Current Options and New Developments in Hemophilia

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Disclosures

- Research support to OHSU from:
  - Baxter
  - Pfizer
  - NovoNordisk

Agenda

- Early treatment of hemophilia
- Factor repletion
- Treatment of patients with inhibitors
- Adjuvant therapies
- Future directions
  - Gene therapy
  - Premature termination codon suppression

Abbreviations used

- FVIII = factor VIII
- recFVIII = recombinant factor VIII
- pdFVIII = plasma-derived factor VIII
- FIX = factor IX
- recFIX = recombinant factor IX
- pdFIX = plasma-derived factor IX
- FVIIa = activated factor VII
- recFVIIa = recombinant activated factor VII

History of hemophilia therapy

- Prior to the 1940's-supportive care and whole blood transfusion
  - Low concentration of factors VIII and IX
  - Individuals suffered significant pain and morbidity
  - Average lifespan 27 years
- 1964-Judith Graham Pool described method to produce cryoprecipitate
  - Rich in FVIII and fibrinogen
  - Beginning of home infusions
  - Average lifespan 40 years

History of hemophilia therapy

- 1970's-plasma-derived factor concentrates
  - Pools of 20,000+ donors
  - Made school, work, and travel possible
  - Average lifespan 60 years

However….

- Hepatitis B and C were known to be in plasma supply
- Thought to be an "acceptable" risk in light of drastic improvement in quality of life
- First individual with hemophilia that died from HIV infection reported in 1982
  - Only retrospectively was it discovered that plasma-derived factor was vector
  - HIV was isolated in 1984
  - Heat treatment of plasma-derived factor became standard practice in 1985

Recombinant factor VIII

- FVIII gene sequenced and cloned in 1984
- Because of complex glycosylation and post-translational modification of FVIII protein, recombinant FVIII can only be produced in mammalian cell culture
- First clinical trial of recombinant FVIII concentrate reported in 1990
- First recombinant FVIII concentrate marketed for clinical use in 1992

Recombinant FVIII generations

- First generation
  - Required bovine or human albumin to stabilize FVIII molecule
- Second generation
  - Albumin required during manufacturing process, but removed from final product
- Third generation
  - Albumin free during entire manufacturing process
  - Purification process removes impurities from medium and cell culture

Current FDA-approved recombinant FVIII concentrates

Product	Manufacturer	Viral inactivation steps	Stabilizer	Proteins in product	Viral safety studies
First Generation					
Recombinate	Baxter	1	Human albumin	Bovine serum albumin	Yes
Second Generation					
Kogenate FS	Bayer	2	Sucrose	Human plasma protein	Yes
Helixate FS	Bayer (CSL)	2	Sucrose	Human plasma protein	Yes
Refacto	Pfizer	2	Sucrose	Human serum albumin	Yes
Third generation					
Advate	Baxter	2	Trehalose	None	Yes
Xyntha	Pfizer	5	Sucrose	None	Yes

Viruses in recFVIII concentrates

- Viral transmission has never been reported in any recombinant FVIII product
- Theoretical risk exists in first and second generation products
- Non-viral pathogens such as prions possible in first and second generation products

Recombinant FIX

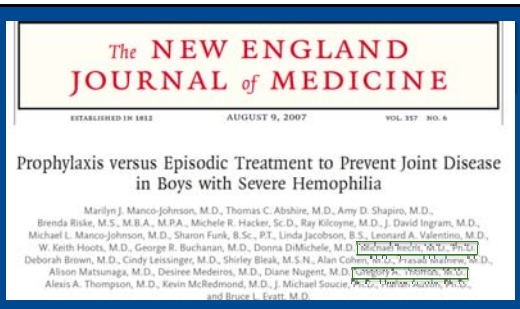
- Human FIX gene was cloned in 1982
- Minor differences in post-translational sulfation between recFIX and pdFIX
- 30% lower *in vivo* recovery of recFIX

Replacement strategies

- Episodic (on-demand)
  - Missing factor is replaced at onset of bleeding
    - 1 unit/kg FVIII = 2% increase in plasma FVIII activity
    - 1 unit/kg FIX = 1% increase in plasma FIX activity
- Prophylactic
  - Administered to prevent bleeding
  - Recommended by:
    - National Hemophilia Foundation
    - World Federation of Hemophilia

Prophylaxis

- Primary prophylaxis
  - Factor given to prevent complications
- Secondary prophylaxis
  - Factor given after onset of complications to prevent recurrence



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Prophylaxis versus Episodic Treatment to Prevent Joint Disease in Boys with Severe Hemophilia

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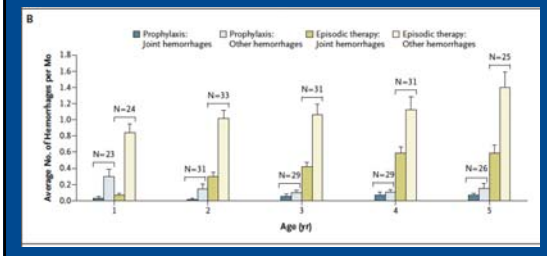
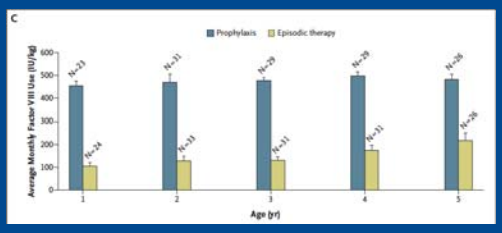
Main results

- At 6 years of age:
  - 93% of boys receiving prophylaxis had normal joints
  - 55% of boys receiving episodic infusions had normal joints
  - Relative risk of joint damage in boys receiving episodic infusions was 6.1
- High titer inhibitors detected in two boys receiving prophylactic infusions
- CNS hemorrhage occurred in three boys receiving episodic infusions

Bleeding episodes



Factor usage



Inhibitors

- Most serious treatment related complication
  - 15-50% in hemophilia A
  - 1-3% in hemophilia B (associated with anaphylaxis)
- Strongest determinant of risk of inhibitor development is mutation type
  - Large deletions
  - Inversions
  - Nonsense mutations
  - Splice site mutations
- No evidence for difference between inhibitor development in those exposed to plasma versus recombinant concentrate

Inhibitors and acute bleeding

- Activated prothrombin complex concentrates
  - Only FEIBA available in United States
  - Contains activated clotting factors, including FVIIa
  - Dosed at 75-100 units/kg every 12 hours
  - Most common side effect is thrombosis
- Recombinant activated factor VIIa
  - Dosed at 90-120 mcg/kg every 2 hours
  - Licensed for treatment of:
    - Hemophilia A or B with inhibitors
    - FVII deficiency
    - Glanzmann thrombasethenia (absence of gp IIb/IIIa)

Inhibitor reduction

- Immune tolerance reduction
- Immunomodulation
- Immunoadsorption and plasmapheresis

Immune tolerance induction (ITI)

- Repetitive doses of factor ± immunosuppressive therapy
- May have initial rise in antibody titer (anamnestic response)
- Immune tolerance must be maintained by continued exposure to infused factor
- Predictors of success of ITI
  - Inhibitor titer < 10 BU at start of ITI
  - Peak inhibitor titer < 200 BU
  - Presence of inhibitor < 2 years prior to start of ITI
  - Low risk mutations (small insertions/deletions, nonsense mutations)

immunomodulation

- First reported in 1965, using mercaptopurine in conjunction with high dose factor replacement
- Agents reported
  - Corticosteroids
  - Cyclophosphamide
  - Azathiaprine
  - Vincristine
  - Cyclosporin
  - Tacrolimus
  - Mycophenolate mofetil
  - Rituximab
  - IVIg

Immunoadsorption/plasmapheresis

- Extracorporeal strategies for removing pathological antibodies rapidly, though transiently
- Plasmapheresis replaces patients plasma with donor plasma, thereby reducing inhibitor titer
- Allows for use of FVIII or FIX concentrate for acute bleeding episodes or invasive procedures
- Immunoadsorption binds Fc portion of IgG to protein A

Adjuvant bleeding therapies

- Desmopressin
- Antifibrinolytic agents
  - ε-aminocaproic acid
  - Tranexamic acid
- Topical agents
  - Fibrin sealants
  - Floseal matrix
  - Topical thrombin

Desmopression

- For use in von Willebrand disease or mild/moderate hemophilia A
- Mechanism of action
  - Increased von Willebrand factor levels with resultant increased FVIII levels
  - Stimulation of platelet adhesion
  - Increased expression of tissue factor
- Dosing
  - Intravenous-0.3 mcg/kg iv every 12 hours up to 3 doses
  - Intranasal-every 12 hours up to three doses
    - < 50 kg: one puff
    - > 50 kg: two puffs

Antifibrinolytics

- Inhibit proteolytic activity of plasmin, therefore inhibiting fibrinolysis
- Indicated for mucosal bleeding:
  - Oral
  - Nasal
  - Menstrual
- Contraindicated in:
  - DIC
  - Thromboembolic disease
  - Upper urinary tract bleeding

Topical agents

- Fibrin sealants
  - Mixture of fibrinogen and thrombin concentrates
  - May contain FXIII or aprotinin
  - From human or bovine plasma
  - > 20% incidence of antibodies against thrombin
- FloSeal Matrix-bovine gelatin/human thrombin
- Thrombin-JMI-bovine thrombin
- BioGlue-bovine albumin/glutaraldehyde
- CoSeal-PEG polymer that cross-links proteins
- Quickclot-zeolite (dehydrates blood)

Future directions

- Factor repletion
- Genetic therapies
  - Gene therapy
  - Premature termination codon suppression

Future of factor repletion

- Main area of research has been prolonging half-life of factor concentrates
- Approaches explored to date:
  - Sustained delivery
  - Chemical modification of factor molecule
  - Genetic alteration

Sustained delivery of FVIII

- Release protein into blood stream more slowly, thereby increasing bioavailability
- Pegylated liposomal FVIII - Bayer
  - Prolonged mean number of days between bleeding episodes
  - No change in measured half-life
  - Pulled from phase 2 trial

Chemically modified FVIII

- Blocks receptor-mediated clearance
- Conjugates FVIII with hydrophilic polymer
  - Polyethylene glycol
  - Sialic acid
- Creates a molecular "shield" around FVIII molecule
- This strategy has been accomplished in many complex proteins
- Unknowns about this strategy
  - Clearance over an extended period of time
  - Alterations in immunologic properties

Genetic alteration of FVIII molecule

- Prevention of proteolysis
  - A2 subunit covalently cross-linked to A3 subunit
  - Activated protein C cleavage sites blocked
- Interference of FVIII clearance
  - Has not yet been accomplished
  - All attempts have resulted in decreased activity
- Generation of fusion proteins
  - FVIII molecule combined with either albumin or Fc fragment of IgG
  - Fc:FVIII fusion protein now in phase 3 trials (Biogen)

Gene therapy

- Goal is to replace dysfunctional gene with an exogenous functional gene
- Hemophilia is perfect condition for gene therapy
  - Relatively prevalent
  - Molecular pathway well studied
  - Caused by single gene mutations
  - Wide therapeutic window
  - FVIII and FIX do not need to be expressed by endothelial cells or hepatocytes
  - Monitoring of factor levels simple

Gene therapy

- Challenges
  - FVIII is a large molecule with a large gene making insertion into gene delivery systems difficult
  - Murine, canine, and non-human primate studies have not predicted the lack of efficacy and adverse events seen in human trials
  - Inhibitor development has been demonstrated

Premature termination codon suppression

- Nonsense mutations of either FVIII or FIX typically cause severe disease
- Caused by:
  - Base pair substitutions
  - Insertions
  - deletions

] Leading to the production of truncated proteins

Premature termination codon suppression

- PTC124 (Ataluren) oral agent that promotes a read-through of nonsense codons.
- Phase I trials have demonstrated the safety of PTC124 in healthy volunteers
- Phase II trials have demonstrated efficacy of PTC124 in treatment of cystic fibrosis
- Currently a phase II trial of 28-day treatment cycles of PTC124 in hemophilia is underway
  - Alternative to intravenous infusions of factor
  - Theoretically decrease risk of of inhibitor formation that complicates factor infusion and gene therapy

Hemophilia research at OHSU

- Basic lab
  - Bone mineral deficiencies in FVIII deficient mice
- Clinical trials
  - PTC124
  - Fc:FVIII fusion molecule
  - Long-acting FVIIa
- Physical therapy protocols
  - Gait abnormalities in bleeding disorders
  - Carbon fiber splints in bleeding disorders