Lyophilized Plasma for Austere Conditions

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Introduction

- Epidemiology and etiology of coagulopathy
- Damage control resuscitation
- Lyophilized plasma products in use
- Development and optimization of LP
- Advantages beyond coagulation
Acute Traumatic Coagulopathy (ATC)

- Tissue Hypoperfusion
- Severe Injury

ATC

- Acidosis
- Hemodilution
- Hypothemia

TIC

- Inflammatory Response
- Hyperfibrinolysis
- Endothelial Dysfunction
- Dysfibrinogenemia
- Platelet Dysfunction

Uncontrolled Hemorrhage
Kaplan–Meier Curves

Damage Control Resuscitation

• **Primary Goals**
  – Hemorrhage control
  – Avoid lethal triad

• **Risk factors for MT**
  – SBP < 110
  – HR > 105
  – HCT < 32
  – pH < 7.25

• **Transfusion Goal** – 1:1:1

85% chance of Massive Transfusion
Damage Control Resuscitation

- Last in first out
- FWB only if components not available or patient not responding
- Platelets obtained in theater
- Additional fibrinogen required
- rFVIIa option if coagulopathy persists
- TXA for patients requiring MT
Advantages of Dried Plasma

- Logistically superior
- Stored as a powder for years
- Rapidly reconstituted in water
  - FFP 30 minutes to thaw
- Could be widely available
  - FFP availability problematic in rural areas, pre-hospital and far forward
- Retains factor function
  - FFP 60 – 70% activity after thawing
South African Dried Plasma

- South African Bioplasma FDP
  - Lyophilized, pooled, solvent detergent treated
  - 1500 donors, ABO universal, frozen < 6 hours
  - Stored below 25°C
  - Prospectively studied in CPB patients and equivalent to plasma

German LyoPlas

- Single donor (Blood type compatibility)
- Stored up to 15 months
- 200,000 TFNs – 0.023% major complications similar to FFP
French Flyp

- Up to 11 donors/unit (Universal)
- Stored up to 24 months
- Available to US SF on IRB protocol
- 1000 transfusions with no major adverse events
TABLE 1. Transfusion Data Before Administration of FDP

<table>
<thead>
<tr>
<th>Blood Products, Fluids, and Agents Given</th>
<th>Median</th>
<th>Range</th>
<th>Percent of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells (units)</td>
<td>3</td>
<td>1–13</td>
<td>32</td>
</tr>
<tr>
<td>Whole blood (units)</td>
<td>4</td>
<td>1–7</td>
<td>5</td>
</tr>
<tr>
<td>Crystalloid (L)</td>
<td>1</td>
<td>0.2–5</td>
<td>56</td>
</tr>
<tr>
<td>Colloid (mL)</td>
<td>500</td>
<td>100–8,000</td>
<td>15</td>
</tr>
<tr>
<td>rFVIIa (mg)</td>
<td>2</td>
<td>1–7</td>
<td>9</td>
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<tr>
<td>Fibrinogen (g)</td>
<td>1.5</td>
<td>1–3</td>
<td>6</td>
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![Prothrombin time graph](image)

Swine Studies with LP

- US products in early FDA trials
- Whole blood steriley removed from swine
- Plasma component separated
- Lyophilized by HemCon® Medical Technologies, Inc.
- Powdered plasma returned
- Reconstituted prior to transfusion
Preparation of LP

- LP very alkalotic
- Requires addition of acid to reconstitution fluid
- Vitamin C utilized
- Reconstitute to original volume
Methods: Swine Model

- Multi-center pre-clinical trial
- OHSU, USAISR, Mass General
- Previously validated model
- 32 Yorkshire crossbred swine
- Anesthetized, mechanically ventilated
- Carotid and jugular catheters placed

- Syverud et al, Resuscitation 1988
- Wladis et al, Shock 2001
- Kiraly et al, J Trauma 2006
- Cho et al, Shock 2008
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<tr>
<th>Baseline</th>
<th>Injury Phase</th>
<th>Hemorrhage Phase</th>
<th>Operative Phase</th>
<th>Hemostatic Resuscitation Phase</th>
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</table>


Shuja, et al. *J Trauma* 2008
• 60% of estimated blood volume removed
• Hypothermia, acidosis, coagulopathy induced
  – Active cooling
  – Saline infusion
Baseline          Injury        Hemorrhage         Operative
Phase            Phase          Phase            Phase

Femur fracture
Controlled hemorrhage
Grade V liver injury

Shock
3:1 NS

Hemostatic Resuscitation Phase
- 4 randomized groups
  - FFP
  - LP
  - FFP : PRBC
  - LP : PRBC

- Volume equal to controlled hemorrhage
- Re-warmed to 37°C
- Labs drawn hourly

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<td></td>
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<td>4hr</td>
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</tr>
</tbody>
</table>

- Femur fracture
- Controlled hemorrhage
- 3:1 NS
- Grade V liver injury
Residual Activity

Blood loss after liver injury

Spoerke et al. *Arch Surg* 2009;144:829-834.
IL-6

Optimization of LP

- Minimize fluid for reconstitution
  - 50% equivalent
- Optimize reconstitution fluid
  - H$_2$O best
- Optimize anti-oxidants
  - Vitamin C dosed to correct pH
  - Effects unchanged 7.5 mM – 22.5 mM
Back to the Future
How does plasma work mechanistically?

- Over 1000 proteins in plasma
- Many are biologically active
- Many have unknown functions in health and disease
Working Hypothesis:

• We have formed the fundamental hypothesis: that if we prevent or repair endothelial dysfunction early after traumatic injury – we can improve immune function and coagulation disturbances associated with trauma.

• We hypothesize that plasma decreases inflammation and repairs endothelial injury and dysfunction.

• We further hypothesize this effect of plasma transfusion leads to increased survival and improved secondary outcomes related to injury- ie organ failure and long term disabilities.
Working Biological Model
Endotheliopathy of Trauma

Assay of Endothelial Permeability

- Seed Cells
- Induce Permeability VEGF/ Hypoxia
- Add FITC- Dextran
- FITC-Dextran Read at Ex. 485/Em 530
FFP Inhibits Vascular Endothelial Permeability and Tightens the Junctions Between Endothelial Cells

Net: Decreased Permeability

Net: Increased Resistance

**FFP Protects the Endothelial Glycocalyx**

The glycocalyx is a ubiquitous barrier that protects the underlying endothelium. 

Lung Injury in HS is inhibited by FFP

Lung Tissue

Pulmonary Vascular Leak

Pulmonary Inflammation

N=5 mice/group

Lung Vascular Permeability in HS is Inhibited by FFP
Spray Dried Plasma: Resusix combines dried plasma with Pathogen reduction

- Entegrion Inc. and Velico Inc.
- Plasma is pooled (150 donors, type AB)
- Pathogen reduced via solvent detergent treatment
  - TnBP and Octoxinol
- Spray Dried
  - Powder has 2-5% of original moisture content
  - Allows the plasma to be concentrated
In vitro: SDP is functionally equivalent to FFP in inhibiting endothelial permeability and inflammation

- Spray-dried plasma and fresh frozen plasma modulate permeability and inflammation in vitro in vascular endothelial cells
  - TRANSFUSION Volume 53, January 2013 Supplement

- In vitro Assays show both SDP and FFP equivalently
  - Inhibit endothelial cell permeability
  - Inhibit leukocyte binding
  - Reconstitute endothelial adherens junctions after VEGF treatment
In Vivo - SDP is Equivalent to FFP

FFP and SDP are equivalent in reversing the endotheliopathy of trauma in vivo in a mouse model of hemorrhagic shock and trauma.

Fresh frozen plasma and spray-dried plasma mitigate pulmonary vascular permeability and inflammation in hemorrhagic shock

Daniel R. Potter, PhD, Gail Baimukanova, MD, PhD, Sheila M. Keating, PhD, Xutao Deng, PhD, Jeffrey A. Chu, Stuart L. Gibb, PhD, Zhanglong Peng, PhD, Marcus O. Muench, PhD, Marina E. Fomin, PhD, Philip C. Spinella, MD, Rosemary Kozar, MD, PhD, and Shibani Pati, MD, PhD, San Francisco, California

J Trauma Acute Care Surg
Volume 78, Number 6, Supplement 1
FFP and SDP equivalently inhibit pulmonary vascular permeability induced by hemorrhagic shock in mice.
CD68 positive monocytes and macrophage infiltrates are decreased by SDP and FFP resuscitation but not LR.
Overview of the endothelial cell junctions

Dejana, E., Nature Reviews Molecular Cell Biology 5. 261-270
Tight and Adherens Junctions are Preserved in by FFP and SDP but not LR
Summary – Benefits of Plasma in Trauma

- Correction of coagulopathy of trauma
- Repletion of volume deficit
- Anti – Inflammatory
- Decreases vascular permeability
- Repairs the endothelium
- Decreases organ injury
- Superior to crystalloid
Dried Plasma Conclusions

• Logistically superior
• Functionally equivalent or superior to FFP
• Dried plasma can be pooled, pathogen reduced and stored for extended periods of time
• Dried plasma should be used in lieu of FFP in austere conditions
Thank you!