12th Annual Canadian Blood Services International Symposium

Plasma: Transfuse it, Fractionate it or Forget it?

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Canadian Blood Services
it's in you to give
What Can We Learn from Animal Models of Coagulopathy and Bleeding (about plasma transfusion)?

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Bleeding

- A clinical problem feared by physicians and patients alike
- Transfusion services provide blood products vs. bleeding
- Bleeding poses complex pathophysiological problems
  - Loss of oxygenation via RBC
  - Reduced blood volume and pressure
  - Loss of coagulation via platelets and plasma
- Blood loss must be halted by coagulation
Plasma contains all the soluble coagulation factors

- Platelets also provide a surface for assembly of complexes (e.g. tenase and prothrombinase), release biological modifiers, provide aggregated bulk
- Another cell provides the source of Tissue Factor
Can’t stop bleeding? Coagulopathy…maybe

- Coagulopathy: A deficit in the blood’s ability to clot OR
- A deficit in the ability of plasma to clot (as opposed to thrombocytopenia) OR
- An abnormality manifested by an elevated laboratory plasma clotting time

- Arises from:
  - Single factor deficiency
  - Multiple factor deficiency
  - Pan-factor deficiency

The NEW ENGLAND JOURNAL of MEDICINE

REVIEW ARTICLE
CRITICAL CARE MEDICINE
Bleeding and Coagulopathies in Critical Care
Beaverly J. Hunt, M.D.
Lack coag factors? Give plasma?

• Transfusion of plasma may be useful in adults who are:
  – **Bleeding** or undergoing invasive procedures
  – On warfarin & **bleeding** or in need of an urgent invasive procedure
  – Undergoing massive transfusion (**due to bleeding**) with coagulation abnormalities
  – Deficient in factors for which no concentrate is available (**at risk of bleeding**) (at risk of bleeding)
  – Suffering from TTP and needing plasma exchange (**this one is not about bleeding**)

• So what’s the problem? “The **lack of good-quality evidence** is most marked in the use of blood components to manage major bleeding.”
Animal models

• No level 1 evidence of plasma as an efficacious Tx for bleeding
• Either plasma is ineffective or clinical studies have failed to show that efficacy
• Will trials be mounted?
• Animal models can provide pre-clinical biological evidence to aid assessment of human in vitro studies and clinical observations
• Questions (arising from plasma quality work):
  – Does plasma transfusion affect bleeding?
  – If so, what constituent factors in the plasma are most important?
Animal models and bleeding

- Pan-factor deficiency arises in trauma
- Frith D et al. Thromb Res 2012 reviewed animal models of trauma-induced coagulopathy
  - Majority of models combined hemodilution, traumatic injury and uncontrolled hemorrhage
  - 0/43 studies tested plasma as an intervention
- Letourneau P et al. J Trauma 2011 hemodiluted rats with human FFP (65 ml/kg, Day 0 or Day 5), provoked uncontrolled hemorrhage via liver laceration, fluid-resuscitated with Hextend, found survival > Day 0 vs. Day 5 group
- Imam A et al. J Neurotrauma 2014 broke ribs, damaged liver & brain, bled swine 40% blood volume, 2 hours shock, resuscitated with FFP or saline, found brain lesion smaller with FFP
- Way too many variables for us!!
How to answer our plasma transfusion questions?

• Needed a new and SIMPLE model
• Mouse models
  – Previous experience
  – Small blood volume
  – Relatively inexpensive
  – Ethically acceptable
  – Controlled conditions
  – Accessible, dispensable “limb”
  – Gene knockout mice
BECA: Blood Exchange-induced Coagulopathy Approach

- A novel mouse model
- OUT: Whole blood (0.5 ml) from donor mouse
- IN: Washed RBC in 5% Human Albumin Solution (HAS) (0.5 ml)
- Repeat 4X
- Test recipient (BECA) mouse
  - Complete Blood Count
  - PT
  - Blood loss and bleeding time
  - Effect of plasma transfusion
Blood Exchange-induced Coagulopathy Approach (BECA)

- Time (minutes):
  - -15, -10, -5, 0, 30, 45, 46, 47, 49, 64, 65

- PROCEDURE:
  - Anesthetize
  - Cannulate
  - Begin Blood exchange
  - 4 x 0.5 ml exchange
  - Complete Blood exchange
  - Interference via cannula
  - Transect tail
  - Collect shed blood in warm water
  - Remove tail from water
  - Terminal Cardiac Puncture

- DONOR MOUSE:
  - Wash Red Cells
  - Anesthetize
  - Cardiac Puncture
  - Euthanize

- TREATED MOUSE

*not to scale*
## Table 1: Comparison of hematological values between control and BECA mice

<table>
<thead>
<tr>
<th>TEST</th>
<th>Control mice (n=6)</th>
<th>BECA mice (n=8)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>39 ± 3</td>
<td>17 ± 2</td>
<td>&lt; 0.0001 (UTT)</td>
</tr>
<tr>
<td>Hemoglobin (g/l)</td>
<td>134 ± 8</td>
<td>60 ± 8</td>
<td>&lt; 0.0001 (UTT)</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin (MCH, pg)</td>
<td>19.0 ± 0.9</td>
<td>18.6 ± 0.3</td>
<td>0.20 (UTT)</td>
</tr>
<tr>
<td>Erythrocytes (x10^{12}/L)</td>
<td>7.85 ± 0.48</td>
<td>3.60 ± 0.56</td>
<td>&lt; 0.0001 (UTT)</td>
</tr>
<tr>
<td>Leukocytes (x10^9/L)</td>
<td>2.9 ± 2.7</td>
<td>0.44 ± 0.23</td>
<td>0.0047 (M-W)</td>
</tr>
<tr>
<td>Lymphocytes (x10^9/L)</td>
<td>2.7 ± 2.5</td>
<td>0.39 ± 0.20</td>
<td>0.0047 (M-W)</td>
</tr>
<tr>
<td>Platelets (x10^9/L)</td>
<td>820 ± 280</td>
<td>280 ± 130</td>
<td>0.0004 (M-W)</td>
</tr>
<tr>
<td>Fibrinogen (% of post-BECA/pre-BECA value)</td>
<td>NA</td>
<td>20 ± 6</td>
<td>0.008 (M-W)</td>
</tr>
</tbody>
</table>

Values are reported as the mean ± the standard deviation; p values are reported for comparisons of control versus BECA mice values (or pre- versus post-BECA fibrinogen levels in the same mice) by unpaired t-test (UTT) for normally distributed data sets with similar standard deviations or otherwise by Mann-Whitney test (M-W).

- **BECA procedure**
  - ▼ RBC 2X,
  - ▼ platelets 3X,
  - ▼ fibrinogen 5X

- If mouse blood volume is 1.5 ml, 0.5 ml exchange (1/3) would lead to a calculated reduction in fibrinogen or any other non-replaced component (e.g. plasma proteins) to 19.36% of starting values.
BECA induces coagulopathy and bleeding

- BECA mice exhibit ▲PT (1.3 X) versus control
- BECA mice exhibit ▲blood loss (9X) from tail transection
- Next: Test effects of plasma transfusion
Transfusion of normal, FVIII -/-, or Fg -/- plasma in BECA

A

- 300 μl (12 ml/kg body weight) of WT FFP reduced blood loss or bleeding time versus HAS (5% Human Albumin Solution)
- **Answer to Q1**: Yes, plasma transfusion reduces bleeding in this model.
- FFP from FVIII -/- mice was equally effective vs. WT FFP
- FFP from fibrinogen (Fg) -/- mice was ineffective
- **A start on answering Q2**: Fg levels are more important than FVIII levels in determining plasma efficacy at reducing bleeding
But...what if Fg-/- plasma had compensatory changes in other plasma proteins?

- Mixing normal and Fg -/- plasma gave expected dose response
- Adding back purified human fibrinogen to Fg -/- plasma fully restored its ability to limit bleeding
- Fg alone could not restore hemostasis, so Fg and at least one other plasma protein are rate-limiting
- Labile proteins were NOT limiting, since 5 day thawed plasma was still effective
Could we reproduce these results in a different model?

- Dr. Heyu Ni laboratory
- Intravital microscopy to observe the cremaster muscle microvasculature (arterioles)
- Laser beam to injure the vessel wall
- Fluorescently labeled platelets to assess the extent of thrombus (intravascular, non-occlusive blood clot) formation

Blue R et al, Blood 2009; 114:195-201
BECA + laser injury model

- Created coagulopathy via BECA and quantified kinetics of clotting
- WT mFFP or FVIII-/- mFFP not Fg-/- mFFP restored clottability
Is tail transection a good model?

- Variation in blood loss
  - Wide range of blood loss in similarly handled mice
  - Mimics clinical experience
  - We typically assess 15 mice per group

- Humans don’t have tails, use a different vessel/limb
  - Actually transect 2 veins and an artery
  - Saphenous vein puncture (Pastoft AE Haemophilia 2012)
  - Saphenous vein or artery model equally sensitive to anticoagulation (LMW heparin) as tail transection (vein + artery) but differential response to anti-platelet agent (Vaezzadeh N et al J Thromb Haemostasis 2014;112(2):412-8)
Why is BECA sensitive to Fg, not FVIII?

- 16 - 20% FVIII is *enough* to maintain hemostasis
  - Data from Hemophilia A patients & FVIII -/- mice
  - 0.3 ml FVIII -/- plasma transfused into plasma volume of 1.2 ml
  - 20% FVIII X 1.2/1.5 = 16% (just dilution of circulating FVIII)
  - Other factors 16% + 0.3/1.5 = 16% + 20% = 36%
  - 30% pan-factor levels generally considered sufficient

- 20% Fg is *not enough* to maintain hemostasis
  - 2.4 mg/ml = mean [Fg] in mouse plasma
  - 20% = 0.48 g/l; after Fg concentrate 0.86
  - (although mixing mice and men) below 1 g/l Fg transfusion trigger
Conclusions

• Existing animal models are focused on trauma-induced coagulopathy, typically in larger laboratory animals
• Our lab has focused on delineating the relationship between plasma quality and bleeding reduction in mice in a novel model (BECA)
• We have shown Fg is more important than FVIII as a plasma quality marker despite regulatory focus on FVIII
• Next steps: Explore prothrombin complex concentrates, defined mixtures of human coagulation factors as plasma alternatives
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