Session 1 Topics

- Review of coagulation and the vascular phase of hemostasis
- Unfractionated heparin
- Low molecular weight heparin

Coagulation Pathway

Vascular Phase of Hemostasis

- Platelet adhesion to injured vascular surfaces
  - Requires von Willebrand factor and fibrinogen
  - Is irreversible
- Platelets undergo release reaction
  - Liberate ADP
- ADP induces platelet to platelet aggregate formation
- Conversion of arachidonic acid to thromboxane promotes platelet aggregate formation
- White thrombus, platelet plug forms

Unfractionated Heparin Chemical Composition

- Sulfated glycosaminoglycan
- Polysaccharide chains with molecular weights of 30,000 – 100,000
- Chains alternating N-acetylglucosamine and glucuronic acid
- Purified heparin polysaccharide chains of 3,000 – 35,000 with average molecular weight of 12,000

Action of Unfractionated Heparin

- Has no anticoagulant action of itself
- Binds to and accelerates action of antithrombin
- Induces a conformational change in the antithrombin molecule
- Heparin splits off the antithrombin when antithrombin binds thrombin or Xa
- Heparin then attaches to another antithrombin -a true catalyst
Other Action of Heparin

- Accelerates the neutralization of thrombin by Heparin Cofactor II
- Heparin Cofactor II also accelerated by heparin sulfate from endothelial cells

Heparin Sources

- Bovine lung tissue
- Porcine intestinal mucosa
- Bovine lung rarely used due to increased incidence of heparin induced thrombocytopenia (HIT)

Pharmacokinetics of Heparin

- Intravenous administration – immediate anticoagulation half life of one hour; anticoagulant effect for 2 – 6 hours
- Subcutaneous administration – peak anticoagulation at 4 hours with effect up to 12 hours
- In high doses, most is excreted in the urine unaltered

Use of Heparin

- Immediate anticoagulant
- Activity is variable, requires monitoring
- Used for initial treatment of DVT and PE
- Used in open heart surgery
- Used to cap intravenous lines

Factors Affecting Anticoagulant Effect

- Level of Antithrombin
- Heparin binding to acute phase proteins
- Release of platelet factor 4

Tests for Monitoring Heparin

- Thrombin time – rarely used due to extreme sensitivity
- Activated clotting time (ACT) – widely used in OR for monitoring high levels of heparin used in cardiopulmonary bypass
- Activated partial thromboplastin time (APTT) – most widely used
- Specific assay based on Xa inhibition with chromogenic substrate
Monitoring Heparin Therapeutic Range

- Therapeutic range 0.3 – 0.7 units/ml by specific assay
- APTT clotting time corresponding to range of 0.3-0.7 units/ml

Establishment of Therapeutic Range for APTT

- Brill-Edwards Method
  - Measure heparin level in at least 30 patients on heparin only
  - Do APTTs on all specimens
  - Calculate dose response curve by regression analysis
  - Therapeutic range is APTT range that corresponds to 0.3 – 0.7 units/ml
  - Determined from dose response curve using regression analysis
- Problems for small hospital
  - Getting enough patients
  - Doing heparin assay

Example of Brill-Edwards Method

<table>
<thead>
<tr>
<th>Specimen</th>
<th>APTT</th>
<th>Heparin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>0.15</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>0.38</td>
</tr>
<tr>
<td>3</td>
<td>55</td>
<td>0.45</td>
</tr>
<tr>
<td>4</td>
<td>88</td>
<td>0.85</td>
</tr>
<tr>
<td>5</td>
<td>75</td>
<td>0.70</td>
</tr>
<tr>
<td>6</td>
<td>65</td>
<td>0.55</td>
</tr>
<tr>
<td>7</td>
<td>35</td>
<td>0.25</td>
</tr>
<tr>
<td>8</td>
<td>47</td>
<td>0.35</td>
</tr>
<tr>
<td>9</td>
<td>68</td>
<td>0.72</td>
</tr>
<tr>
<td>10</td>
<td>98</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Brill-Edwards cont . . .

APTT at 0.30 units 47 sec

APTT at 0.7 unit 75 sec

Thus therapeutic range is 47 – 75 sec approximate

Specific Heparin Assay

- Based on inhibition of Xa
- Heparin forms complex with antithrombin (heparin-AT)
- Heparin-At +Xa → AT-Xa + Xa
- Free Xa + chromogenic → substrate peptide + Paranitroanaline

Heparin Assay
Heparin Neutralization

- Protamine sulfate in vivo and in vitro
- Polybrene in vitro only

Allergic Reactions to Protamine Sulfate

- Patients with allergy to fish
- Prior exposure to protamine
  - Prior neutralization of heparin
  - Protamine in some insulin preparations
- Men who have had a vasectomy

Adverse Effects of Heparin

- Hemorrhage
- Heparin induced thrombocytopenia (HIT)
- Osteoporosis

Overdose of Heparin

A 58 year old female was undergoing an elective colonoscopy. The patient suddenly began to bleed from a venipuncture site and an IV site. The physician noticed bleeding from an anal fissure. The patient had no prior history of any type of bleeding disorder and had never experienced excessive bleeding with several surgical procedures. The laboratory results were as follows:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin Time (9-12 sec)</td>
<td>&gt;100 sec</td>
</tr>
<tr>
<td>With equal volume of normal plasma</td>
<td>70 sec</td>
</tr>
<tr>
<td>Prothrombin Time (11-13sec)</td>
<td>80 sec</td>
</tr>
<tr>
<td>With equal volume of normal plasma</td>
<td>40 Sec</td>
</tr>
<tr>
<td>Activated Partial Thromboplastin Time(25-36 sec)</td>
<td>&gt;100 sec</td>
</tr>
<tr>
<td>With equal volume of normal plasma</td>
<td>65 sec</td>
</tr>
<tr>
<td>Platelet Count 140,000-400,000/mm3</td>
<td>255,000/mm3</td>
</tr>
<tr>
<td>Fibrinogen (200-400mg/dl)</td>
<td>75 mg/dl</td>
</tr>
<tr>
<td>Reptilase time (10-13 sec)</td>
<td>11 sec</td>
</tr>
</tbody>
</table>

Overdose of Heparin Discussion

Patient had an IV that was to be capped with 1 ml of 1000 unit/ml heparin. RN used vial with 10,000 unit/ml heparin by mistake. 4 hr later thrombin time was 12 sec. Heparin assays were not common place at this time but would have been most helpful.

Heparin Induced Thrombocytopenia

- Type I platelet count drops slightly but returns to normal while heparin is continued; not antibody mediated
- Type II severe antibody mediated thrombocytopenia with life threatening arterial and venous thrombosis if heparin is continued
Heparin Induced Thrombocytopenia Type II

- Potentially fatal complication of heparin therapy due to the development of a heparin dependent antibody against platelet factor 4
- Thrombocytopenia results from activation of platelets by complex of IgG, heparin and platelet factor 4
- Platelet activation causes release of ADP which causes platelet aggregate formation

Diagnostic Features of HIT Type II

- Platelet count of less than 100,000/mm³ or count of less than 50% of the platelet count before heparin was started
- Occurs 5-12 days after heparin is started
- Abnormal results in tests for antibody to platelet factor 4 or heparin mediated platelet activation
- Occurrence of arterial or venous thrombus formation while on heparin

Tests for HIT Type II

- Elisa assay for antibody to platelet factor 4
- Release of radio-labeled serotonin from normal donor platelets
- Platelet aggregation
- Daily platelet counts recommended for all patients on heparin

Heparin Induced Platelet Aggregation

Case of Heparin Induced Thrombocytopenia (HIT)

A 65 year old male had undergone heart surgery 7 days ago. His platelet counts were as follows:

<table>
<thead>
<tr>
<th>Day</th>
<th>Platelet Count (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admission</td>
<td>275,000/mm³</td>
</tr>
<tr>
<td>Day 1 post op</td>
<td>175,000/mm³</td>
</tr>
<tr>
<td>Day 2 post op</td>
<td>180,000/mm³</td>
</tr>
<tr>
<td>Day 3 post op</td>
<td>170,000/mm³</td>
</tr>
<tr>
<td>Day 4 post op</td>
<td>185,000/mm³</td>
</tr>
<tr>
<td>Day 5 post op</td>
<td>160,000/mm³</td>
</tr>
<tr>
<td>Day 6 post op</td>
<td>172,000/mm³</td>
</tr>
<tr>
<td>Day 7 post op</td>
<td>185,000/mm³</td>
</tr>
</tbody>
</table>

Case of HIT (cont.)

Platelet factor 4 antibody – heparin dependent antibody present. Heparin was discontinued and patient started on Argatroban. Platelet count 24 hours later was 150,000/mm³. Patient made an uneventful recovery.
Treatment of HIT Type II
• Stop heparin
• Use direct thrombin inhibitors
  – Argatroban
  – Lepirude (Refludan®)
• Warfarin is contraindicated

Low Molecular Weight Heparin
• Made by enzymatic or chemical depolymerization
• Lower molecular weight 4,000 – 5,000
• Loss of most anti IIa activity but retain anti Xa
• Predicable anticoagulant activity

Administration and Monitoring
Low Molecular Weight Heparins
• Given subcutaneously
• Peak level at 4 hours post injection
• Half life of 4.5 hours with significant activity at 12 hours

Laboratory Monitoring
Low Molecular Weight Heparin
• Usually not required
• Assayed by inhibition of Xa using a chromogenic substrate
• Same assay as unfractionated heparin but different calibrator
• APTT not reliable but may be slightly prolonged

Conditions Requiring Monitoring
of Low Molecular Weight Heparin
• Patients with renal insufficiency
• Exceptionally large or small patients
• Newborn and children
Advantages of Low Molecular Weight Heparins

• Predictable anticoagulant action
• Does not require monitoring for most patients
• Lower incidence of HIT
• Lowest incidence of osteoporosis

Disadvantages of Low Molecular Weight Heparin

• Not easily reversed by protamine sulfate
• Longer half life than unfractionated heparin
• More expensive

Low Molecular Weight Heparins in Common Use

• Enoxaparin (Lovenox®)
• Dalteparin (Fragmin®)
• Both calibrated against the same standard

Questions?

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Thank You!