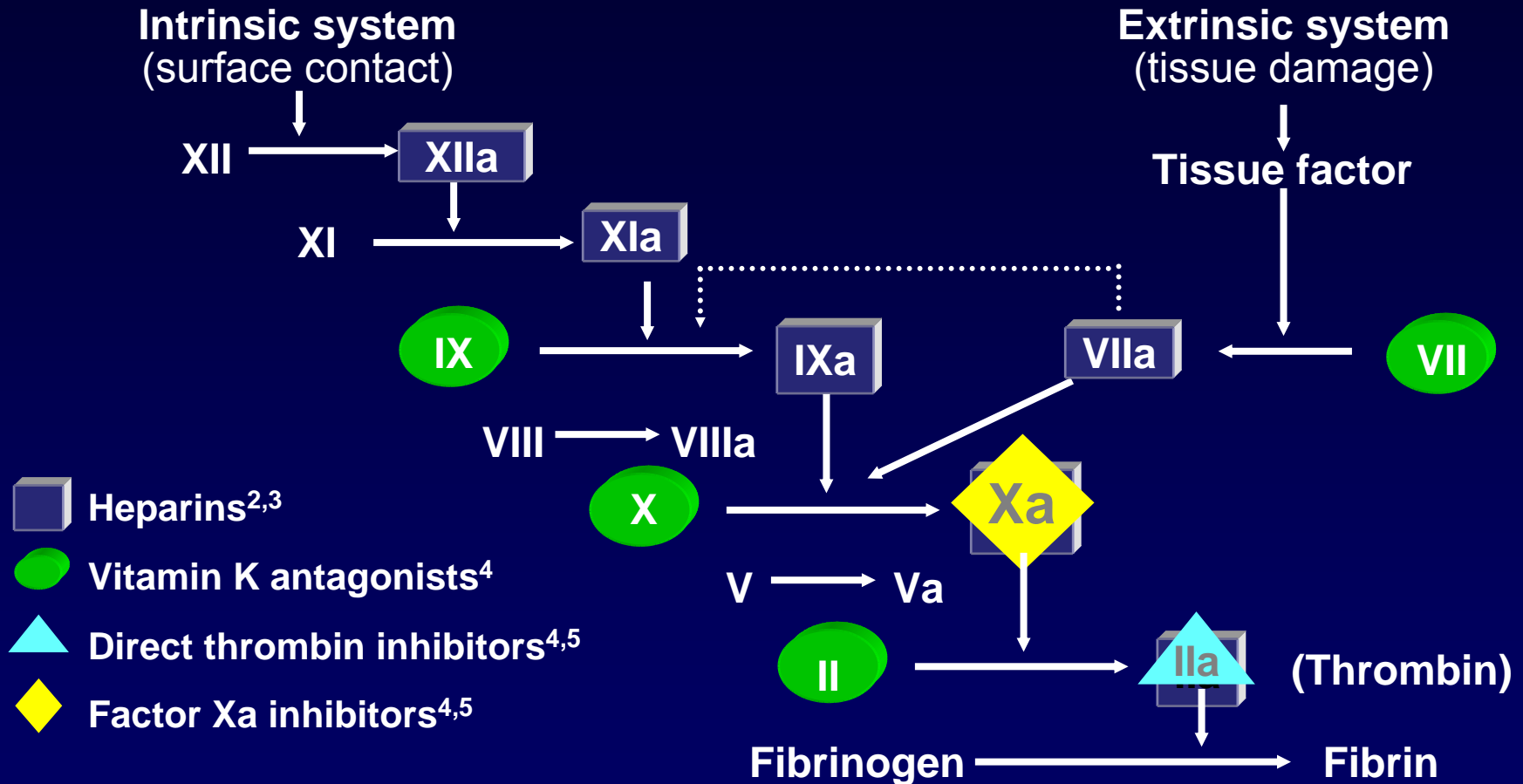


Anticoagulants and Thrombolytics

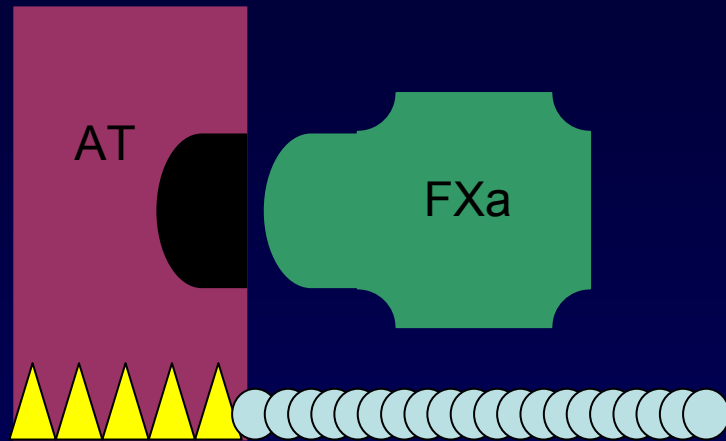
Teresa L. Carman, M.D.
Vascular Medicine

Mechanisms of Anticoagulation

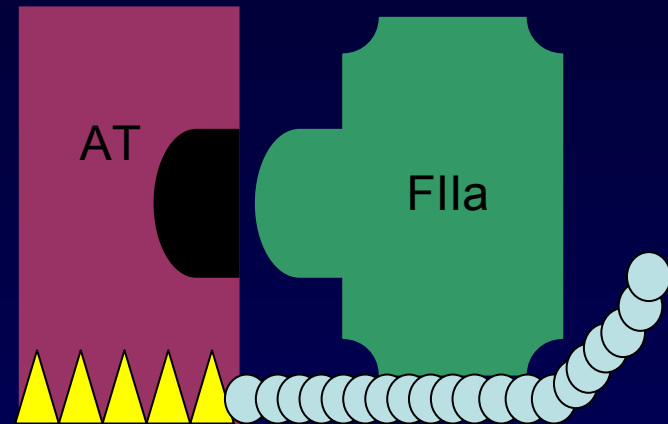


1. Adapted from Petitou M et al. *Nature*. 1991;350(suppl):30-33; <http://www.nature.com/>
2. Hirsh J, Fuster V. *Circulation*. 1994;89:1449-1468.
3. Hirsh J et al. *Chest*. 2001;119(1 suppl):64S-94S.
4. Nutescu EA, et al. *Pharmacotherapy*. 2004;24(7 Pt 2):82S-87S.
5. Weitz JI, Hirsh J. *Chest*. 2001;119(1 suppl):95S-107S.

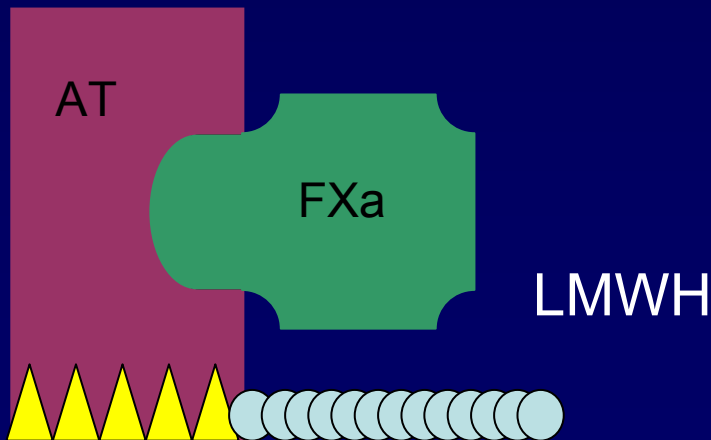
Indirect anti-Xa Anticoagulants



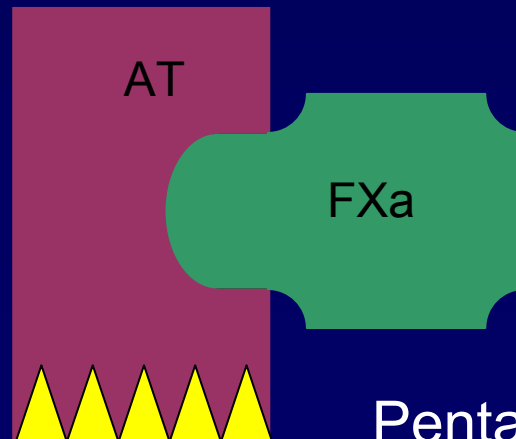
**Pentasaccharide
Sequence**



**> 18 saccharide
units**



LMWH



Pentasaccharide

Heparin

- Glycosaminoglycan mixture
- Derived from porcine intestine or bovine lung
 - Molecular weight – 3K-30K
- Only 1/3 of molecules have the pentasaccharide sequence required for antithrombin binding

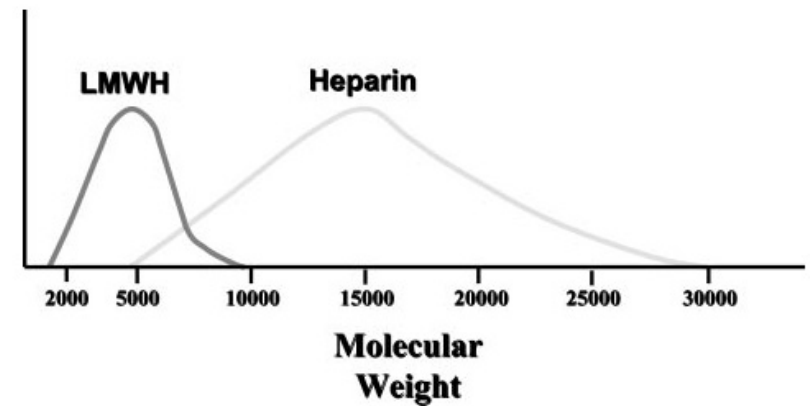


FIGURE 2. Molecular weight distributions of LMWHs and heparin.

Heparin Activity

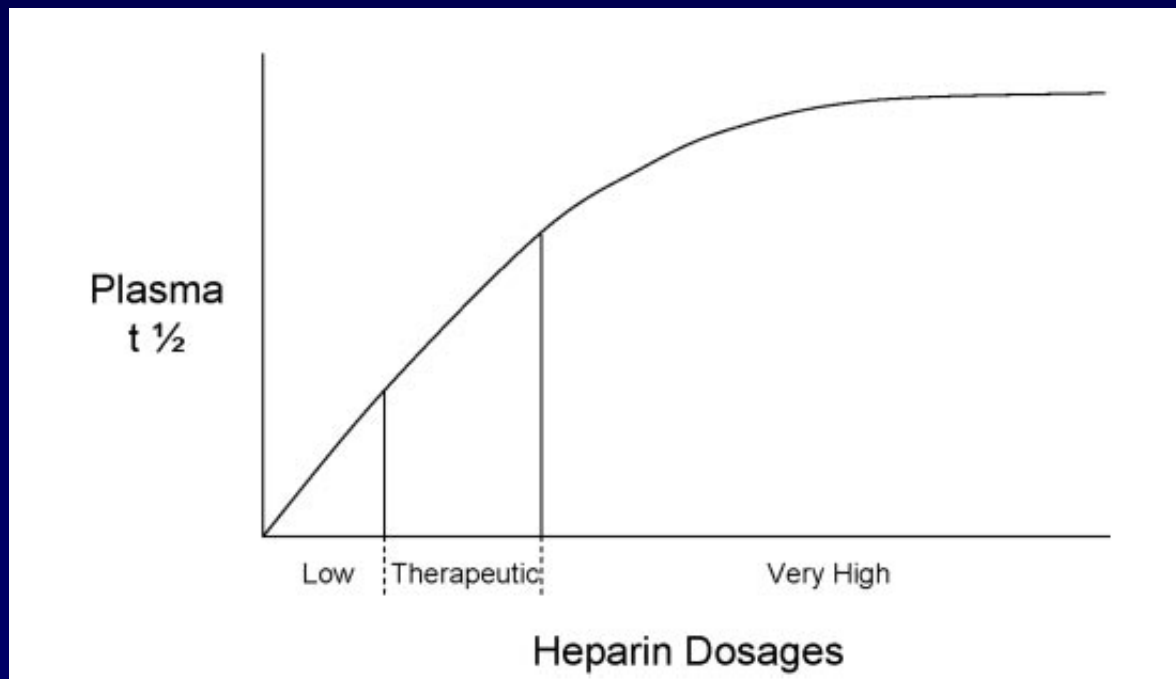
- **Augments antithrombin mediated mechanisms of anticoagulation**
 - Clearance of activated serine proteases (FXa, FIIa, FIXa, FXIa, FXIIa)
- Pentasaccharide independent mediated inhibition thru heparin cofactor II (Very high doses)
- Very high doses binds FIXa and secondarily inhibits FXa generation (supraphysiologic doses)
- **Prevents fibrin formation as well as thrombin mediated activation of platelets, FV and FVIII**

Heparin Pharmacology

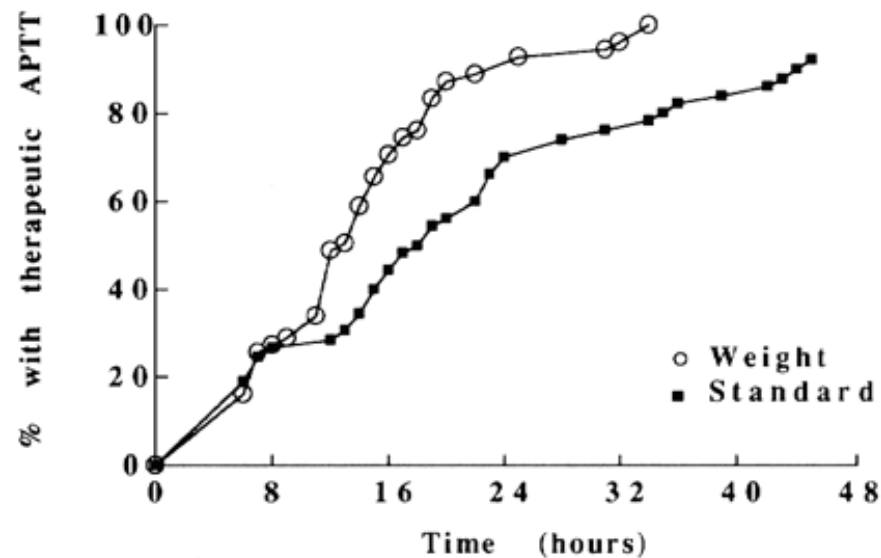
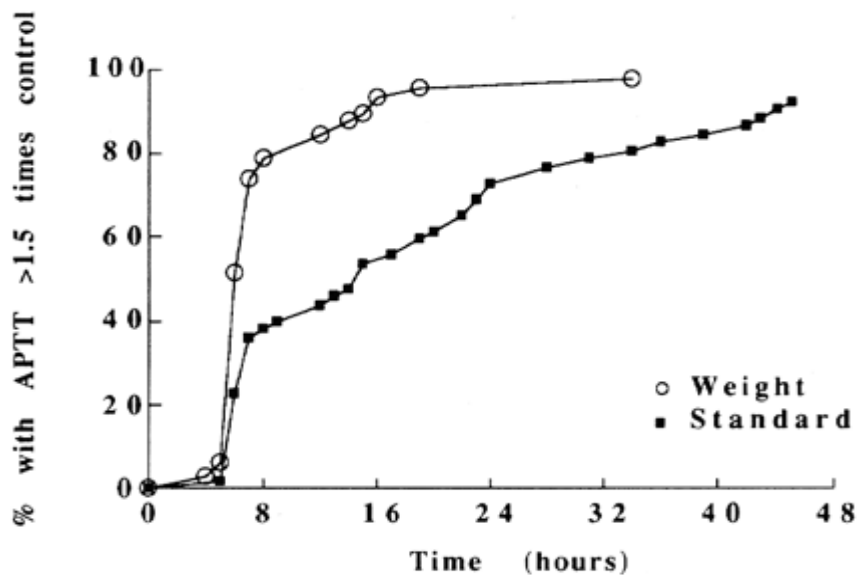
- **IV Therapeutic use**
 - 80 U/Kg bolus and 18 U/Kg/Hr infusion (VTE)
 - 5000 Unit bolus and 1000 unit/hr infusion
- **SC dose** \approx 10% higher than the IV requirements
 - 5000 IU IV bolus then 250 U/Kg q 12 hours with mid-interval aPTT monitoring and dose adjustment
 - 333 U/kg initial dose; 250 U/Kg q 12 hours (no monitoring)*
- **Prophylaxis**
 - 5000U sq q 8-12 hours

Heparin

- Clearance is thru endothelial cell receptors and macrophages (little renal clearance and no adjustments are required)
- Activity and clearance determined by the size of the molecules and total dose (approx 60-90 minutes)



Time to Therapeutic



Time	Nomogram		P Value
	Standard	Weight-based	
Within first 24 hours, %			
Within therapeutic range	35	57	<0.001
Subtherapeutic	58	15	<0.001
Above therapeutic range	7	27	<0.001
Within first 48 hours, %			
Within therapeutic range	44	65	<0.001
Subtherapeutic	49	18	<0.001
Above therapeutic range	8	18	<0.001

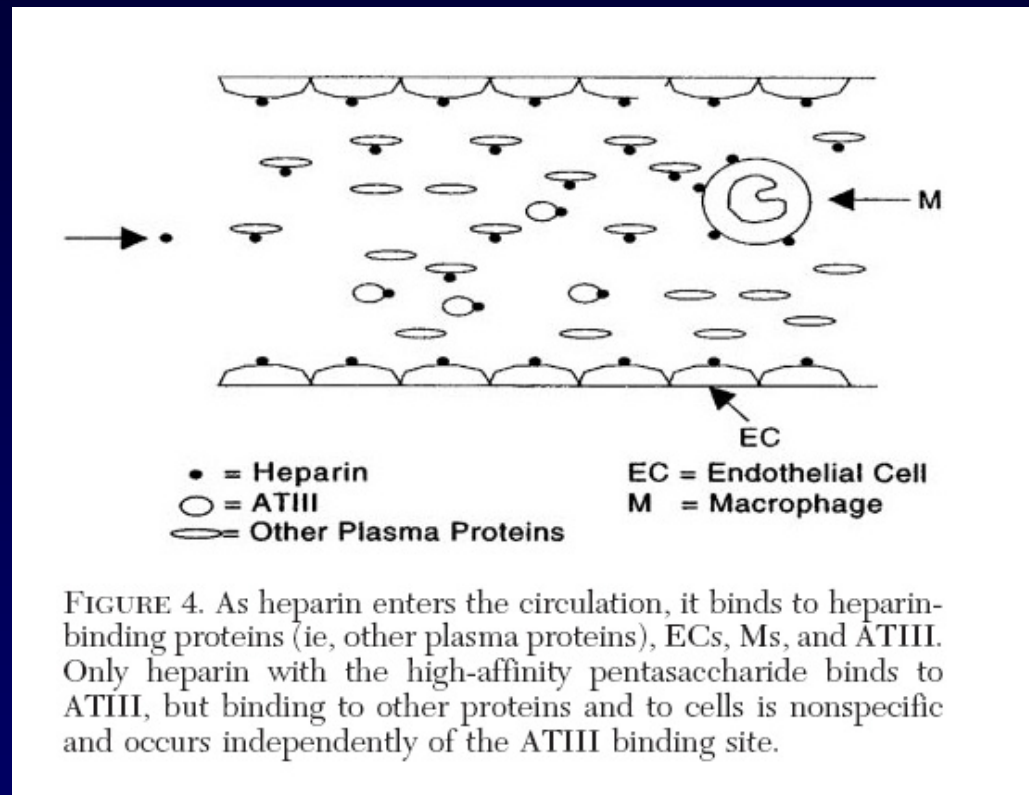
Subtherapeutic aPTT

	Nomogram		<i>P</i> Value
	Standard	Weight-based	
First APTT* on therapy >1.5 times control, %	32	86	<0.001
APTT >1.5 times control within 24 hours, %	77	97	0.002
APTT in therapeutic range within 24 hours, %	75	89	0.08
Bleeding episodes, <i>n of n</i>			
Minor	2 of 52	2 of 63	1.0
Major	1 of 52	0	0.45
Recurrent venous thromboembolism, <i>n of n (%)</i>	8 of 32 (25)	2 of 41 (5)	0.02

* APTT = activated partial thromboplastin time.

Heparin

Non-specific binding affects heparin bioavailability:
platelets,
macrophages,
endothelial cells,
and plasma proteins



Suboptimal Bioavailability

- Approximately 33% of molecules have pentasaccharide and are functional
- Non-specific adsorption onto proteins, cells, platelets – limits availability
- 80 U/Kg and 18 U/Kg/hr – best choice for dosing

Monitoring

- **Requires aPTT and platelet monitoring**
 - aPTT 1.5- 2.5 X control (lab reagent and coagulometer specific)
 - Correlated to a chromogenic heparin assay of 0.3-0.7 anti-factor Xa units
 - Anti-Xa monitoring required in patients with very high or low dose requirements
 - Abnormal baseline aPTT
 - Lupus anticoagulants
 - “Failed therapy”

Heparin Limitations

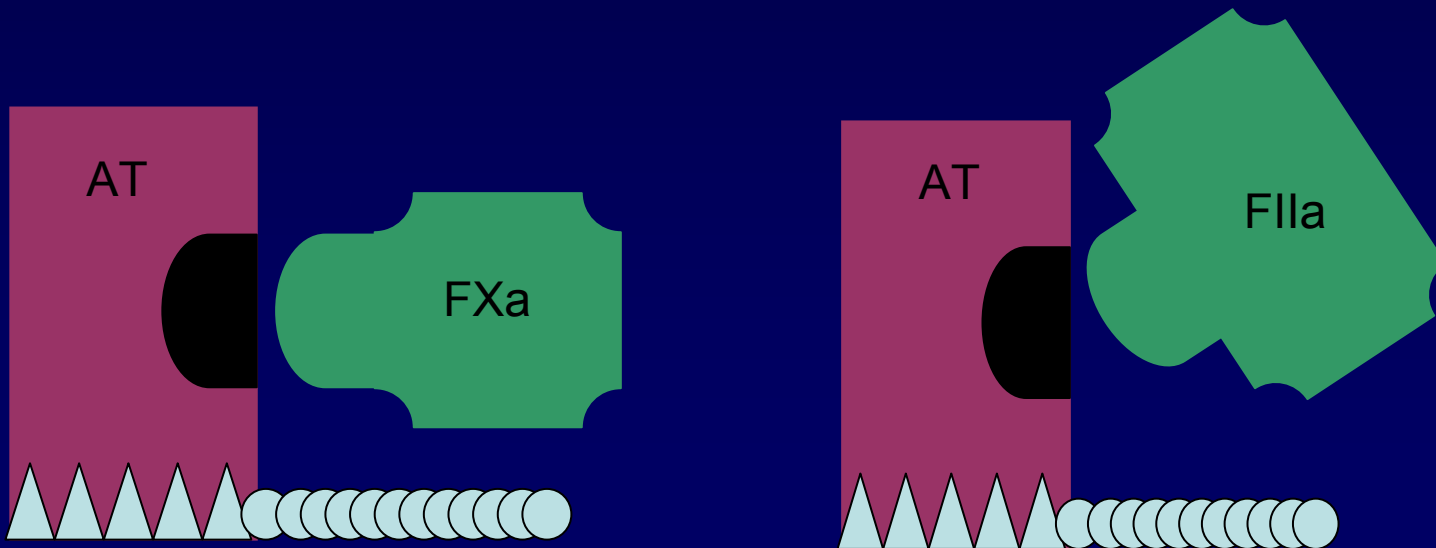
- No activity against clot bound thrombin (IIa) or Xa
- Heparin resistance
 - Antithrombin deficiency, increased heparin-binding, elevated FVIII or fibrinogen, increased heparin clearance
- Significant risk for heparin:platelet factor 4 antibody formation (HIT)
- Bone effects
 - Osteoblast and osteoclast effects
- Benign (non-clinically significant) elevations of transaminases

Revering Effect

- Protamine serves as an antidote
 - 1mg/100 units
 - (Max 50 mg IV over 1-3 minutes)
 - Administer slowly to avoid hypotension and bradycardia
 - Protamine dosing
 - Infusion rate (IR) + ($\frac{1}{2}$ X IR) + ($\frac{1}{4}$ X IR) + ($\frac{1}{8}$ X IR)
 - 1200 + 600 + 300 + 150 = 2250 \approx dose
20mg

LMWH

- Chemical or enzymatic fragmentation of UFH
- AT mediated clearance mechanism
 - Smaller molecules - fewer available for FII binding
 - FXa inhibition predominates (2.4 - 4:1)



LMWH

Table 5—Methods for Preparation of LMWHs and Danaparoid

Agent	Method of Preparation
Dalteparin (Fragmin)	Nitrous acid depolymerization
Danaparoid sodium (Orgaran)	Prepared from animal gut mucosa; contains heparan sulfate (84%), dermatan sulfate (12%), and chondroitin sulfate (4%)
Enoxaparin sodium (Lovenox/Clexane)	Benzylation followed by alkaline depolymerization
Nadroparin calcium (Fraxiparin)	Nitrous acid depolymerization
Tinzaparin (Innohep)	Enzymatic depolymerization with heparinase

Table 6—Biological Consequences of Reduced Binding of LMWH to Proteins and Cells

Binding Target	Biologic Effects	Clinical Consequence
Thrombin	Reduced anti-IIa activity relative to anti-Xa activity	Unknown
Proteins	More predictable anticoagulant response	Coagulation monitoring unnecessary
Macrophages	Cleared through renal mechanism	Longer plasma half-life permits once-daily administration
Platelets	Reduced formation of HIT antibodies	Reduced incidence of HIT
Osteoblasts	Reduced activation of osteoclasts	Lower risk of osteopenia

LMWH

- Mean molecular weight 4000-5000 daltons
- Anti-Xa:Anti-IIa approximately 2:1-4:1
- 3-6 hour $t_{1/2}$
- Peak anti-Xa activity at 3-5 hours
- Renal clearance

LMWH in VTE

- Dalteparin* (Fragmin ®, Pfizer)
 - 100 IU/kg q 12 hours
 - 200 IU/kg q 24 hours
 - **VTE in cancer:** 200 IU/Kg q 24 hours; 150 IU/Kg q 24 hours
 - 2500 IU q day (low-moderate risk)
 - 5000 IU q day (high risk)
- Enoxaparin (Lovenox ®, Sanofi-Aventis)
 - 1 mg/kg q 12 hours (outpatient or inpatient)
 - 1.5 mg/kg once daily (inpatient)
 - 30 mg sq q12 hours (orthopedic)
 - 40 mg sq q day (medical, abdominal surgery)
- Tinzaparin (Innohep ®, DuPont)
 - 175 IU/kg once daily
- Nadroparin (Fraxiparin - available in Canada)

*Not FDA approved for VTE treatment

Enoxaparin (RI)

Dose adjustment for renal impairment

CrCl < 30 ml/min – 59% dose reduction

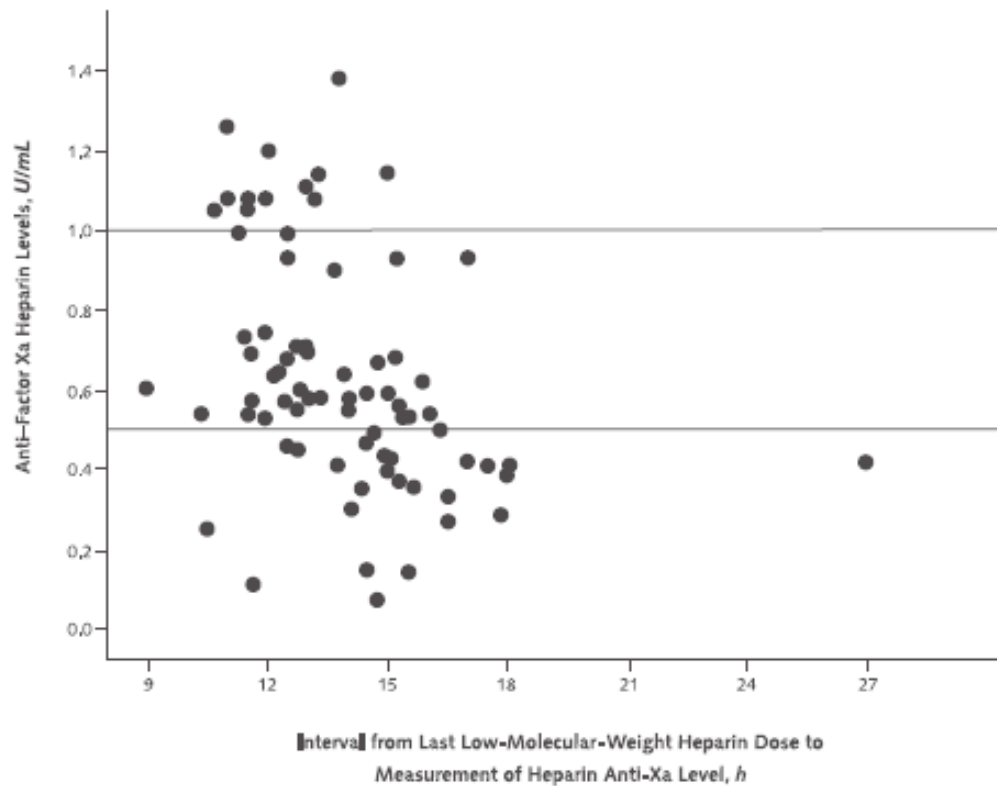
- Abdominal surgery prophylaxis, hip or knee replacement, acute medical illness
 - 30 mg sq once daily
- Venous thromboembolism, USA, NQWMI,
 - 1 mg/kg once daily

LMWH Monitoring

- **Monitoring not typically required**
 - Considered in renal insufficiency, morbid obesity, and pregnancy
 - Anti-Xa activity (timed 4 hours after the dose)
 - Enoxaparin**
 - Q 12 hour dosing 0.6 – 1.0 IU/ml
 - Daily dosing 1.0 – 2.0 IU/ml
 - Tinzaparin**
 - > 0.85 U/ml (target range)
 - Dalteparin**
 - > 1.05 U/ml (target range)

Clearance

Figure. Association between residual anti-Xa level and interval from last dose of low-molecular-weight heparin.



Neutralization

- Unclear neutralization but historically approximately 60% neutralization by protamine
 - 1 mg per 100 anti-Xa units given within the past 8 hours
 - Smaller doses if the administration was > 8 hours prior

LMWH

Benefits

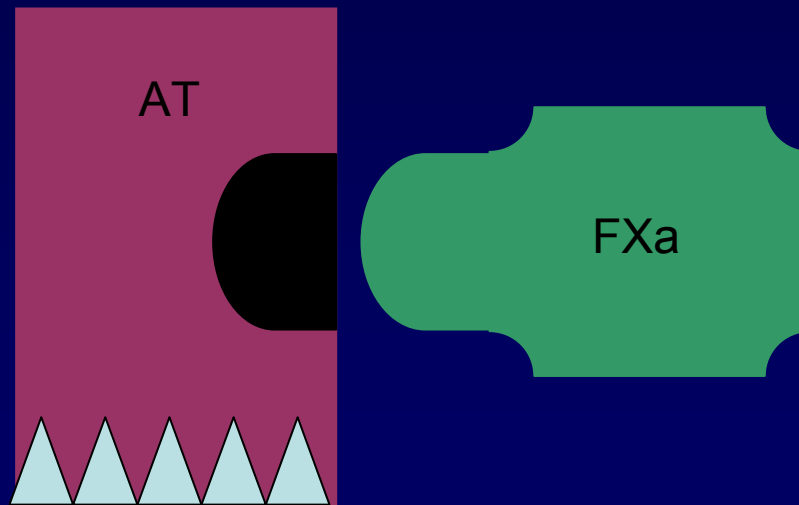
- Efficacy and safety comparable to UFH
- SC administration
- Predictable dose response
- Longer $t_{1/2}$
- No aPTT monitoring required
- Less non-specific binding
- Lower risk for HIT
- Lower incidence of bone effects

Cautions

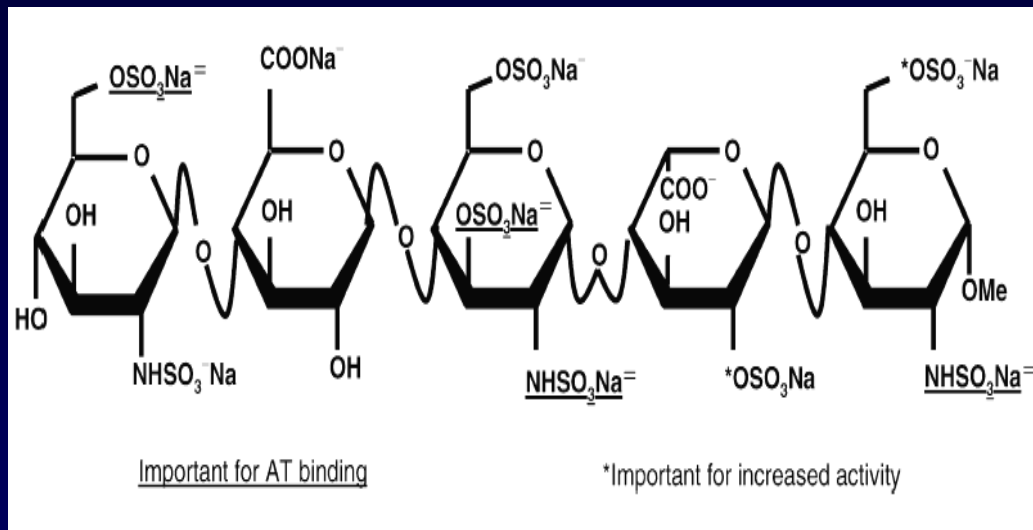
- Renal clearance
- Must still follow platelet counts
- Anti-Xa monitoring when required
- Cross reactivity with HIT antibodies
- Limited reversibility with protamine (60-70%)

Oligosaccharides

- Synthetic agents
- Selective AT-mediated FXa inhibition and clearance



Pentasaccharides



Fondaparinux (Arixtra® GlaxoSmith Kline)

Fondaparinux

- Synthetic pentasaccharide analogue
 - Binds selectively and reversibly to AT
 - 300-fold increase in affinity for FXa
 - 17-21 hour half-life
 - Prolonged effect for 2-4 days
 - Renal elimination – contraindicated with CrCl < 30 ml/min
 - No specific antidote, does not bind to protamine.
rFVIIa increased prothrombin activation in one study

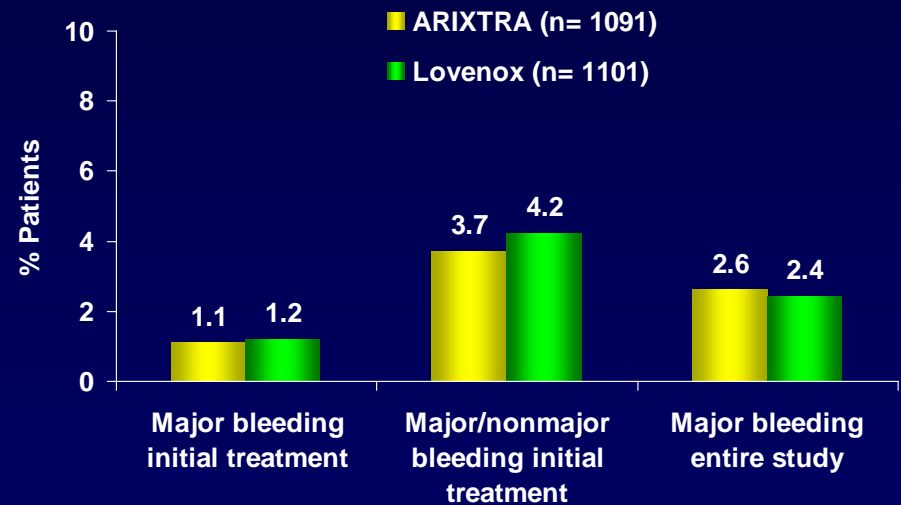
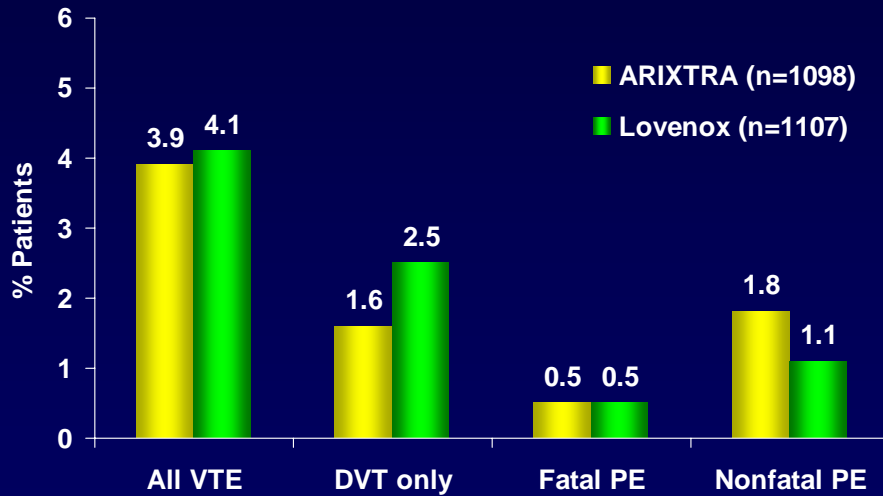
Fondaparinux

- Does not significantly bind to plasma proteins
- No known effect on platelet function
- Does not significantly bind platelet factor 4
 - not associated with development of HIT(T)*
- Long t $\frac{1}{2}$ - Once daily dosing
- Must hold 36-48 hours prior to surgery
- No APTT monitoring
 - Anti-Xa assays may be performed but have not been used in clinical trials
 - 0.2-0.4 ug/ml with 2.5 mg dose
 - 0.5-1.5 ug/ml with 7.5 mg dose

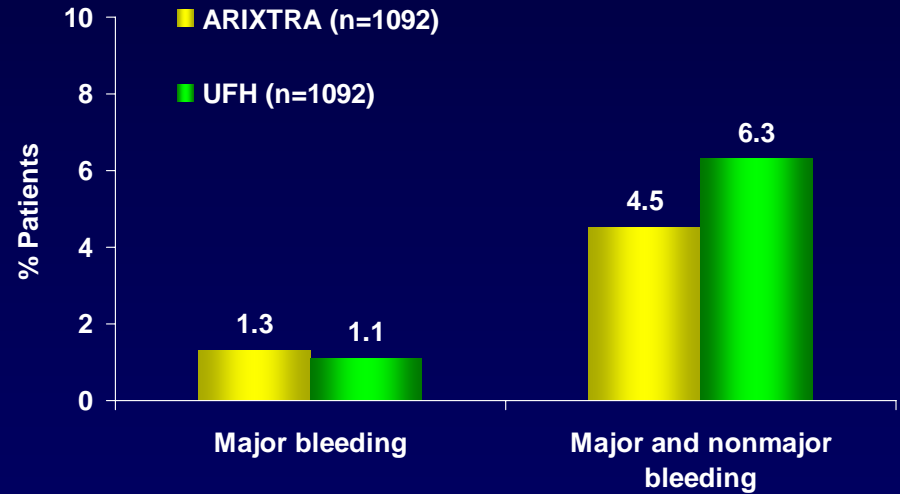
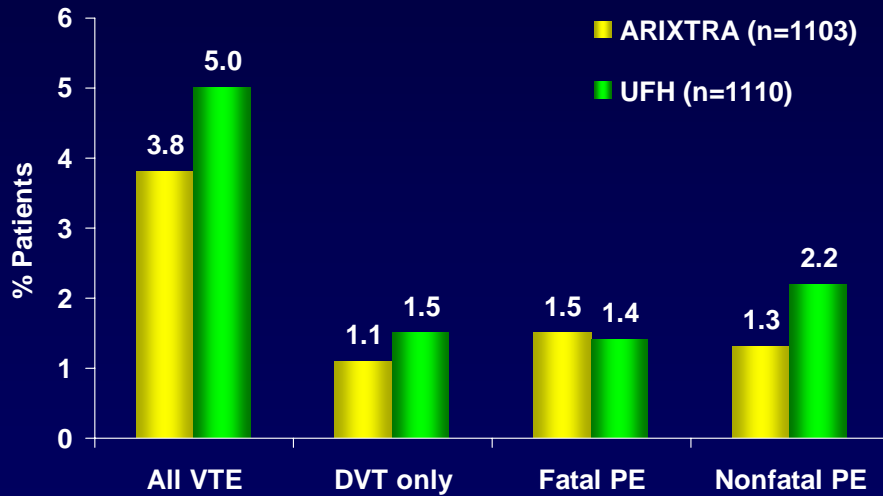
Fondaparinux

- Approved for DVT prophylaxis in hip fracture, THA, and TKA
 - 2.5 mg sq daily (begin 6 hours post-op)
 - 50% reduction in VTE compared with enoxaparin
 - Turpie AG. Arch Intern Med 2002;162:1833
- Approved for DVT or PE treatment
 - Rembrandt, Matisse DVT and Matisse PE trials
 - 5 mg daily (< 50 Kg); 7.5 mg daily (50-100 Kg); 10 mg (>100 Kg)

DVT Treatment



PE Treatment



Idraparinux

- Synthetic pentasaccharide analogue
- Increased affinity for AT
- T $\frac{1}{2}$ approximately 130 hours
- Weekly SC administration
- Undergoing/completed phase III investigation in DVT, PE and atrial fibrillation
- Biotinylated idraparinux
 - Added biotin segment to allow for rapid neutralization by avidin

Idraparinux

- Van Gogh
 - Idraparinux compared to LMWH/VKA
- Van Gogh DVT
 - 2904 randomized patients
 - Recurrent VTE 2.9% vs 3.0% ($p=0.00056$)
 - Bleeding 4.5% vs 7% ($p=0.004$)
- Van Gogh PE
 - 2215 randomized patients
 - Recurrent VTE 3.4% vs 1.6% ($p=0.59$)
 - Bleeding 5.8% vs 8.2%

Cautions

Spinal/Epidural Hematomas

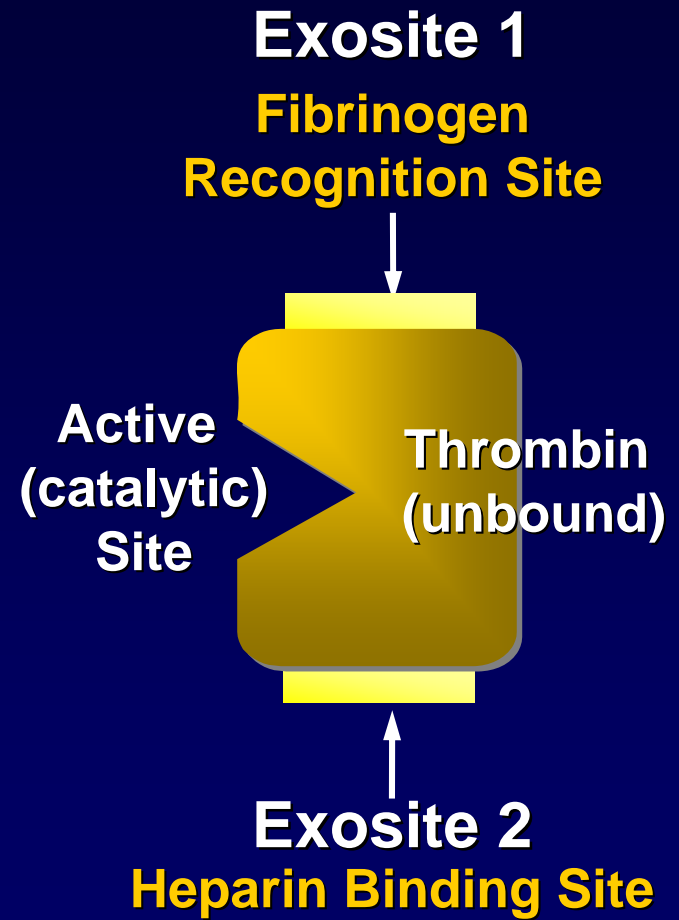
When epidural/spinal anesthesia or spinal puncture is employed, patients anticoagulated or scheduled to be anticoagulated with low-molecular-weight heparins, heparinoids or fondaparinux sodium are at risk of developing an epidural or spinal hematoma, which can result in long-term or permanent paralysis. The risk of these events may be higher with postoperative use of indwelling epidural catheters or concomitant use of drugs affecting hemostasis. Patients should be frequently monitored for signs and symptoms of neurological impairment. (See BOXED WARNING)

Direct Thrombin Inhibitors

- Direct inhibitors of FIIa (thrombin)
- Binds to free and clot bound thrombin
 - Prevents thrombin dependent conversion of fibrinogen to fibrin
 - Inhibits positive feedback mechanism on FV, FVIII and FXIII
 - Limits thrombin activation of platelets
- Does not induce nor cross-react with heparin:PF4 antibodies

Direct Thrombin Inhibitors (DTIs): Action Site

- Active (catalytic) site
- Exosite 1
 - Fibrinogen Recognition Site
 - Also responsible for interaction with thrombomodulin, Protein C, and platelet activation
- Exosite 2
 - Binds antithrombin
 - Heparin binding site



DTI Specificity for the Active Site

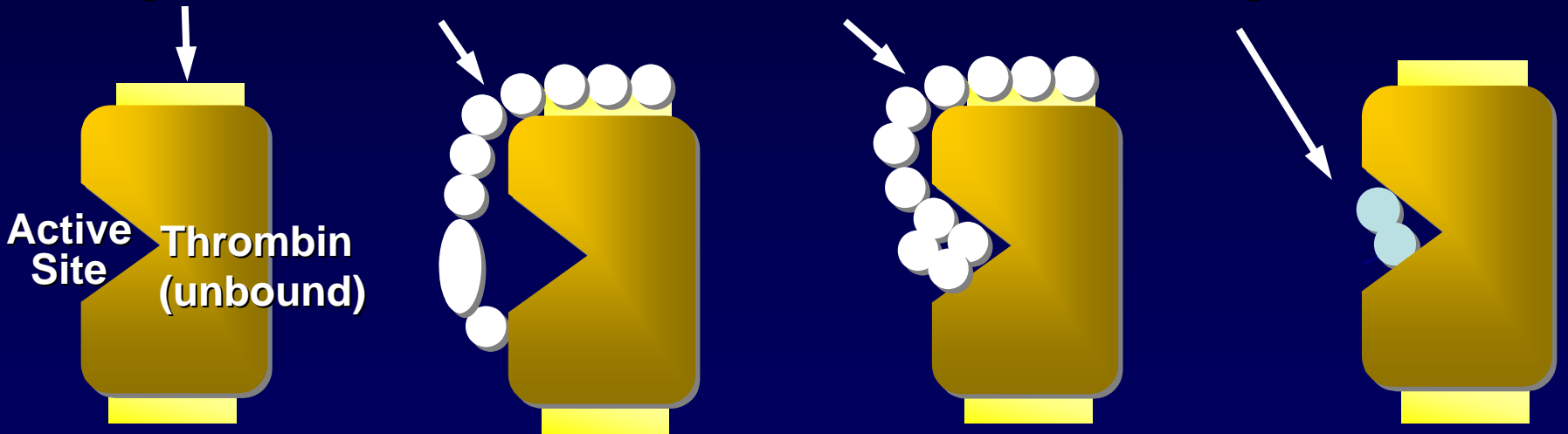
Exosite 1

**Fibrinogen
Recognition Site**

Hirudin

Bivalirudin

**Argatroban,
Melagatran**



Exosite 2

**Irreversible Binding
at Active Site**

**Reversible Binding
at Active Site**

**Reversible Binding
at Active Site**

Direct Thrombin Inhibitors

Currently 3 developed agents in the US

- Lepirudin (Refludan ®, Berlex) – HIT(T)
- Argatroban (GlaxoSmithKline) – HIT/HITT/PCI
- Bivalirudin (Angiomax ®, Medicines Company)
– PCI/PCI with HIT
- Desirudin (in Europe)

- Undergoing study in PTA/cardiopulmonary bypass

Differentiation of DTIs

	Lepirudin	Bivalirudin	Argatroban
Molecular wt (daltons)	6980	2180	508
Half-life	50-65 min	25 min	40-50 min
Elimination	Renal	Proteolytic/ Renal	Hepatic
Monitoring	Yes aPTT/ECT	Yes aPTT/ACT	Yes aPTT/ACT
INR Effect	Yes (+)	Yes (+)	Yes (++)
Route of administration	IV or SQ	IV	IV
Antibodies	++	no	no

Dosing Regimens

- **Lepirudin**
 - 0.15 mg/kg/hr gtt with or without 0.4 mg/kg bolus
 - aPTT 1.5-2.5 X pt baseline or mean lab normal range
- **Argatroban***
 - 1 - 2 mcg/kg/min infusion
 - aPTT 1.5 – 2.5 X mean lab normal range
 - Factor X level < 45% correlates with INR > 2
- **Bivalirudin**
 - 0.7 mg/kg bolus than 1.75 mg/kg/hr for the procedure
 - 0.1 – 0.2 mg/kg/hr***
 - aPTT 1.5-2.5 X pt baseline or lab mean normal range***

Warfarin

- Most readily available vitamin K antagonist
- Interferes with Vitamin K dependent γ -carboxylation
 - Factors II, VII, IX, and X
 - Protein C and S, Protein Z
- Water soluble, readily absorbed from the GI tract

Warfarin

- Slow onset and offset
 - T $\frac{1}{2}$ 29-45 hours
- Variable dose response
 - Circulates bound to albumin
 - Pharmacokinetics may be altered by drugs, diet and illness
 - Genetic variability in metabolism (CYP2C9 genes VKORC)

Warfarin

- Anticoagulant effect (VII, IX) vs. antithrombotic effect (FX, II)
- Anticoagulant effect can be seen within 2 days
- Antithrombotic effect takes minimum of 4-5 days d/t the $t_{1/2}$ of prothrombin (60 hours)
- Requires careful monitoring and dose adjustment
 - $INR = (\text{patient PT} / \text{mean normal PT})^{ISI}$

Warfarin Dosing

2.1 Initiation and Maintenance Dosing

2.1.1. In patients beginning vitamin K antagonist (VKA) therapy, we recommend the initiation of oral anticoagulation with doses between 5 mg and 10 mg for the first 1 or 2 days for most individuals, with subsequent dosing based on the international normalized ratio (INR) response (Grade 1B). At the present time, for patients beginning VKA therapy without evidence from randomized trials, we suggest against the use of pharmacogenetic-based initial dosing to individualize warfarin dosing (Grade 2C).

2.2 Initiation of Anticoagulation in Elderly or Other Populations

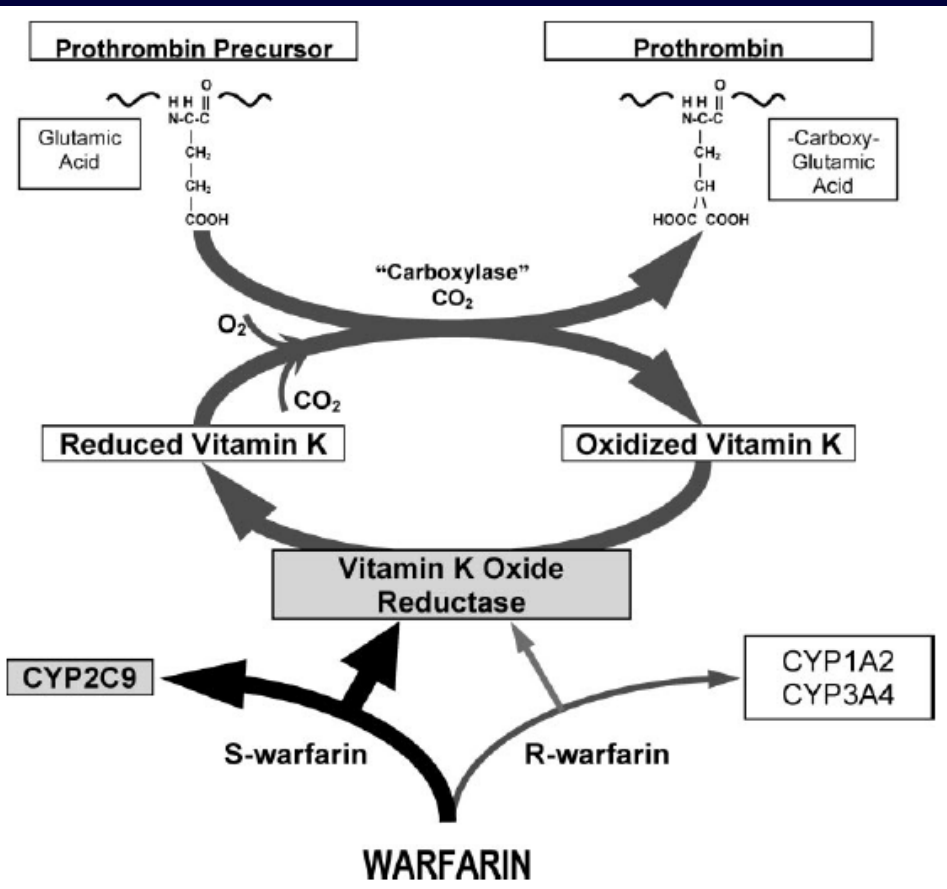
2.2.1. In elderly patients or patients who are debilitated, are malnourished, have congestive heart failure (CHF), have liver disease, have had recent major surgery, or are taking medications known to increase sensitivity to warfarin (eg, amiodarone), we recommend the use of a starting dose of ≤ 5 mg (Grade 1C) with subsequent dosing based on the INR response.

2.3 Frequency of Monitoring

2.3.1. In patients beginning VKA therapy, we suggest that INR monitoring be started after the initial two or three doses of oral anticoagulation therapy (Grade 2C).

2.3.2. For patients who are receiving a stable dose of oral anticoagulants, we suggest monitoring at an interval of no longer than every 4 weeks (Grade 2C).

Warfarin Reversal



- Vitamin K
 - Elevated INR and increased risk for bleeding (?)
 - Rapid reversal is required
- FFP
 - Active bleeding
 - Urgent peri-op management
- Bypassing agents
 - Prothrombin complex concentrate
 - rFVIIa

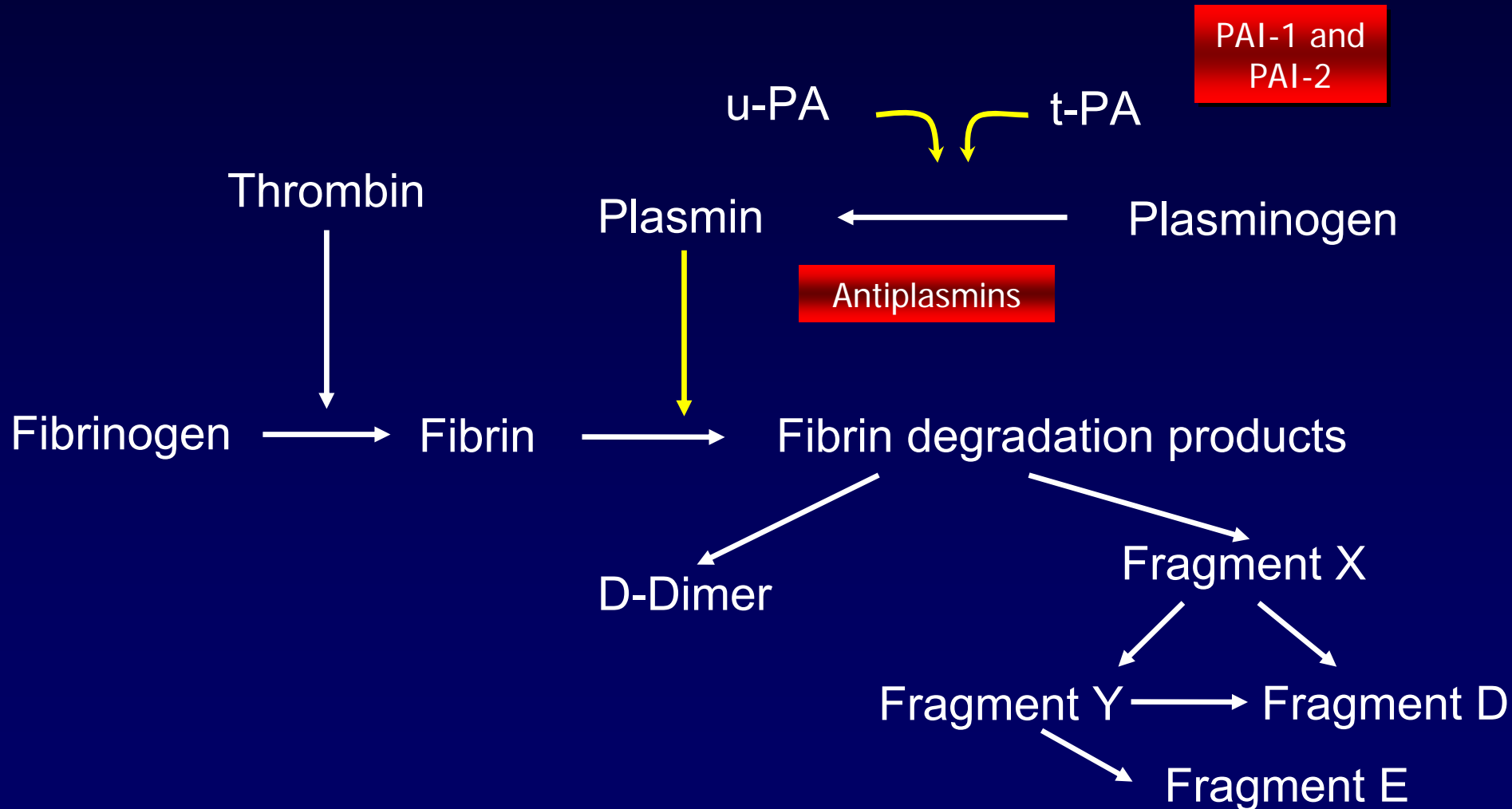
Vitamin K Use

INR	Clinical Scenario	Action
>3 and < 5	No bleeding and no or low risk for bleeding	Lower dose or omit dose, if only sl elevated no dose change may be required
5 - 9	No bleeding and no or low risk for bleeding	Hold 1-2 warfarin doses follow and resume when INR <3
5 - 9	No bleeding and rapid reversal required for surgery	Vit K 2-4 mg PO; repeat in 24 hours if still > 5; (**may need FFP depending on the timing of surgery)
5 - 9	Minor bleeding or increased risk for bleeding	Vitamin K 1-2.5 mg PO; repeat in 24 hours if still elevated
> 9	No bleeding and low risk for bleeding or minor bleeding	Stop warfarin; Vit K 3-5 mg PO; repeat in 24 hours if INR > 6
> 9	Serious bleeding	IV Vit K, FFP, PCC, rFVIIa

Future Therapies

- Oligosaccharides with AT-mediated anti-Xa activity
- Direct anti-Xa – apixaban, rivaroxaban
- FVIIa/TF inhibitor (rNAPc2)
- TFPI
- FVIIai
- Thrombin inhibitor with AT-mediated activity - dabigatran

Fibrinolysis

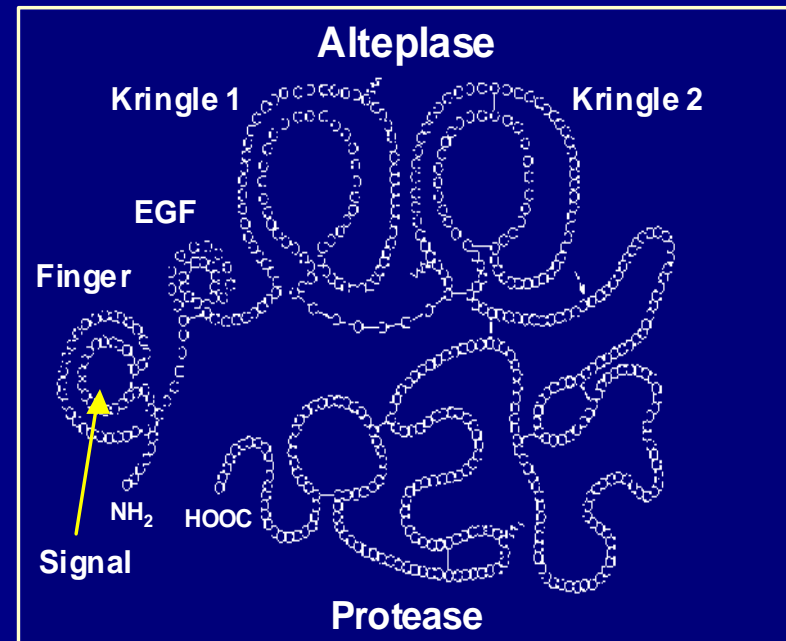


Classes of Fibrinolytics

- Indirect plasminogen activators
 - Streptokinase
 - Staphylokinase
- Plasminogen activators
 - ru-PA (urokinase)
 - rt-PA (alteplase)
 - r-PA (retaplastase)
 - TNK-ase (tenecteplase)
 - Desmoteplase
- Direct fibrinolytics
 - Alfimeprase
 - Plasmin
- Under development
 - Lanoteplase
 - Monteplase
 - Pamiteplase
 - Saruplastase
 - scu-PA (pro-urokinase)
 - Anistreplase

Functional Domains

- Finger domain – fibrin binding
- EGF – clearance by hepatic receptors
- Kringle 1 – hepatic endothelial cell clearance
 - minor fibrin binding site
- Kringle 2 – protease stimulation; fibrin/fibrinogen binding site
- Protease domain – splitting of plasminogen; PAI-1 inhibition
- CHO side chains – plasma clearance



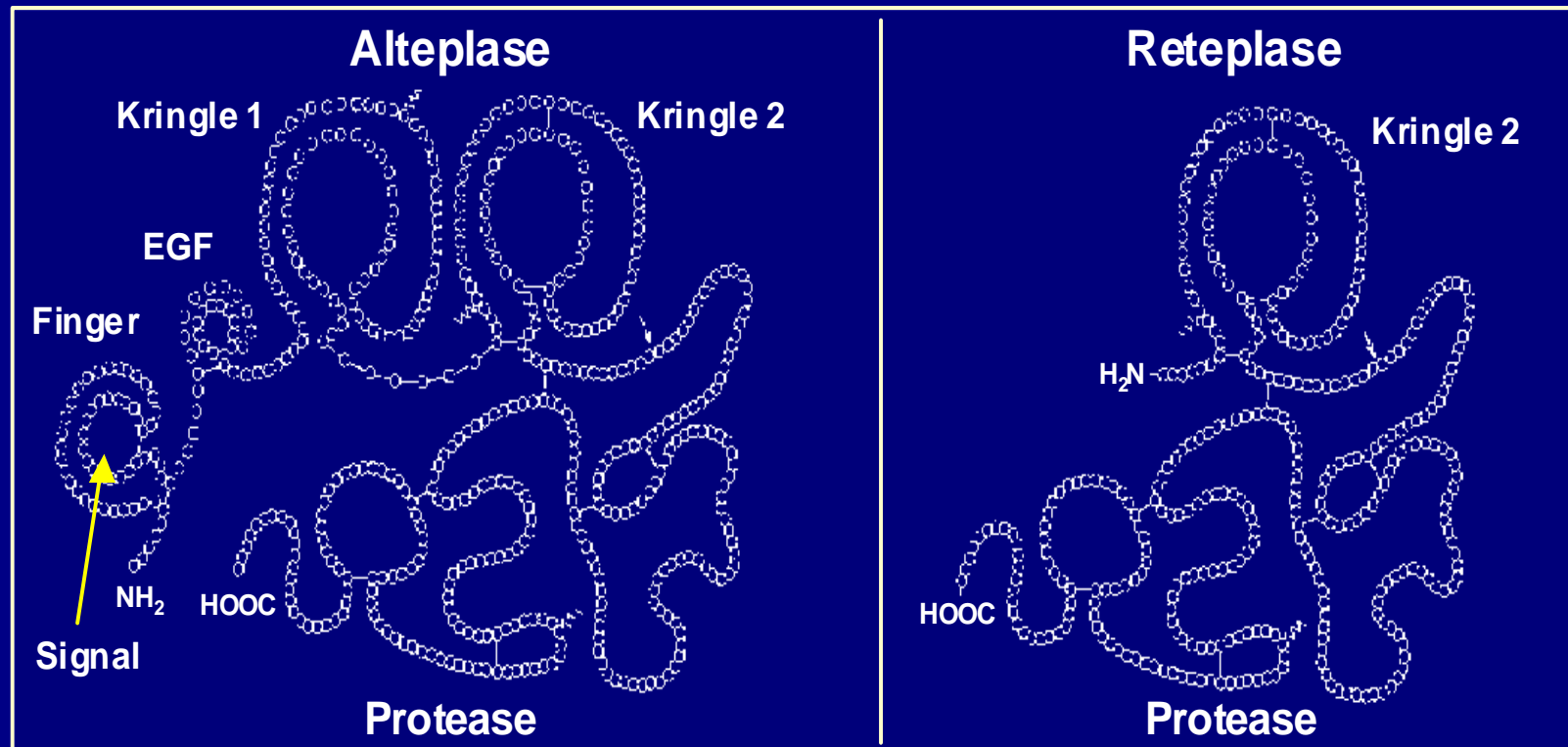
527 amino acids, MW 65,000
Mammalian cells (glycosylated)

Alteplase (rt-PA)

- High affinity binding to fibrin
- Fibrin-bound plasminogen activation >> fluid-phase plasminogen activation
- T $\frac{1}{2}$ 4-6 minutes
 - Requires longer infusion times
 - 100 mg over 2 hours (PE)
- Inactivated by PAI-1
- Currently utilized in MI, stroke, pulmonary embolism, and catheter occlusion,
 - Off-label use in arterial occlusion and venous thrombosis (0.5-1 mg/hour)

Functional Domain Modification

Deletion mutations



527 amino acids, MW 65,000

Mammalian cells (glycosylated)

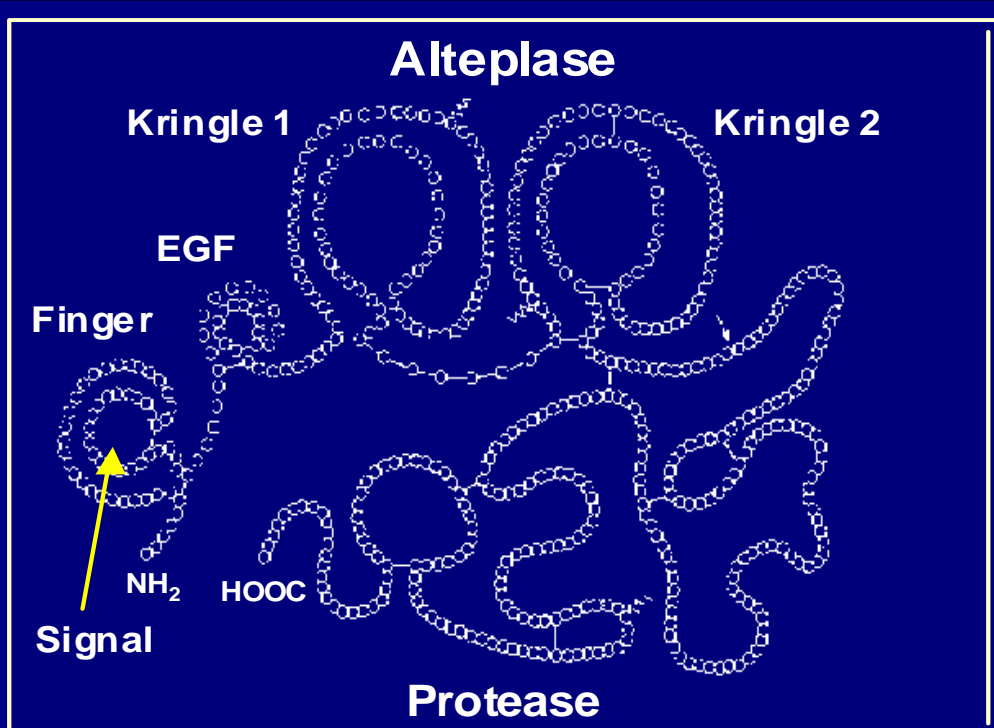
355 amino acids, MW 39,000

E coli (not glycosylated)

Retaplastase (r-PA)

- Fibrin-specific plasminogen activator
- Decreased fibrin binding compared to rt-PA
- T $\frac{1}{2}$ - 14 to 18 minutes
- Double bolus administration
 - 10 mg bolus repeated in 30 minutes
- Approved for myocardial infarction
 - Not approved but studied in PE, arterial and venous thrombosis

Functional Domain Modification



527 amino acids, MW 65,000
Mammalian cells (glycosylated)



Aminoacid point mutations

Tenecteplase (TNK-ase)

- 3 - aminoacid point mutations
- Longer t $\frac{1}{2}$ (17-20 min)
- Increased fibrin specificity
 - Less systemic plasminogen/plasmin activation
 - Decreased bleeding complications
- Increased resistance to PAI-1

- Administered by single bolus
- Approved for MI
- PE – use in case reports and case review
(J Thromb Thrombolysis 2007)

Desmoteplase

- 4 plasminogen activators identified in vampire bat saliva
- Desmoteplase is a recombinant analogue DSPA α
- Requires fibrinogen or fibrin as a catalyst for activity
- 10^5 fold increased activity with fibrin
- Little systemic conversion of plasminogen to plasmin
- Stroke trials



Vampire Bat (*Desmodus rotundus*)