Historical view of coagulation

Extrinsic pathway
- VWF
- TF
- X
- Xa
- Va
- Lipid
- IIa

Intrinsic pathway
- XI
- XII
- PC
- Xa
- Va
- Lipid
- IIa

Now recognized that cells do more than just provide a phospholipid surface.
Cells considered to direct haemostasis.

Cell Based Model

Basic haemostasis
- Haemostasis = balance between 'procoagulant' and 'anticoagulant' forces
- Usually divided into 2 phases:
  - Primary haemostasis = formation of platelet plug
  - Secondary haemostasis = activation of coagulation cascade
Primary haemostasis

- Platelet plug formation at sites of injury
- Occurs within seconds of injury
- Important in stopping blood loss from capillaries, small arterioles, and venules

Secondary haemostasis

- Reactions of plasma coagulation system that result in fibrin formation
- Requires several minutes for completion
- Fibrin strands strengthen the primary haemostatic plug
- Important in larger vessels and
- Prevents bleeding hours or days after the injury

**Extrinsic Pathway**
- Tested by PT
  - Fibrinogen → Fibrin
  - Blood Clot

**Intrinsic Pathway**
- Tested by APTT
  - Fibrinogen → Fibrin
  - Blood Clot

**Common Pathway**
- Tested by TT
  - Fibrinogen → Fibrin
  - Blood Clot

**Role of Platelets**
In vitro Effects of Detergent Sclerosants on Coagulation, Platelets, and Microparticles

Kurosh Parsi
ACP NZ 2010

The effects of STS and POL on:
- Clotting tests
- Clotting factors
- Red cell lysis
- Platelet lysis
- Platelet derived microparticles

Clotting times
- Platelet-rich plasma (PRP)
- Platelet-poor plasma (PPP)

Haemolysis
- Whole blood
- Washed red cells

Clotting factors
- Factor assayed on pooled normal plasma
  - Activity at 100% initially
  - Mixed with 0.3% STS/POL
  - Duration: 5 and 30 minutes

Platelet lysis
- Turbidity measurements
- PMP
- Flow cytometry

Measurement of
- Clotting times
- Platelet-rich plasma (PRP)
- Platelet-poor plasma (PPP)

Measurement of
- Clotting factors
- Factor assayed on pooled normal plasma
  - Activity at 100% initially
  - Mixed with 0.3% STS/POL
  - Duration: 5 and 30 minutes

Platelet lysis
- Turbidity measurements
- PMP
- Flow cytometry

Thrombin Time (TT)
PPP

Legend:
- STS
- POL
Prothrombine Time (PT) PPP

Legend:
- STS
- POL

Cutting Test Detects
PT Extrinsic Pathway
Fibrinogen and Factors VII, X, V, II

XACT PRP

Legend:
- STS
- POL

Cutting Test Detects
XACT Procoagulant phospholipid, factors V and II

STS Effect on XACT

Legend:
- STS initially
- STS after 2.5h incubation

Cutting Test Detects
XACT Procoagulant phospholipid, factors V and II

APTT PPP

Legend:
- STS
- POL

Cutting Test Detects
APTT Intrinsic Pathway
Fibrinogen, factors XII, XI, PKK, HMWK, IX, VIII, X, V, II

SACT PRP

Legend:
- STS
- POL

Cutting Test Detects
SACT Surface activated clotting time
Procoagulant phospholipid

POL Effect on XACT

Legend:
- POL initially
- POL after 2.5h incubation

Cutting Test Detects
XACT Procoagulant phospholipid, factors V and II
Effect on Clotting Factors

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

Effect of Sclerosants on Clotting Factors

<table>
<thead>
<tr>
<th>FACTOR</th>
<th>PNP with 0.3% POL</th>
<th>PNP with 0.3% STS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T = 5 min</td>
<td>T = 30 min</td>
</tr>
<tr>
<td>II</td>
<td>102%</td>
<td>97%</td>
</tr>
<tr>
<td>V</td>
<td>70</td>
<td>59</td>
</tr>
<tr>
<td>X</td>
<td>76</td>
<td>75</td>
</tr>
<tr>
<td>VII</td>
<td>82</td>
<td>79</td>
</tr>
<tr>
<td>VIII</td>
<td>117</td>
<td>106</td>
</tr>
<tr>
<td>IX</td>
<td>111</td>
<td>119</td>
</tr>
<tr>
<td>XI</td>
<td>152</td>
<td>126</td>
</tr>
<tr>
<td>XII</td>
<td>135</td>
<td>135</td>
</tr>
</tbody>
</table>

Legend:
- After 5 min
- After 10 min
- After 20 min
- After 40 min
- After 60 min

Effect on Platelets

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

Effect of on Platelets (PRP)

Legend:
- After 5 min
- After 10 min
- After 20 min
- After 40 min
- After 60 min

STS Effect on Platelets (PRP)

Effect on Platelet Derived Microparticles

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
Effect on PLT Microparticles

Summary
- High Concentration (>0.6%)  
  - STS prolongs all clotting times  
  - Possess anticoagulant properties (STS > POL)  
  - STS significantly destroyed FV and FVII  
  - Lyse PLT  
  - Lyse PMP
- Low Concentrations (STS 0.1-0.3%)  
  - Shortened XACT and SACT  
  - Procoagulant properties (POL > STS)  
  - Activated PLT  
  - Released PDMP

Interaction of STS with the coagulation system

Antithrombotic Mechanisms
- Help to limit coagulation where it is not required
- Inactivate specific clotting factors such as:  
  - Antithrombin (AT) - FXa and FII  
  - Proteins C and S (PC, PS) - FVa and FVIII  
  - Tissue factor pathway inhibitor (TFPI) - TF

Normal Thrombus Generation

Scleroantithrombotic

Antithrombotic Mechanisms

Antithrombotic Mechanisms

Antithrombotic Mechanisms

Antithrombotic Mechanisms

Antithrombotic Mechanisms
Antithrombotic Mechanisms

- Therapeutic anticoagulant drugs act via similar mechanisms
  - Heparin binds to AT
  - Increases its ability to inactivate factors X, thrombin
  - For heparin to work you need AT

Methods

- Freeze dried samples spiked with POL and STS
- PC and AT determined using chromogenic assays
- Free PS determined by immuno-turbidimetric method

Effect on Protein C (PC)

Effect on Protein S (PS)

Effect on Antithrombin (AT)
**Apparent rise in AT**
- Compared samples containing 1.5% STS
  - In bovine serum albumin (BSA) VS hydrolysed gelatin (no plasma)
  - BSA fully neutralises STS
- Sample containing BSA displayed no AT
- Sample containing hydrolysed gelatin produced 46% AT activity
- Rise in AT activity due to the direct effect of STS on thrombin used in this assay

**Effect on APC induced anticoagulation**
- Normal plasma
- POL and STS
- Tested with and without APC present in CaCl₂
- APTT measured

---

**STS Effect on APC**

![STS Effect on APC graph]

Legend:
- APC and Normal Plasma
- Normal Plasma

**POL Effect on APC**

![POL Effect on APC graph]

Legend:
- APC and Normal Plasma
- Normal Plasma

---

**Activated PC resistance (APCR)**

APC Ratio = clotting time with APC / clotting time without APC

![Activated PC resistance (APCR) graph]

APC Ratio = \(\frac{62.5}{30} = 2.08\)

62.5 (s) / 30 (s)

**Activated PC resistance (APCR)**

APC Ratio > 2  Normal
APC Ratio < 1.8 indicates abnormality
- lack of sensitivity of FV to APC or APC Resistance
- APCR
  - FV Leiden mutation or
  - other factors that reduce the sensitivity of FV to APC eg liver disease or OCP
Activated PC resistance (APCR)

- Normal and APC resistant plasma containing 0.15%-0.25% sclerosants were mixed with APC.
- dRVVT-LR based assay was used.
- Ratios of clotting times with APC to those without APC were derived.

Comparison with heparin

- Sclerosants at various dilutions were added to normal plasma +/- heparin.
- APTT was measured to compare the anticoagulant activity of heparin against activity of sclerosant.

Interaction with Heparin

- Sclerosants added to normal plasma with and without heparin present.
- APTT measured.
Objectives

- To investigate the in vitro effects and interactions of STS and POL on:
  - RBC
  - Platelets
  - PMPs
  - Endothelial cells

- Protective effects of plasma components including albumin

Summary STS High Cn

- Anti-thrombotic
  - Destroys factors V and VII
  - Potentiates the effect of APC
  - Mimick Anti-FII and anti-FXa effect of AT
  - Directly destroys clotting factors

Net effect
ANTI-TROMBOTIC

Summary POL HIGH Cn

- POL by contrast:
  - Does not demonstrate an inhibitory effect on APC
  - Increases APC resistance
  - Does not inhibit clotting factors in vitro

Net effect
Neutral
? +/- Pro-thrombotic

Summary STS and POL Low Cn

- Anti-thrombotic
  - nil

- Pro-thrombotic
  - Activate PLT
  - Release PMP
  - Shortens XACT, SACT

Net effect
PRO-TROMBOTIC

Scleroneutralization

Parsi K, Exner T, Ma DO, Connor DE, Joseph JE, Herbert A
The Lytic Effects of Detergent Sclerosants on Erythrocytes, Platelets, Endothelial Cells and Microparticles are Attenuated by Albumin and other Plasma Components in Vitro
Endovasc Surg. 2008;36:216-223
**Haemolysis**

- Sclerosants were added to
  - washed red cells
  - plasma containing sedimented blood cells
- Absorbance measured at 520nm, peak absorption for free Hb released from lysed cells

**Sclerosants Neutralized by Albumin**

- Mixture of Bovine Serum Albumin (BSA) and sclerosants added to washed red cells
- Absorbance measured at 405nm

**STS is Neutralized by Albumin**

- Less albumin, More lysis
- More albumin, More cells

**POL is Neutralized by Albumin**

- Less albumin, More lysis
- More albumin, More cells
Summary

- Haemolysis in plasma was caused by:
  - STS > 0.25%
  - POL > 0.45%
- Albumin neutralized haemolysis induced by the sclerosants
- Sclerosants had a similar lytic effect on platelets at high concentrations also inhibited by albumin

Take home message!

1ml of 3% STS will be neutralized by 5mls of blood
1ml of 3% POL will be neutralized by 2.5ml of blood

<table>
<thead>
<tr>
<th>Diam. mm</th>
<th>Length cm</th>
<th>STS (%)</th>
<th>POL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>3.2</td>
<td>100x</td>
<td>100x</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>33.3x</td>
<td>28.6x</td>
</tr>
<tr>
<td>5</td>
<td>25.5</td>
<td>50x</td>
<td>163x</td>
</tr>
</tbody>
</table>

POL is inhibited more than STS!

POL was more active than STS in both saline and 4% BSA.
Platelet microparticle (PMP)

- PMP formation was assessed in response to sclerosants
  - In PRP
  - Washed platelet samples
- Flow cytometry performed using Annexin V and CD41a as markers

Results

- In PRP, STS and POL concentration of 0.2% induced PMP
- In washed platelets, STS and POL concentration of 0.01% induced PMP

Endothelial cell lysis

- HUVECs were cultured to confluence
- Mixtures of sclerosants and BSA were added to HUVECs
- Lysis was determined by absorbance at 540nm
Results

- POL had less lytic effects on endothelial cells
- Both plasma and BSA reduced the lytic effect of STS on the endothelial cells

Conclusion and Summary

- Sclerosants at therapeutic concentrations lyse BLOOD CELLS and ENDOTHELIAL CELLS
- Both sclerosants induce release of PMP
- Albumin significantly inhibits both sclerosants
- POL is more potent than STS if in the absence of plasma components

Interaction of detergent sclerosants with the coagulation system: an update

Kurosh Parsi,1,2 Thomas Exner,3 David Ma,1,2 and Joanne Joseph 1,2

1 Haematology Research Lab, St Vincent's Hospital, Sydney
2 University of New South Wales, Sydney

"You are completely free to carry out whatever research you want, so long as you come to these conclusions."

Kurosh Parsi
ACP NZ 2010