Therapeutic Uses for Blood Products and Enzyme Therapeutics

BIT 230
Walsh Chapter 9
Blood and Blood Products

- Source of traditional biologics
- Blood composed of
  - red blood cells
  - white blood cells
  - platelets
  - plasma (contain cellular elements) - therapeutic proteins come from plasma
Therapeutic Blood Products

- Clotting factors
  - Factor VIIa, VIII, IX and XIII
- Platelet concentrate
- Hemoglobin
- Whole blood (hemorrhage)
- Red blood cells
Risk with Blood Product Use

- Transmission of infectious diseases
  - Hepatitis B and C
  - HIV
  - Cytomegalovirus (CMV) (cold symptoms or more serious in people with HIV)
  - *Treponema pallidum* (syphilis)
  - Trypanosomes
Strict GMP guidelines for using blood products

- Careful screening of all donors and donations
- Use of pathogen removing methods or inactivation during processing steps
- Stringent screening of all finished products
- TRACEABILITY important! - no test 100% accurate (1/42,000 HIV negative blood unit donated is actually positive)
Treatment of Blood Products

- Can’t heat inactivate or chemically treat blood product to remove pathogens
- Rely on careful screening procedures
- Chromatography steps effective in separation of pathogens from the blood product
Use of Platelets

- Key role in blood clotting process
- Administered either prophylactically or therapeutically to prevent or minimize blood loss- people suffering from thrombocytopenia (low number of thrombocytes, alternate word for platelets)
Human Serum Albumin (HSA)

- Most abundant blood protein (Table 9.4 page 356 table of known plasma proteins)
- 65.5 kDa protein
- 60% of total plasma protein
- Responsible for most of blood osmotic pressure (retaining sufficient fluid in blood vessels)
- makes blood “thicker than water”
Therapeutic HSA

- Available in aqueous form or concentrated form
- plasma extender in hemorrhage, shock, burns and edema
- used after surgery
- market value about $1 billion
Hemostasis

- Maintain constant blood volume;
- Mechanisms for hemostasis:
  - congregation and clumping of platelets at site of vascular injury
  - localized constriction of blood vessel at damage site
  - induction of the blood coagulation cascade
Coagulation Cascade

- Fibrinogen converts into fibrin
- Fibrin aggregate at injury site, causing thrombus (clot) formation
- Seals off damages area to prevent further blood loss
- Process: 12+ blood clotting factors involved in cascade
## Coagulation Factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Fibrinogen</td>
</tr>
<tr>
<td>II</td>
<td>Prothrombin</td>
</tr>
<tr>
<td>III</td>
<td>Tissue factor or thromboplastin</td>
</tr>
<tr>
<td>IV</td>
<td>Calcium ions</td>
</tr>
<tr>
<td>V</td>
<td>Proaccelerin (Labile factor)</td>
</tr>
<tr>
<td>VII</td>
<td>Proconvertin (Stable factor)</td>
</tr>
<tr>
<td>VIII</td>
<td>Antihemophilic factor A, Antihemophilic globulin</td>
</tr>
<tr>
<td>IX</td>
<td>Antihemophilic factor B, Plasma thromboplastin component, Christmas factor</td>
</tr>
<tr>
<td>X</td>
<td>Stuart-Prower factor</td>
</tr>
<tr>
<td>XI</td>
<td>Plasma thromboplastin antecedent, Hemophilia C, Rosenthal syndrome</td>
</tr>
<tr>
<td>XII</td>
<td>Hageman factor</td>
</tr>
<tr>
<td>XIII</td>
<td>Fibrin stabilizing factor, Laki-Lorand factor</td>
</tr>
</tbody>
</table>
Coagulation Factors cont’d

- Roman numeral designation (see factors in **Table 9.5 page 359** and next slide)
- Intrinsic and extrinsic pathways
  - Intrinsic - cascade that utilizes only factors that are soluble in the plasma
  - Extrinsic - some factors that are insoluble in the plasma, e.g., membrane-bound factors (factor VII)
See this PowerPoint Presentation

coaulation lecture
Overview of blood coagulation

Vessel Injury
- Adhesion to vWF
- Release of Prostaglandins

Platelet Activation
- Release of ATP, factor V, fibrinogen, HMWK, Ca2+
- Vasoconstriction

Platelet Aggregation
- Platelet Plug
- Thrombin

Coagulation Cascade
- Clot

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Genetic disorders

- Lack of gene expression of clotting factor
- Altered amino acid sequence of clotting factor
- Both intrinsic and extrinsic pathways must work for proper clotting
- Results of mutations bruising or prolonged bleeding
Disorders cont’d

- 90% of characterized disorders deficiency in factor VIII, rest due to factor IX
- treatment administration of whole blood or relevant coagulation factor (purified from whole blood)
- recombinant factors important to minimize risk of exposure to blood pathogens
Factor VIII - Hemophilia

- Hemophilia A
- X-linked recessive disease
- Intact VIII: made up of 2 products, factor VIII and von Willebrand factor (vWF)
- 1-2 million Da
- fully intact VIII needed to enhance activation rate of factor IX of intrinsic system
Hemophilia

- Level of intact factor VIII affects severity of disease (need at least 5% expression)

- Rarer von Willibrand’s disease- lack both components of factor VIII complex

- Administer weekly factor VIII complex for life
rVIII

- Expressed VII:C cDNA in CHO and BHK cells
- Clinical studies show equivalent effects of recombinant and native isolated factor VIII
- Some problems due to immunogenic response
- Same production issues as with any biologic
Factor IX

- Hemophilia B
- rarer disease
- treatment is administration of concentrated factor IX
- produced recombinantly in CHO cells
- also X-linked recessive
Anticoagulants

- Inappropriate clotting dangerous
- Occurs in blood vessels, impedes blood flow
- results in heart attack (coronary thrombosis resulting in m.i.) or stroke (blood vessel that supplies the brain)
Major Anticoagulants

- Heparin
- Warfarin
- Hirudin
- Ancrod
- Protein C
- Dicoumerol
Heparin

- Proteoglycan - make up of large carbohydrate component, so more like a poly-sugar than a protein
- injected drug
- found in mast cells in endothelium of blood vessels
- activates antithrombin (plasma protein)- inactivates clotting factor
- One of few carbohydrate product with therapeutic application
Vitamin K Antimetabolites

- Dicoumarol and warfarin- given orally
- prevent vitamin K-dependent carboxylation of factors II, VII, IX and X
- hinders effective functioning of the coag cascade
- Side effect: prolonged bleeding
- need to choose dosage carefully
Hirudin

- Directly inhibits thrombin by binding to it
- Isolated from leech saliva in *Hirudo medicinalis*
- Short 65 aa polypeptide
- Does not require a co-factor
- Weak immunogen
- Not as much bleeding side effects
- Trade name: Refudan (purifying scheme in Figure 9.15, page 378)
Thrombolytic Agents

- Thrombosis function to plug damaged blood vessel
- after repair, blood clot is removed via enzyme degradation (fibrinolysis)
- when inappropriate clot forms, damage minimized if you can speed up removal of clot
- Agents developed to do so
Thrombolytic Agents

- tPAs (see diagram of action Figure 9.18 Page 382)
  - Activase (Genetech)
  - Ecokinase (Galenus Mannheim)
  - Retavase (Boehringer-Mannheim/Centocor)
  - Rapilsyn (BM)
- TNKase
- Streptokinase
- Urokinase
Tissue plasminogen activator

- Plasmin catalyzes degradation of fibrin in clots (dissolving clot)
- plasmin derived from plasminogen
- plasminogen synthesized and released from kidneys; 90 kDa protein
- tPA potently activates plasminogen
Early tPA

- Studied in 40’s; normal low level made it difficult to characterize
- Bowes melanoma cell line secretes large amounts of tPA
- cloned in “83 and produced in CHO cells
- Genentech got first tPA license in 1987 (Activase)
- need to treat within hours of m.i.
Recombinant tPA

- Several modified forms to improve efficacy (faster-acting, prolonged half-life)

- produced in *E. coli* (Ekokinase, Retavase and Rapilysin)

- no glycosylation leads to prolonged serum half-life
Enzymes as Therapies

- Table 9.12, page 389
- Enzymes given intravenously
- encapsulate or covalently modify enzymes to counteract antigenicity or short half-lives
- capsule needs to be non-immunogenic material
- Liposomes- facilitate entry into cell (used in transfection protocols)
Glucocerebrosidase

- Used to treat Gaucher’s disease
- Disease affects lipid metabolism - these are lipids such as sphingosine, found in brain and neural tissue, associated with myelin sheath of nerves
- Symptoms: enlargement of liver and spleen, mental retardation
Glucocerebrosidase cont’d

- Genzyme Corp. in Boston - marked in 1991 - brand name Ceredase - taken from placenta
- administration to Gaucher’s patients relieve symptoms of disease
- Cerezyme - recombinant version produced in CHO cells
- $200 million market
Digestive aids

- Used in compromised digestive functions, such as cystic fibrosis
- another e.g. - lactase, aids in lactose intolerance
- pepsin- degradation of dietary protein
- pancreatin - degradation of dietary carbohydrate, fat and protein