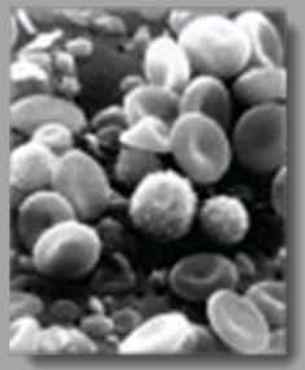


12th Annual Canadian Blood Services International Symposium

Plasma: Transfuse it, Fractionate it or Forget it?

Canadian Blood Services
it's in you to give

Advancing Transfusion Medicine

What Can We Learn from Animal Models of Coagulopathy and Bleeding (about plasma transfusion)?

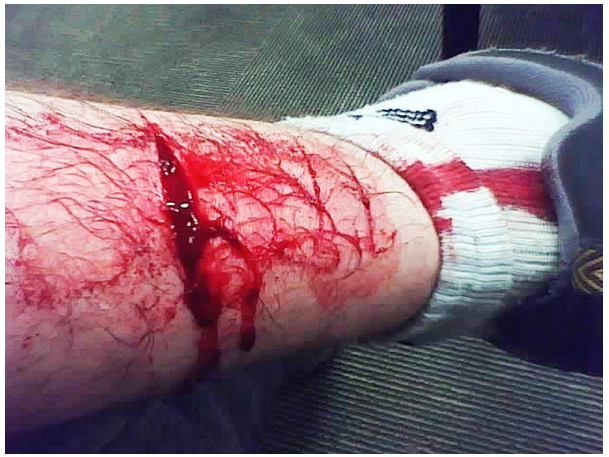
William Sheffield
Associate Director, Research
CBS Centre for Innovation, and
Professor, Pathology and Molecular Medicine, McMaster University

CBS Symposium, Toronto 2014-09-13

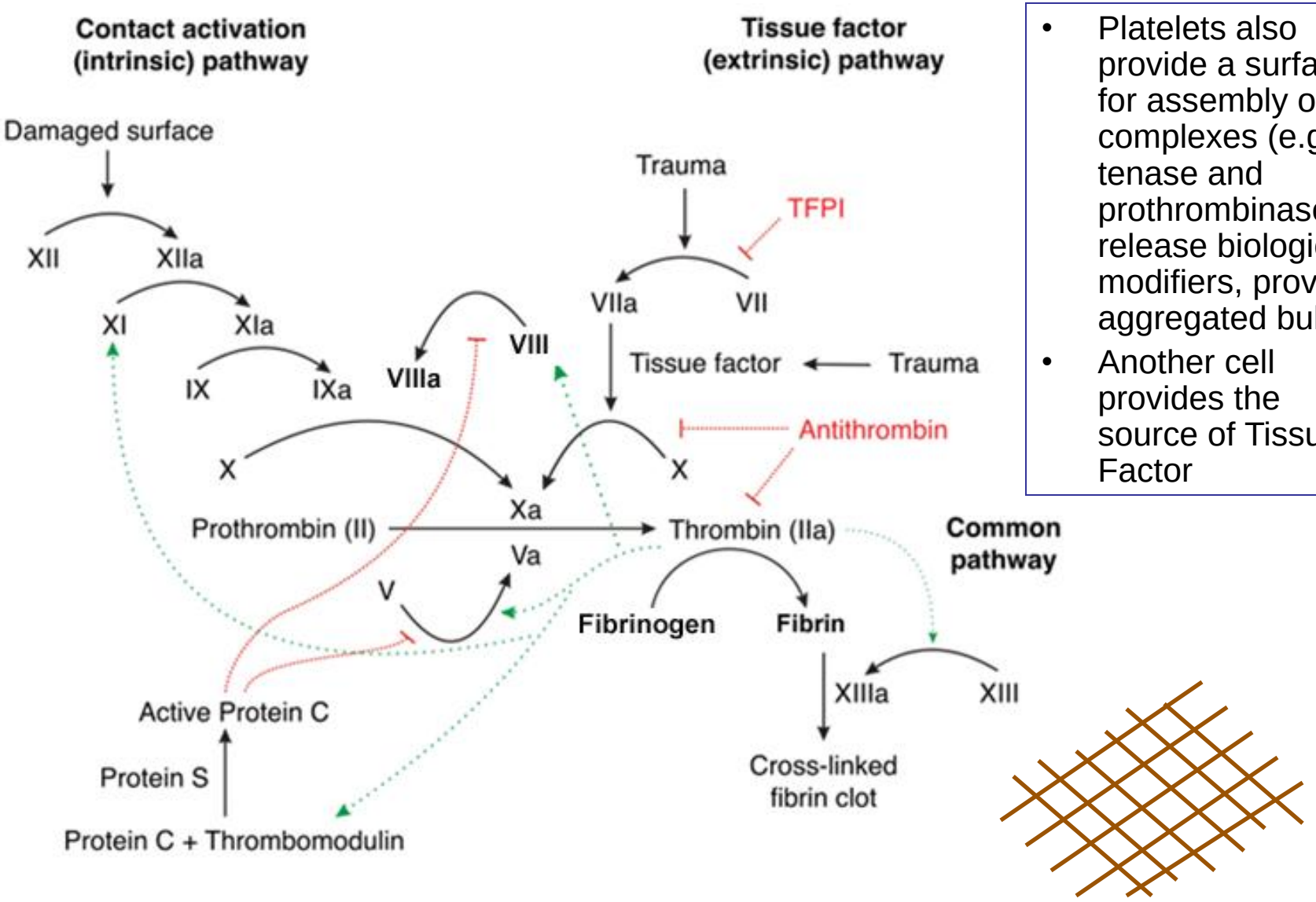
Canadian Blood Services
it's in you to give

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

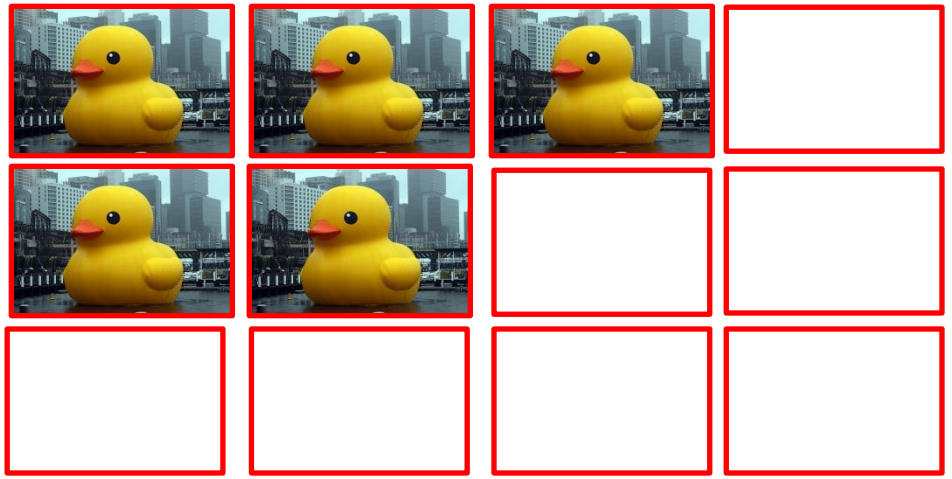


- Platelets also provide a surface for assembly of complexes (e.g. tenase and prothrombinase), release biological modifiers, provide aggregated bulk
- Another cell provides the source of Tissue Factor

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



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)
  - 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."


CIRCULAR OF INFORMATION
For the Use of Human Blood Components

The NEW ENGLAND JOURNAL of MEDICINE

REVIEW ARTICLE

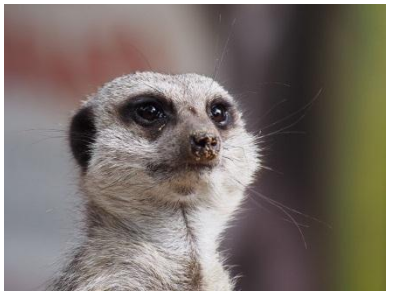
CRITICAL CARE MEDICINE

Bleeding and Coagulopathies in Critical Care

Beverley J. Hunt, M.D.

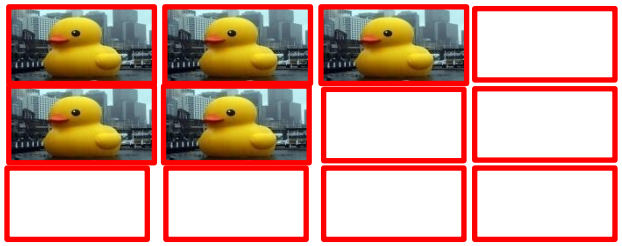
Animal models

- 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?



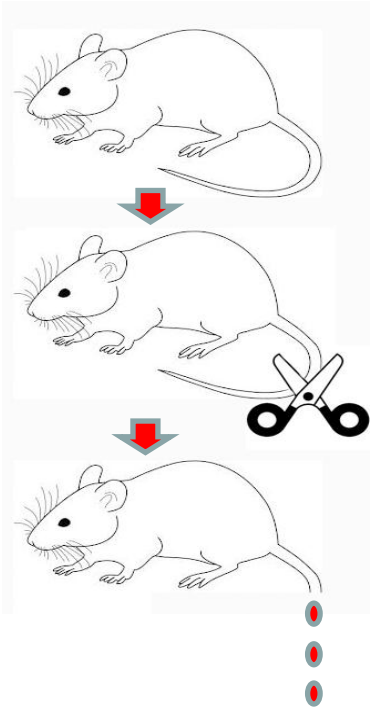
Animal models and bleeding

- 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!!



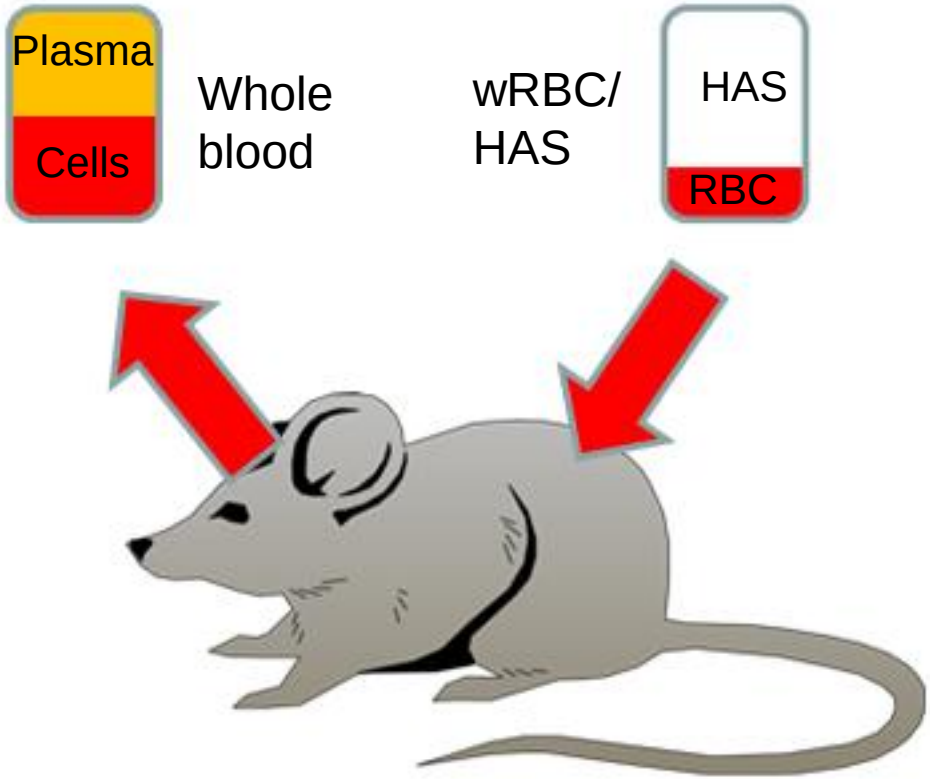
How to answer our plasma transfusion questions?

- 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

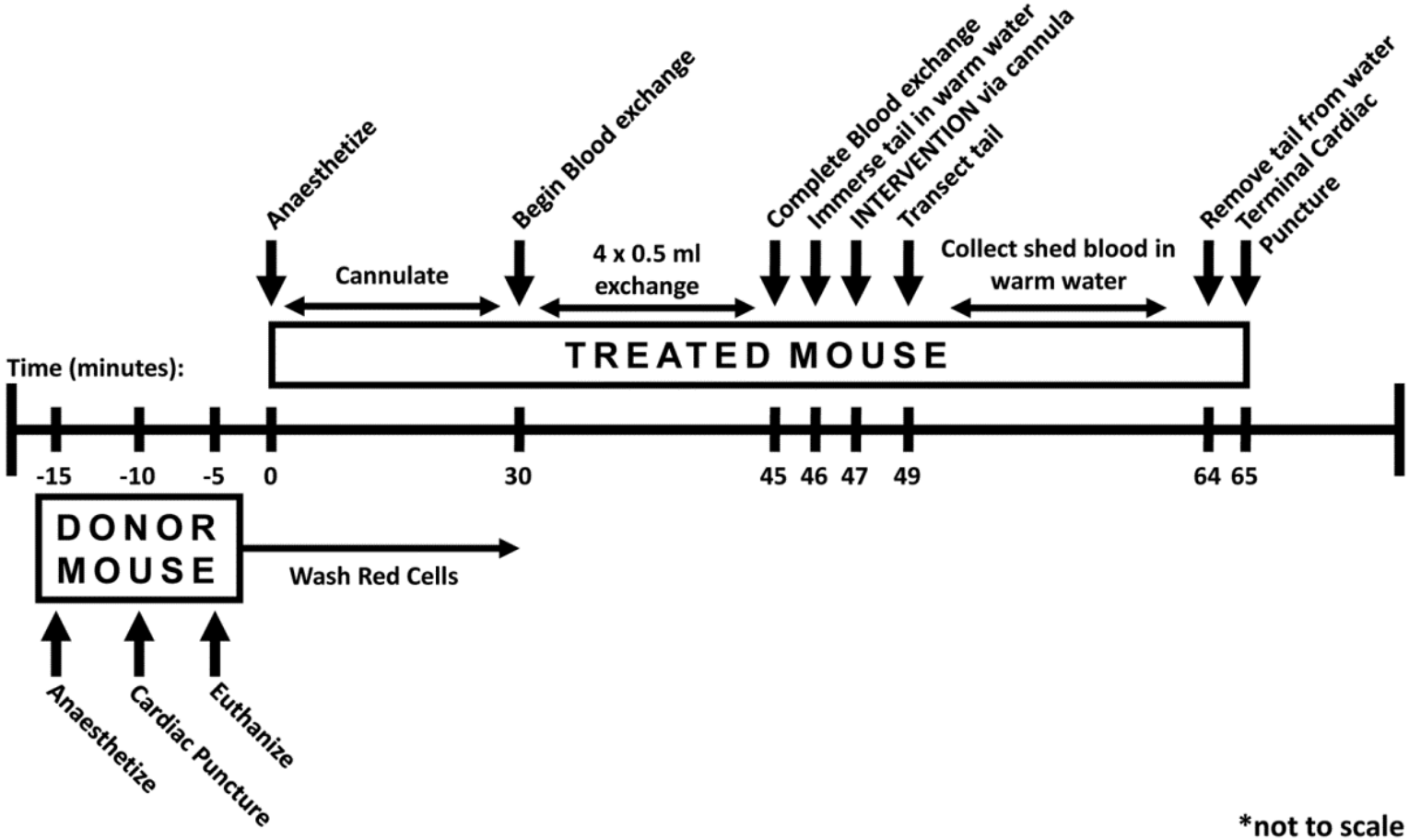


BECA: Blood Exchange-induced Coagulopathy Approach

- 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)



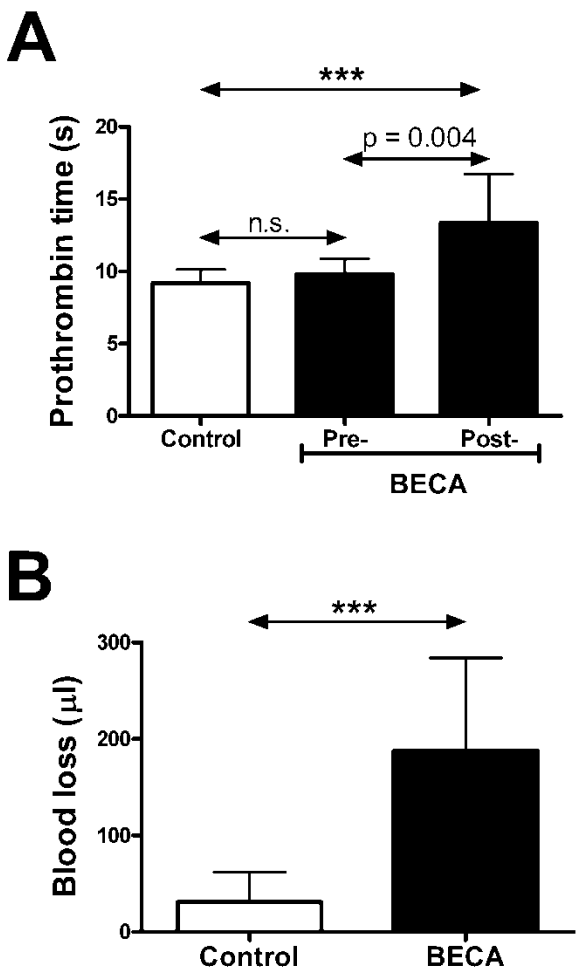
BECA versus control mice

Table 1: Comparison of hematological values between control and BECA mice			
TEST	**Control mice (n=6)**	**BECA mice (n=8)**	**p**
Hematocrit (%)	39 ± 3	17 ± 2	< 0.0001 (UTT)
Hemoglobin (g/l)	134 ± 8	60 ± 8	< 0.0001 (UTT)
Mean corpuscular hemoglobin (MCH, pg)	19.0 ± 0.9	18.6 ± 0.3	0.20 (UTT)
Erythrocytes ($x10^{12}$/L)	**7.85 ± 0.48**	**3.60 ± 0.56**	**< 0.0001 (UTT)**
Leukocytes ($x10^9$/L)	2.9 ± 2.7	0.44 ± 0.23	0.0047 (M-W)
Lymphocytes ($x10^9$/L)	2.7 ± 2.5	0.39 ± 0.20	0.0047 (M-W)
Platelets ($x10^9$/L)	**820 ± 280**	**280 ± 130**	**0.0004 (M-W)**
Fibrinogen (% of post-BECA/pre-BECA value)	NA	**20 ± 6**	0.008 (M-W)
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).			

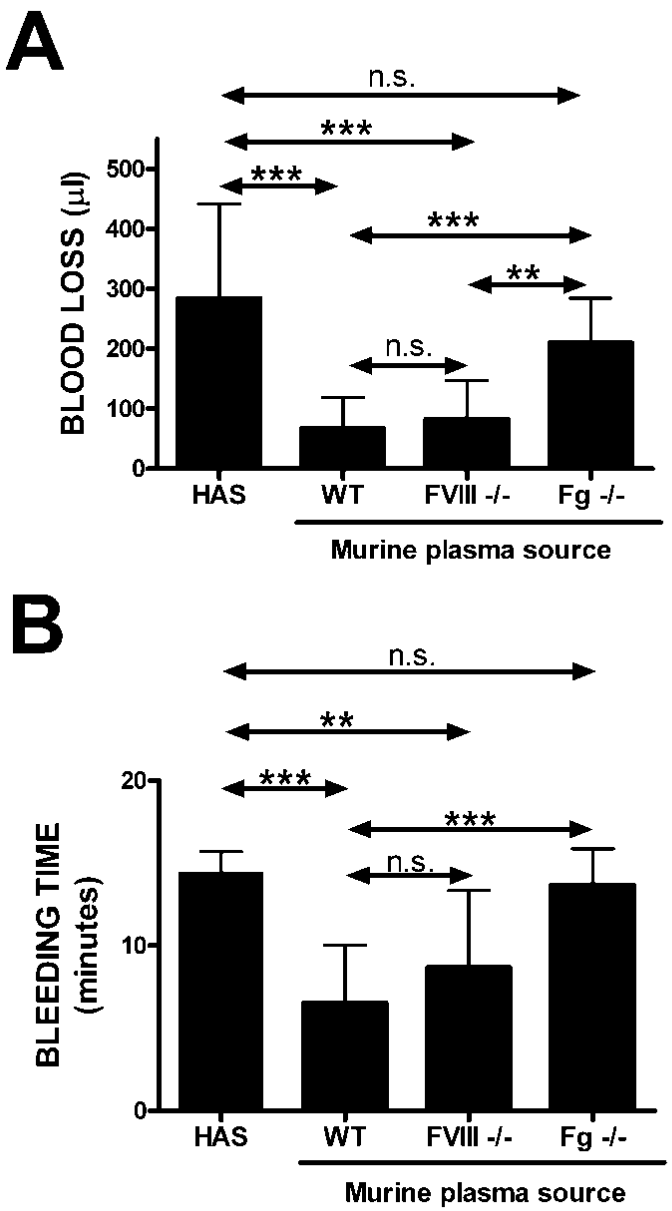
- BECA procedure
  ▼ RBC 2X,
  ▼platelets 3X,
  ▼fibrinogen 5X
- If mouse blood volume is 1.5 ml, 0.5 ml exchange (1/3) would lead to a calculated reduction in fibrinogen or any other non-replaced component (e.g. plasma proteins) to 19.36% of starting values.

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



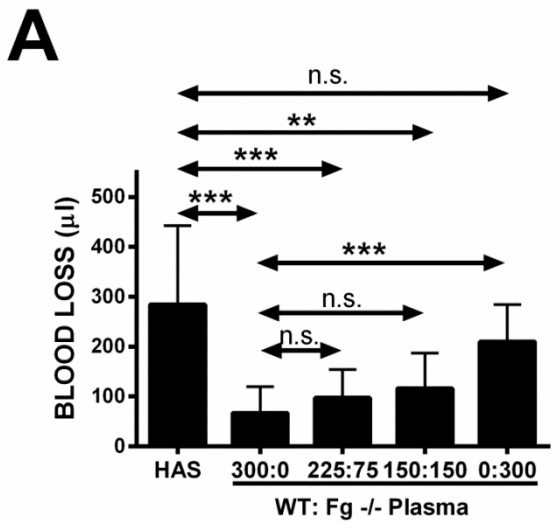
Transfusion of normal, FVIII -/-, or Fg -/- plasma in BECA

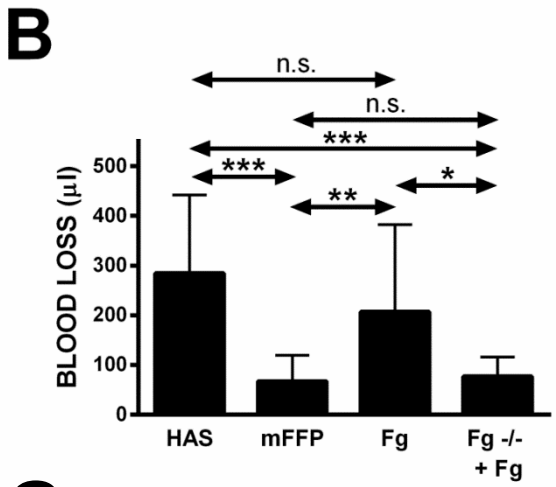


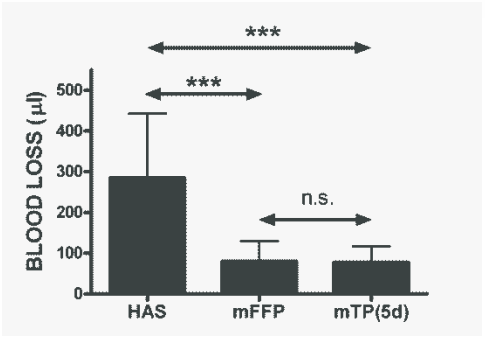
- 300 µl (12 ml/kg body weight) of WT FFP reduced blood loss or bleeding time versus HAS (5% Human Albumin Solution)
- Answer to Q1: Yes, plasma transfusion reduces bleeding in this model.
- FFP from FVIII -/- mice was equally effective vs. WT FFP
- FFP from fibrinogen (Fg) -/- mice was ineffective
- 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

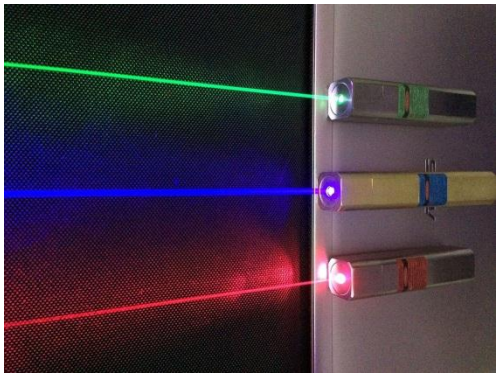


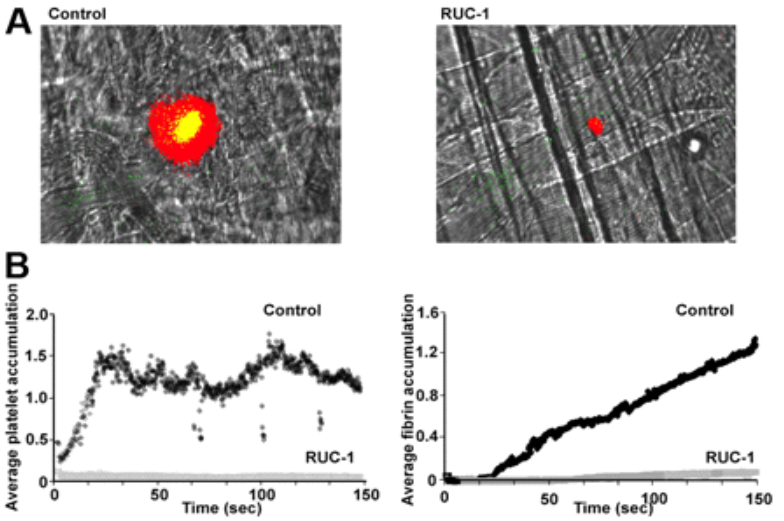




Could we reproduce these results in a different model?

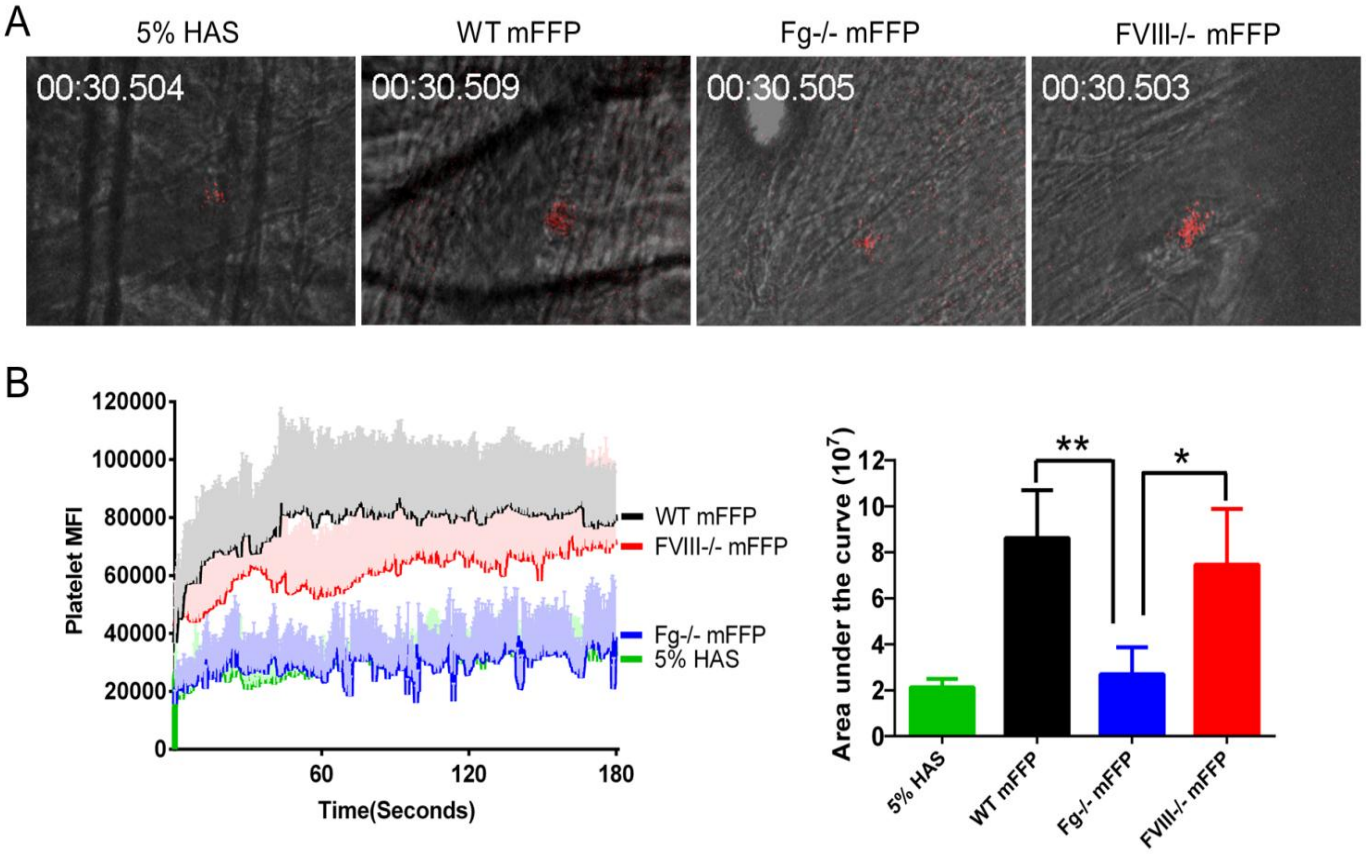
- Dr. Heyu Ni laboratory
- Intravital microscopy to observe the cremaster muscle microvasculature (arterioles)
- Laser beam to injure the vessel wall
- Fluorescently labeled platelets to assess the extent of thrombus (intravascular, non-occlusive blood clot) formation





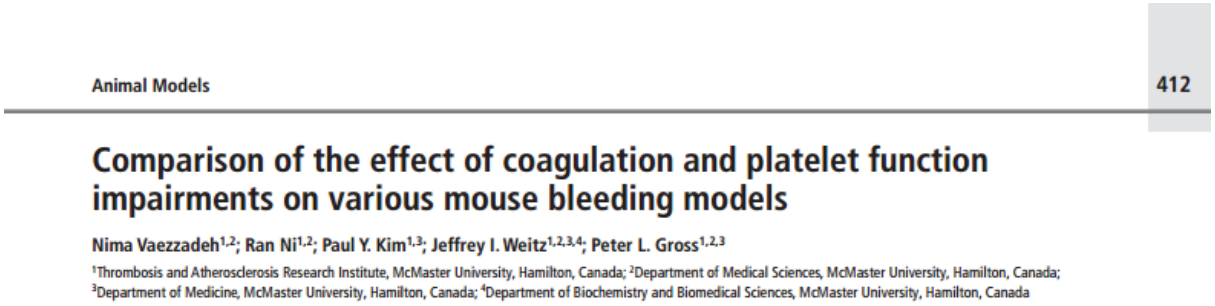
Blue R et al, Blood 2009; 114:195-201

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?



Animal Models 412

Comparison of the effect of coagulation and platelet function impairments on various mouse bleeding models

Nima Vaezzadeh[1,2]; Ran Ni[1,2]; Paul Y. Kim[1,3]; Jeffrey I. Weitz[1,2,3,4]; Peter L. Gross[1,2,3]

[1]Thrombosis and Atherosclerosis Research Institute, McMaster University, Hamilton, Canada; [2]Department of Medical Sciences, McMaster University, Hamilton, Canada; [3]Department of Medicine, McMaster University, Hamilton, Canada; [4]Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Canada

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

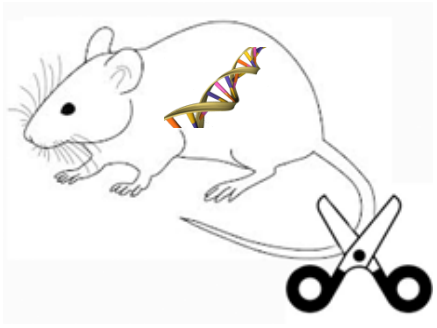
- Humans don't have tails, use a different vessel/limb
  - Actually transect 2 veins and an artery
  - Saphenous vein puncture (Pastoft AE Haemophilia 2012)
  - Saphenous vein or artery model equally sensitive to anticoagulation (LMW heparin) as tail transection (vein + artery) but differential response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

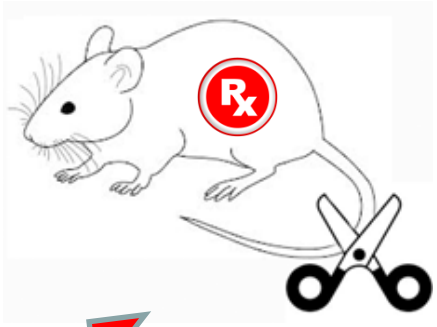
Why is BECA sensitive to Fg, not FVIII?

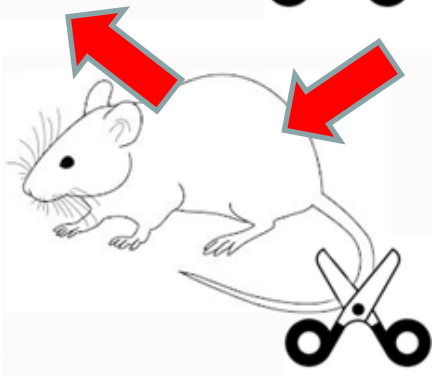
- 16 - 20% FVIII is *enough* to maintain hemostasis
  - Data from Hemophilia A patients & FVIII -/- mice
  - 0.3 ml FVIII -/- plasma transfused into plasma volume of 1.2 ml
  - 20% FVIII X 1.2/1.5 = 16% (just dilution of circulating FVIII)
  - Other factors 16% + 0.3/1.5 = 16% + 20% = 36%
  - 30% pan-factor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







Acknowledgements

- Louise Eltringham-Smith
- Sharon Gataiance
- Melissa Lambourne
- Xi Lei
- Adili Reheman
- Dr. Heyu Ni
- Kimberly Talbot
- Scott Meixner
- Dr. Ed Pryzdial
- Funding: CBS Intramural Operating Grants 2012-2016

Please note: Except where noted, images were created from research data de novo or were obtained from CBS or are public domain images obtained from commons.wikimedia.org.