in all assays except in FIBTEM where the amplitude remained within the normal range of 9–25 mm (Fig. 1(b) and (c)). This indicated an inhibition of platelet contribution to clot firmness. In tissue factor (TF)-based assays (EXTEM, APTEM and FIBTEM), weak clots formed at 0.3% STS but clot formation was inhibited at higher concentrations.

High concentrations (≥0.3% STS, ≥0.6% POL)

Both agents inhibited clot formation and indefinitely prolonged r/CT and k/CFT (Fig. 1(b) and (c)). CFR was reduced to zero. No value for TEG® CI could be obtained (CI < -3.0), indicating a hypocoagulable state.

Other findings

Addition of aprotinin (APTEM) did not influence the maximum amplitude as compared with EXTEM and, hence, no fibrinolysis was detected. Direct prothrombin activation (ECATEM) yielded results similar to other non-TF-based assays.
Clot lysis time (CLT)

t-PA at 25 ng ml\(^{-1}\) within a 50% diluted plasma clot induced clot lysis within 6 h. STS at 0.1% shortened CLT to 4 h and at 0.15% to <2 h (Fig. 2). POL had less effect.

Interaction with exogenous t-PA

t-PA was inactivated by STS at ≥0.4% in plasma while POL had less effect (Fig. 3). STS interfered more with t-PA activity in protein-poor buffer, whereas, in plasma (which is protein rich), 10-fold higher levels of STS were required due to protein binding.

Fibrinogen

STS at 0.3% reduced fibrinogen levels in plasma by 20%, and at ≥0.6% destroyed this protein completely (Fig. 4). POL had minimal effect.

FXIII

STS at 0.075% reduced FXIII to less than 50%, and at 0.15% completely destroyed this protein in WB (Fig. 5). POL had minimal effect. Similar results were obtained in PRP and PPP samples (results not shown). Control experiments showed no interaction with the assay system (results not shown).

Fibrinolytic activity

STS at >0.4% solubilised non-cross-linked fibrin, but at 3% demonstrated a significant lytic effect (Fig. 6(a)). STS had a similar effect to t-PA but was 100,000 times weaker on a direct concentration basis. POL had little effect on non-cross-linked fibrin. Both agents showed no significant effect on cross-linked fibrin agarose gels (Fig. 6(b)) or cross-linked fibrin powder (results not shown).

D-dimer

In VIDAS\textsuperscript{®} assay, STS at ≥0.6% significantly elevated the D-dimer levels to 2.5 mg l\(^{-1}\) (normal range 0–0.5 mg l\(^{-1}\)) while POL had no effect (results not shown). This elevation was not detected by AxSYM\textsuperscript{®} or STA\textsuperscript{®} Liatest\textsuperscript{®}.

Removal of a previously reported STS-induced precipitate\textsuperscript{2} did not influence the D-dimer elevation. STS dilutions in saline, in VIDAS\textsuperscript{®} kit diluent (~0.6 mg l\(^{-1}\)) and in heat-defibrinated PNP showed no measurable D-dimer levels. However, STS spiking of the VIDAS\textsuperscript{®} kit diluent to which purified fibrinogen was added caused elevations of D-dimer (up to 7.4 mg l\(^{-1}\) with 1% STS). Therefore, fibrinogen was required for the STS-induced elevation of D-dimer, as measured by the VIDAS\textsuperscript{®} assay.
while the amplitude in FIBTEM remained within normal amplitude). There was a reduction of amplitude in EXTEM shown as they are typical. (6b) fibrin. Only results obtained with 3% sclerosants are linked with that of t-PA on cross-linked (6a) and non-cross-linked fibrin. This enhances clot stability and increases resistance to fibrinolysis and deformation. Apart from its cross-linking function, FXIIIa covalently binds inhibitors of fibrinolysis, such as antiplasmin, to the fibrin clot. This is possibly the main mechanism by which FXIIIa increases the resistance of the clot to lysis. Destruction of FXIII prevents cross-linking, reduces clot firmness and leads to formation of weak and unstable clots susceptible to lysis.

Detergent sclerosants are used clinically to occlude vessels. The sequence of events leading to vessel occlusion has been unknown and whether clot formation plays a part in this process has been debated. In this study, both agents demonstrated a trimodal effect on clot formation and firmness (Fig. 7). At low concentrations (0.075–0.1%), clot formation was enhanced by both agents as manifested by the formation of strong clots (>20 mm amplitude) in all assays. In addition, strong clots were initiated by both agents in the non-activated NATEM assay, in the absence of any activators, and where the only reagent added was CaCl₂ for re-calcification purposes. The clotting and clot formation times were shortened in NATEM and TEG and the Coagulation Index showed a hypercoagulable state. This procoagulant activity is consistent with the release of platelet-derived microparticles (PMPs) and shortening of phospholipid-dependent Xa and surface-activated clotting times (XACT and SACT) previously reported by this group. At midrange concentrations (0.15% STS, 0.15–0.3% POL), both agents induced weak clots (<20 mm amplitude). There was a reduction of amplitude in EXTEN while the amplitude in FIBTEM remained within normal limits, indicating a lack of contribution from platelets to clot formation and firmness. At higher concentrations (0.6–1.2%), no clots were formed, indicating clotting factor and, in particular, fibrinogen deficiency. This confirms our previous reports that both agents at high concentrations bind phospholipids, destroy a number of clotting factors and inhibit clotting interactions.

We investigated whether clots formed in presence of sclerosants were more prone to lysis. Although both agents increased the clot lysis rates at midrange concentrations, there was no synergistic (or inhibitory) effect between the detergents and t-PA. At higher concentrations, STS (but not POL) had an inhibitory effect on t-PA-induced fibrinolysis most likely due to denaturation of the t-PA protein structure. Plasminogen, the main t-PA substrate, is also destroyed by STS at such high concentrations. Hence, the faster clot lysis rates were not due to a synergistic interaction with t-PA but due to formation of weak clots susceptible to lysis as confirmed by thromboelastometry.

Clot strength is affected by fibrinogen, FXIII and platelets. We found all three to be affected by midrange concentrations of STS while POL had no effect on fibrinogen or FXIII. High fibrinogen concentrations are required for increased clot rigidity and density. In this study, fibrinogen levels were reduced at midrange concentrations of STS and, at ≥0.6%, this protein was completely destroyed. Consistently, the FIBTEM signal was lost at 0.6% STS. This is most likely due to denaturation and unfolding of the fibrinogen protein structure. Furthermore, both cationic and anionic detergents precipitate fibrinogen by forming fibrinogen–detergent complexes.

In this study, FXIII was destroyed by STS at ≥0.15%. FXIIIa covalently cross-links fibrin monomers to form cross-linked fibrin. This enhances clot stability and increases resistance to fibrinolysis and deformation. Apart from its cross-linking function, FXIIIa covalently binds inhibitors of fibrinolysis, such as antiplasmin, to the fibrin clot. This is possibly the main mechanism by which FXIIIa increases the resistance of the clot to lysis. Destruction of FXIII prevents cross-linking, reduces clot firmness and leads to formation of weak and unstable clots susceptible to lysis.

Platelet contribution to clot firmness plays an important role in clot amplitude in thromboelastometry. Comparing the clot firmness in FIBTEM (where platelets are blocked) with EXTEN (includes platelet contribution), midrange concentrations of both sclerosants showed a lack of contribution from platelets to clot firmness. This is consistent with our earlier reports that both agents at similar concentrations (≥0.15%) induce platelet lysis.

We investigated the fibrinolytic activity of sclerosants. Both agents had no destructive effect on cross-linked fibrin and, consistently, no fibrinolysis was detected in APTEM. However, STS destroyed non-cross-linked fibrin and produced a similar dose response curve to t-PA although approximately 100,000 times weaker. This is possibly due to depolymerisation of the non-cross-linked fibrin polymer by STS. Similarly, plasmin has less effect on cross-linked fibrin and the presence of FXIIIa slows down the process of clot lysis induced by t-PA. A similar anionic detergent, sodium dodecyl sulphate (SDS), also exhibits some fibrinolytic activity. The process of cross-linking happens very quickly and non-cross-linked fibrin is normally not present.

**Figure 6** Comparison of fibrinolytic activity of detergent sclerosants with that of t-PA on cross-linked (6a) and non-cross-linked (6b) fibrin. Only results obtained with 3% sclerosants are shown as they are typical. (n = 3, ■ STS × 10⁵, ● POL × 10⁵ and ▲ t-PA).
in a thrombus. Hence, exposure of STS to a fully formed clot would not lead to detectable lysis. POL, a non-ionic detergent, demonstrated no fibrinolytic activity. Non-ionic and zwitterionic detergents are reported to increase the apparent activity of t-PA, although they have little net thrombolytic or fibrinolytic activity.

In this study, high-concentration STS caused an artefactual elevation of D-dimer levels when measured in the VIDAS\textsuperscript{©} ELISA assay. This only occurred when fibrinogen was present in the sample. One possible mechanism is that STS caused a conformational change in fibrinogen, exposing an epitope recognised by monoclonal antibodies used in the VIDAS\textsuperscript{©} assay, but not in the other two commercial assays tested. Fibrinogen can form flexible polymer chains similar to polymeric fibrin by binding end-to-end in D-dimer configuration where the neighbouring D domains form 'DD' regions. D-dimer specific antibodies have been previously reported to react with such soluble fibrinogen aggregates.

Figure 7 Cartoons demonstrating the observed effects of STS (a) and POL (b) on clot formation. The effect demonstrated is at the point of entry of sclerosants into the target vessel where the sclerosant concentration is at its highest and downstream where the concentration drops. High concentration (>0.3%) STS destroys fibrinogen, factor XIII (FXIII), thrombin (T)\textsuperscript{2} and platelets (PLT) and a number of other clotting factors\textsuperscript{2} and in this study inhibited clot formation. High concentration (>0.6%) POL destroys platelets and a number of clotting factors\textsuperscript{2} and in this study also inhibited clot formation. At midrange concentrations (0.15% STS, 0.15–0.3% POL), both agents inhibited the contribution of platelets to clot firmness and generated weak clots. 0.15% STS destroyed FXIII and reduced fibrinogen. Both agents at low concentrations (0.075% and 0.1%) initiated and enhanced strong clot formation. Cn., concentration; FXIIIa, activated FXIII; XL, cross-linked; NXL, non-cross-linked.
prolonged high intravascular concentrations are unlikely. In addition, although relatively high concentrations can be achieved at the point of entry, much lower final concentrations are generated when these agents are mixed with large volumes of blood, diluted and neutralised.

Foam sclerosants are more effective than liquid agents, possibly due to displacement of blood and a reduced exposure to serum albumin and other plasma components. Clinical recurrence, despite the use of high concentrations of sclerosants, is most likely secondary to inadequate vascular injury, formation of intra-vascular thrombus and subsequent recanalisation. Treatment modifications aiming at minimising the intra-vascular blood content may achieve higher final concentrations and a lower recanalisation rate.

Clinically, sclerotherapy has been associated with thrombo-embolic complications, including paradoxical clot embolism and stroke, following the use of low- to midrange thrombo-embolic complications, including paradoxical clot embolism and stroke, following the use of low- to midrange concentrations of sclerosants. Clot formation normally occurs due to interaction of running blood from the adjoining patent vessels reaching the site of vascular injury. The subsequent activation of platelets and the coagulation system would result in clot formation. Formation of such clots is initiated by vascular injury. Based on our present findings, clots can also be initiated directly by low- to midrange concentrations of both sclerosants upon contact with blood and in the absence of vascular damage. The formation of such clots and their viscoelastic properties would depend on the final intra-vascular concentrations achieved. A weak clot deficient in FXIII, as that formed by midrange concentrations of sclerosants, would have the potential for detachment from the vessel wall and embolisation, as well as lysis and clinical recanalisation.

This study was not without limitations. We did not use the euglobulin clot lysis time method as it remains a complex and time-consuming procedure. The in vivo effects of sclerosants on clot formation and lysis, including the effects of foam sclerosants, are currently being investigated by this group in other studies. We report that low-concentration sclerosants initiate a platelet-dependent clot formation process. Platelets play a small role in venous thrombosis and subsequently there have been no systematic studies on the effects of sclerosants on platelets and the influence of platelet inhibitors on sclerotherapy treatment outcomes. Given our current findings, these topics may require further evaluation.

Summary

Sclerosants demonstrated a trimodal effect on clot formation whereby, at low concentrations (0.075–0.1%), they enhanced strong clot formation, at midrange concentrations (0.15% STS, 0.15–0.3% POL) induced weak clots susceptible to lysis and, at higher concentrations, prevented clot formation. Neither agent had a lytic effect on cross-linked fibrin but high-concentration STS destroyed non-cross-linked fibrin. STS caused an artefactual elevation of D-dimer in the VIDAS® assay.

Conflict of Interest

None.

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