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None

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Learning Objectives

Upon completing the reading and answering the learning assessment questions, you should be able to:

1. Understand the mechanism of action of heparin, low molecular weight heparin and synthetic heparinoids.
2. Compare the advantages and disadvantages of heparin, low molecular weight heparin, synthetic heparinoids and direct thrombin inhibitors.
3. Understand when laboratory monitoring of therapeutic anticoagulants is necessary.
4. Learn the clinical indications for use of non-heparin anticoagulants.
INTRODUCTION

In healthy individuals, blood fluidity is maintained via normal hemostasis, which involves a subtle balance between procoagulants and anticoagulants. In the case of injury, clot formation occurs via a complex coagulation pathway. Concomitantly, the anticoagulant pathway is activated, in order to keep the clot localized and ultimately have it dissolved when it is not necessary. Pathologic “thrombosis” occurs when pro-coagulant stimuli overpower the natural anticoagulant and fibrinolytic systems. This may result in venous thromboembolism (VTE) and consequent pulmonary emboli (PE).

Monitoring and prophylaxis of patients at risk of developing PE can prevent mortality and morbidity. Traditional anticoagulation therapy with heparin and warfarin, although time tested and effective, has its own set of complications such as hemorrhage, paradoxical thrombosis, and immune reactions. With easier administration and minimal need for monitoring, newer targeted “ideal” anticoagulants are available and continue to be studied in patient populations. Food and Drug Administration (FDA) approval has been granted to many of these drugs recently. These newer anticoagulants, along with warfarin and unfractionated heparin (UFH), are discussed.

ANTICOAGULANT THERAPY

The anticoagulant therapeutic agents can be classified in several ways. One classification depends on the mode of administration, oral and parenteral, whereas another involves the anticoagulant mechanism of action, such as Vitamin K antagonists (warfarin is the prototype drug), binding to antithrombin (heparin and its derivatives), or direct thrombin inhibitors and factor Xa inhibitors (See Figure 1).

Specifically, this educational review focuses on parenteral antithrombotic agents such as heparin derivatives (low molecular weight heparin (LMWH), ultra low molecular weight heparin (ULMWH), and fondaparinux, which is a synthetic heparinoid as well as an indirect factor Xa inhibitor), parenteral and oral direct thrombin inhibitors (DTI), direct factor Xa inhibitors (which are oral), and danaparoid. Warfarin therapy will not be discussed here.

Figure 1: Coagulation pathway and anticoagulants
HEPARIN

Proprietary prescription heparin is derived from mucosal tissue of porcine intestine and is a carbohydrate polymer composed of approximately 45 monosaccharide units, on an average. Heparin functions by potentiating antithrombin action (Figure 2), but nonspecific interactions can decrease dose predictability and increase risk of bleeding. For example, heparin’s interaction with platelet proteins (ie, platelet factor 4) can trigger antibody formation and immune complex formation, leading to platelet activation and aggregation and, ultimately, thrombocytopenia and thrombosis. This paradoxical effect is referred to as heparin-induced thrombocytopenia (HIT) syndrome. Another disadvantage of long-term heparin therapy is bone loss or osteoporosis as it suppresses osteoblasts and activates osteoclasts. In addition, heparin resistance can occur, requiring higher dosages for therapeutic effect. Heparin is parenterally administered and its bioavailability is affected by age, location of thrombus, weight, and liver or kidney disease. Of note, though, heparin’s action can be completely neutralized by the administration of protamine sulfate, which is very beneficial during bleeding episodes.

Figure 2: Mechanism of action of heparin

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*The inhibition activity of the heparin-AT complex on IIa is 10 times higher as compared to inhibition of factor Xa. At therapeutic concentrations, heparin, in addition to inhibition of thrombin, also causes Xa inhibition as most of its polysaccharide chains are longer than 18 units. Factor Xa can thus be indirectly inhibited by heparin binding solely to AT. Also, heparin binds to non-specific proteins. At therapeutic concentrations, only 33% of the administered heparin is available to assist in anticoagulant activity, as that is the proportion which contains the specific pentasaccharide sequence required to bind to AT.
LOW MOLECULAR WEIGHT HEPARINS (LMWHs)

Chemical and enzymatic depolymerization of UFH yields LMWH fragments, which have one-third the length of the heparin. Compared to UFH, the advantages of LMWH include:

1. Relatively preferential anti-Xa activity when compared to anti-IIa activity, which is safer and more effective
2. Lower dosage required
3. Improved predictability of administered dose
4. Less incidence of HIT
5. Less incidence of osteoporosis
6. Laboratory monitoring not required in most patients

CLINICAL INDICATIONS OF LMWH

The above advantages have made LMWH a popular choice both for VTE prophylaxis and for the treatment of VTE, with or without PE, as well as in acute coronary syndromes such as unstable angina and non Q-wave myocardial infarction (MI).

ANTICOAGULATION MECHANISM OF LMWH

On average, LMWHs consist of 15 monosaccharide unit chains per molecule and irreversibly inhibits factor Xa by binding to and potentiating AT. In contrast to UFH, LMWH functions primarily by inhibiting factor Xa rather than thrombin. In addition, LMWH exhibits limited non-specific binding compared to UFH; therefore, it has more predictable dose-response characteristics and increased plasma half-life. Clinical laboratory testing is usually only necessary in neonates, pregnant women, and those with kidney dysfunction. The decreased incidence of HIT can be explained by the limited binding of LMHW to platelet factor 4 (PF4) as compared to UFH, but the risk is not completely eliminated. The risk of osteoporosis is also lower due to the reduced binding to osteoblasts.

LMWHs are administered subcutaneously (SC), and have a half-life of approximately 3 - 4 hours. The serum peak levels are reached at 2 - 4 hours and the trough occurs just before the next dose at 12 hours. If needed, therapy is best monitored with the Heparin assays (Anti- Xa assay). The specimen used for Anti- Xa assay is generally collected 4 hours after administration, which corresponds to the highest LMWH levels. Baseline laboratories should be obtained before initiation and every 3 - 4 days while on treatment.

ULTRA-LOW MOLECULAR WEIGHT HEPARIN

Selective depolymerization of heparin yields ULMWP. ULMWHs have higher anti-Xa to anti-IIa activity ratios (ranging from 8:1 to 80:1). This makes them efficient antithrombotic agents with fewer adverse risks such as bleeding and HIT. However, no drug from this class is currently available in the United States.
Fondaparinux is a synthetic analog of the unique pentasaccharide sequence in heparin. It binds to AT and causes irreversible inhibition of factor Xa. The drug has no action against thrombin as it cannot bind simultaneously to AT and thrombin due to its small size (See Table 1).

**Table 1: Characteristic features and uses of fondaparinux**

| Clinical use | To treat VTE with or without PE after surgery (5 - 10 mg SC)  
|             | Prophylaxis before major orthopedic and abdominal surgeries (2.5 mg SC) |
| Bioavailability | 100% |
| Half life | 18 hours |
| Dosage | Once daily, available as prefilled syringes, parenteral |
| Laboratory surveillance | Not required, but if necessary, the factor Xa inhibition assay can be used |
| Metabolism | Kidney |
| Contraindication | Severe renal dysfunction  
|             | For prophylaxis, in patients weighing less than 50 kg as increased risk of bleeding  
|             | For spinal or epidural anesthesia as risk of hematoma |
| Advantage | Does not cause HIT as it does not bind to platelets or PF-4  
|           | Can be used in patients with sensitivity to animal products or religious considerations |

Danaparoid is a LMWH, mainly used to treat HIT. It is currently not sold in the US.
**DIRECT THROMBIN INHIBITORS**

DTI mediate their action by binding to thrombin without AT activation. DTIs were primarily developed to overcome limitations of warfarin, heparin, and its derivatives. The advantages of DTIs are the following:

1. Directly inhibit clot bound and free thrombin
2. Do not require cofactor such as AT
3. More predictable anticoagulant response as there is no nonspecific binding to plasma proteins
4. Inhibit thrombin induced platelet aggregation
5. Absence of immune mediated thrombocytopenia
6. Less interference with food or drugs

FDA has approved four parenteral DTIs—hirudin derived recombinant forms [lepirudin (IV/SC), desirudin(SC)], the synthetic hirudin analog bivalirudin (IV), and synthetic argatroban (IV). Dabigatran etexilate is the **only oral** form of DTI approved by FDA.

**DTI MECHANISM OF ACTION**

All DTIs bind directly to the active site of thrombin with variable affinity (Figure 3), but their clinical uses and monitoring needs vary (Table 2). Baseline PT (prothrombin time) and aPTT (activated partial thromboplastin time) should be determined, and dosage can be monitored and adjusted to the targeted aPTT.
Table 2: DTIs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Clinical Uses</th>
<th>Half-life</th>
<th>Site of metabolism</th>
<th>Monitoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepirudin</td>
<td>Treat HIT</td>
<td>2 hours</td>
<td>Kidney</td>
<td>aPTT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Target ratio 1.5-2.5</td>
</tr>
<tr>
<td>Desirudin</td>
<td>Prevent DVT after major surgery</td>
<td>1 - 3 hours</td>
<td>Kidney</td>
<td>Not necessary</td>
</tr>
<tr>
<td>Bivalirudin</td>
<td>In patients with unstable angina undergoing percutaneous coronary intervention (PCI)</td>
<td>25 minutes</td>
<td>Proteolysis in liver and 20% excreted by kidney</td>
<td>Activated clotting time (ACT)*</td>
</tr>
<tr>
<td>Argatroban</td>
<td>To prevent or treat thrombosis in HIT and in patients with or at risk of HIT when undergoing PCI</td>
<td>45 minutes</td>
<td>Liver</td>
<td>aPTT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Target ratio 1.5 - 3.0</td>
</tr>
<tr>
<td>Dabigatran</td>
<td>To prevent stroke and systemic embolism in non-valvular atrial fibrillation (AF)</td>
<td>12 - 14 hours</td>
<td>Kidney</td>
<td>Not necessary</td>
</tr>
<tr>
<td></td>
<td>To treat and reduce the risks of DVT and PE</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*A second dose of bivalirudin is considered if the ACT is not higher than 300 sec, 5 min after the first bolus dose. DTIs are metabolized in the kidneys are contraindicated in patients with kidney disease while argatroban is contraindicated in cases of severe liver failure. aPTT.

Certain DTIs (eg, lepirudin, desirudin) can insight an immune response that decreases excretion of the drug, leading to increased DTI levels. This can increase the risk of bleeding and anaphylactic reactions. In contrast to heparin, no antidote is available for DTIs. Recombinant factor VIIa and prothrombin complex are being explored for rapid reversal of dabigatran in life threatening bleeding.

**FACTOR XA INHIBITORS**

Factor Xa (FXa) inhibitors are a class of anticoagulants that directly bind to and selectively inhibit FXa, blocking the formation of thrombin. FXa inhibitors were primarily developed to overcome limitations of warfarin and heparin for long-term anticoagulation management. The advantages of FXa inhibitors are the following:

1. Oral administration once or twice daily
2. Rapid response of action (peak serum concentrations 1 - 3 hours)
3. Greater antithrombotic activity as positioned upstream in the coagulation pathway
4. No monitoring required
5. Less drug interactions than warfarin
6. Predictable anticoagulant response
7. Both renal and fecal excretion
Rivaroxaban and apixaban are FDA-approved oral direct FXa inhibitors (Table 3). No parenteral direct FXa inhibitors are available.

**Table 3: Comparison of rivaroxaban and apixaban**

<table>
<thead>
<tr>
<th></th>
<th>Rivaroxaban</th>
<th>Apixaban</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical Uses</strong></td>
<td>Prophylaxis of DVT and non-valvular atrial fibrillation</td>
<td>Treatment of atrial fibrillation-induced embolism.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prophylaxis before hip or knee replacement surgery.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treatment and prophylaxis of VTE</td>
</tr>
<tr>
<td><strong>Dosing</strong></td>
<td>15 or 20 mg tablet once or twice daily with food</td>
<td>Once daily</td>
</tr>
<tr>
<td><strong>Half life</strong></td>
<td>7 - 11 hours</td>
<td>12 hours</td>
</tr>
<tr>
<td><strong>Bioavailability %</strong></td>
<td>80</td>
<td>~50</td>
</tr>
<tr>
<td><strong>Metabolism</strong></td>
<td>CYP3A4/5 *, P-glycoprotein</td>
<td>CYP3A4/5 *, P-glycoprotein</td>
</tr>
<tr>
<td><strong>Renal clearance</strong></td>
<td>36%</td>
<td>25%</td>
</tr>
<tr>
<td><strong>Food</strong></td>
<td>Increases the bioavailability</td>
<td>No effect</td>
</tr>
<tr>
<td><strong>Contraindication</strong></td>
<td>Severe renal failure</td>
<td>Severe hepatic failure</td>
</tr>
<tr>
<td></td>
<td>Moderate hepatic impairment</td>
<td></td>
</tr>
</tbody>
</table>

* Cytochrome 3A4P enzyme inducing drugs: bosentan, efavirenz, etravirine, fosphenytoin, nafcillin, nevirapine, oxcarbazepine, phenobarbital phenytoin, primidone, rifabutin, rifapentine. Cytochrome3A4P enzyme inhibiting drugs: etoconazole, ritonavir, clarithromycin, erythromycin. Drugs that inhibit both CYP3A4/5 and P-glycoprotein: ketoconazole, itraconazole, lopinavir-ritonavir, indinavir-ritonavir, ritonavir, conivaptan. Drugs which induce both CYP3A4/5 and P-glycoprotein: carbamazepine, dexamethasone, rifampin, St John's wort. Drugs that either inhibit CYP3A4/5 and P-glycoprotein as opposed to both do not seem to affect or alter rivaroxaban. It is recommended that dose of apixaban be decreased in patients receiving dual inhibitors.

Andexanet alfa, a modified factor Xa recombinant molecule is a potential antidote to factor Xa inhibitors in patients requiring emergency surgery or suffering bleeding.

**LABORATORY MONITORING OF COAGULATION**

The effect of anticoagulant drugs can be measured by coagulation hemostatic tests, as applicable. PT, aPTT, and thrombin time (TT) are common clot-based laboratory tests used to monitor anticoagulation therapy (Table 4).
Table 4: Clot based assays and their reference ranges.

<table>
<thead>
<tr>
<th>Test</th>
<th>Anticoagulant causing prolongation</th>
<th>Pathway measured</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>Warfarin and other Vitamin K antagonists</td>
<td>Extrinsic &amp; common</td>
<td>Generally 10 - 14 seconds, standardized with INR</td>
</tr>
<tr>
<td>aPTT</td>
<td>Heparin and DTIs</td>
<td>Intrinsic &amp; common</td>
<td>25 - 40 seconds</td>
</tr>
<tr>
<td>TT</td>
<td>Heparin and DTIs</td>
<td>Conversion of fibrinogen to fibrin polymer</td>
<td>With lower concentration of thrombin 16 - 25 sec. (\pm 20%) (\pm 5) sec. (\pm 10%)</td>
</tr>
</tbody>
</table>

1:1 Mixing study corrects with warfarin and other Vitamin K antagonists, and remains uncorrected with heparin and direct thrombin inhibitors.

ACT is another clot based assay that has been adapted predominantly for point of care testing, where it is used to monitor high-dose heparin therapy during renal dialysis, percutaneous coronary interventions, and cardiac bypass.

The Factor Xa chromogenic assay can be used to monitor UFH, LMWH, fondaparinux, and FXa inhibitors. This assay utilizes a chromogenic prothrombin (as Factor Xa substrate) to measure the relative amount of anticoagulant in the sample. The remaining chromogenic prothrombin is measured spectrophotometrically and reported in antifactor Xa units/mL. The test has to be calibrated for the specific drug to be tested.

**POINT-OF-CARE TESTING**

In general, point-of-care devices for hemostasis testing can be broadly classified as follows:

1. clot detection analyzers
2. those performing global assessment of clot function
3. platelet function analyzers (not discussed in the current CE)

Point-of-care clot detection devices may provide multiple parameters (PT/INR, PTT or ACT). They are available both as home prothrombin monitors (PT/INR), as well as multi-test systems (PT, PTT, ACT etc) used in the hospital setting. Monitoring should be done by the same device and methodology to have consistent comparability of results. These tests are predominantly being used to monitor patients on warfarin (PT/INR) and in operating rooms.

Thromboelastography (TEG) and Rotational Thromboelastography (ROTEM) are assays that provide information on the function of platelets, coagulation factors, anticoagulant drugs, and clot dissolution (Figure 4). They are useful at the bedside to monitor coagulation status in complex procedures such as cardiac surgeries, liver transplantation, and trauma, and have rapid turnaround time, allowing for immediate perioperative therapeutic decisions. Both TEG and ROTEM systems use slightly different methods and, therefore, should not be considered comparable.
**SUMMARY:**

Heparin and warfarin have been historically used to treat and prevent deep vein thrombi and PE. Limitations and side effects of these drugs have led to the quest for developing an ideal anticoagulant. Heparin-derived LMWH, ULMWH, synthetic heparinoid- Fondaparinux, DTI (hirudin derived and hirudin analogues) and factor Xa inhibitors are now available. The newer anticoagulants have ease of administration, fixed dosages and most do not require laboratory monitoring. Thus, they offer an attractive, feasible, safe alternative to the traditional drugs.

Simultaneous to drug development, there has been a clinical need for home and bedside monitoring of anticoagulated status of patients. While in most situations coagulation testing is best performed in the central laboratory, some situations (such as critical care and clinic/home monitoring programs) can benefit from point-of-care devices that provide rapid results. The continued development of safer, more effective anticoagulants will challenge clinicians and laboratorians to provide accurate, timely monitoring to prevent and treat thromboembolism.
REFERENCES


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