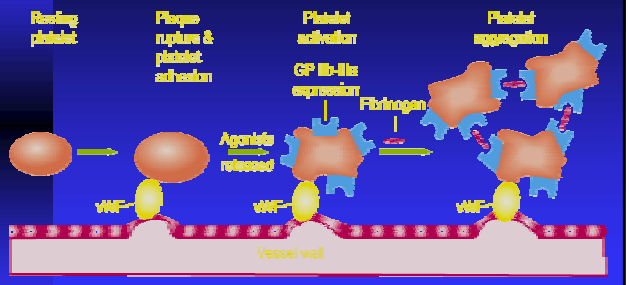
Keywords

- Platelets
- Aspirin resistance
- Aggregation
- Thienopyridines
- IIb/IIIa
- Clopidogrel resistance
- Heparin
- LMWH
- Thrombin
- Bivalirudin
- Ximelagatran

Anthithrombotics and Anticoagulants

David Hasdai, MD

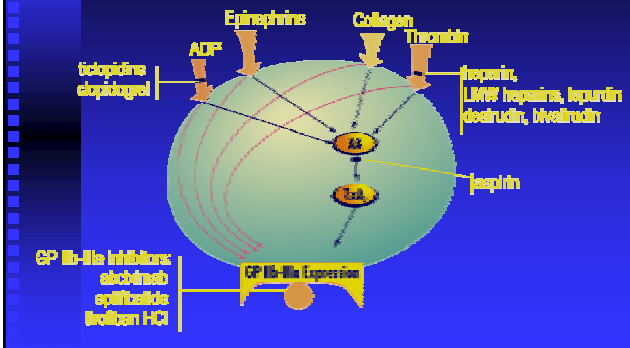
Platelet adhesion, activation, and aggregation



Antiplatelet agents

Aspirin

Therapeutic interventions against platelet activation and aggregation



History of Aspirin

- 1948 Dr. Lawrence Craven notes 400 pts taking aspirin (aspergum) had not had heart attacks *Mississippi Valley Medical Journal*
- 1967 Weiss and Aledort discover aspirin inhibits platelets
- 1982 Sir John Vane awarded Nobel Prize for finding mechanism of dose-dependent inhibition of PG formation
- 1988 FDA approved ASA for reducing risk of recurrent MI, preventing first MI, and preventing recurrent TIA

An original package of Bayer Aspirin sold in the United States from 1909.

Each pill is 5 grains, or ~ 325mg.

Steinhubl SR. TCT 2002

Potential Mechanisms of Aspirin Resistance

- Noncompliance
- Insufficient dose
- Alternative pathway
- Genetic predisposition
- Increased prothrombotic milieu
- Drug interactions

Aspirin Resistance

- Definition – clinical vs. biochemical vs. functional
- Clinical significance – diagnosis and prognosis

Alternative Pathways: Contribution of COX_2

- COX_1 is the major isoform constitutively expressed in mature human platelets
- COX_2 can also contribute to production of TXA_2
- COX_2 upregulation and overexpression could contribute to aspirin resistance as a result of incomplete TXA_2 suppression

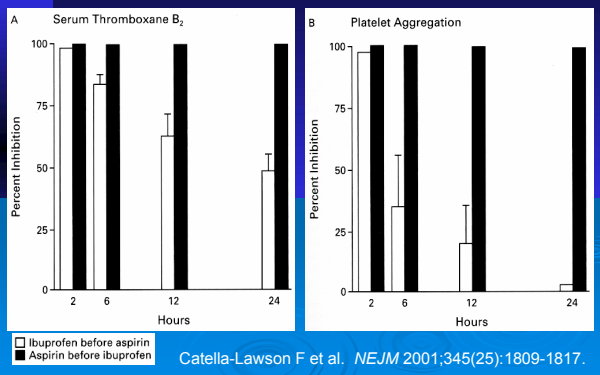
Effect of Aspirin Dose

Aspirin Dose, mg/d	Trials, No.	Patients, No.	Odds Reduction, %
500–1,500	30	18,471	21 ± 4
160–325	12	23,670	28 ± 3
75	4	5,012	29 ± 7

*Data from Antiplatelet Trialists' Collaboration.[13]

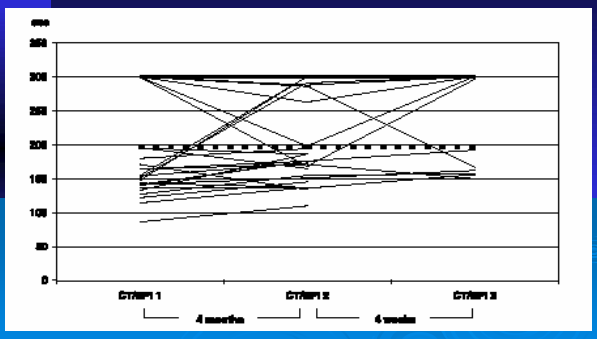
- Higher doses may inhibit endothelial prostacyclin production
- No dose-response effect for thrombosis, but there is a dose-response relation for GI side efx

Aspirin Resistance and NSAIDS



Catella-Lawson F et al. *NEJM* 2001;345(25):1809-1817.

ASA Responsiveness Varies Over Time



Andersen K et al. *Thromb Res* 2003;108:37-42.

Any CK-MB or troponin I elevation in aspirin-resistant and aspirin-sensitive patients

Marker	Aspirin resistant (%)	Aspirin sensitive (%)	p
CK-MB	51.7	24.6	0.006
Troponin I	65.5	38.5	0.012

Chen W-H et al. *J Am Coll Cardiol* 2004.

Natural History of Aspirin Resistance

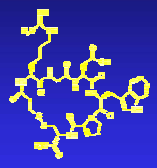
- Prospective, blinded
- 326 stable patients with CAD who were taking ASA for ≥ 7d, not taking other antiplt drugs
- 17 (5.2%) had ASA resistance by optical aggregation at baseline
  - Female
  - Lower Hb
- 24% of resistant pts suffered MI, CVA or death vs 10% of responders (p=0.03)

Gum P et al. *J Am Coll Cardiol* 2003;41(6):961-965.

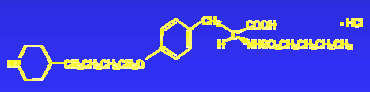
Parenteral inhibitors of GP IIb-IIIa

Antibody
- abciximab (ReoPro®, Centocor/Lilly)

Cyclic peptide
- eptifibatide (INTEGRILIN®, COR/Key)



Nonpeptide
- tirofiban HCl (Aggrastat®, Merck)



Glycoprotein IIb/IIIa Inhibitors

Pharmacokinetics and Monitoring

GP IIb/IIIa Receptor

- Upon activation of the platelet (by one of numerous possible routes) conformational change of the receptor occurs → high affinity ligand binding state
- All ligands (fibrinogen, vWF, fibronectin) are characterized by the arginine-glycine-aspartate (RGD) sequence which has been implicated as the binding site to the GP IIb/IIIa receptor
- Fibrinogen is a divalent ligand – each molecule can bind simultaneously to two GP IIb/IIIa receptors on adjacent platelets resulting in cross-linking

GP IIb/IIIa Receptor

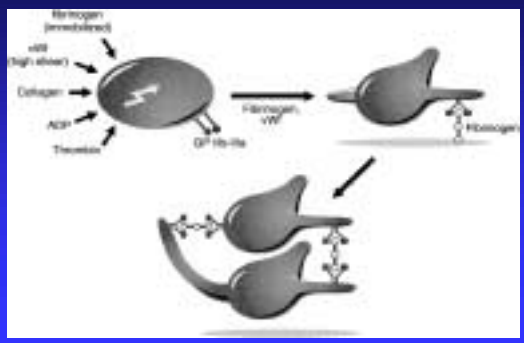
- Mediates platelet aggregation
- Member of the integrin receptor family – can interact with both extracellular and cytoskeletal molecules
- One of the most abundant cell surface receptors (50-80,000 receptors per platelet, 15% of surface protein)
- Ca^{+} ions are critical for maintenance of both structure and function
- In the resting platelet the receptor has minimal binding affinity for ligands – fibrinogen and vWF

Platelet Glycoprotein IIb/IIIa Inhibitors

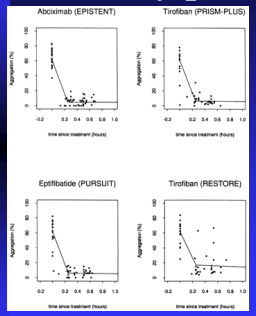
	Abciximab	Eptifibatide	Tirofiban
Specificity			
IIb/IIIa	+++	+++	+++
$\alpha_v\beta_3$	+++	0	0
MAC-1	+	0	0
↓ thrombin generation	++	+	+
Activated clotting time (sec)	30	20	30
Drug:receptor ratio	2	>250	>250
Plasma $T_{1/2}$	Min	2-3 hr	2 hr
Platelet-bound $T_{1/2}$	Hr	Sec	Sec
Reversibility (hr)	12-24	4-6	4-6
Renal adjustment	No	Yes	Yes

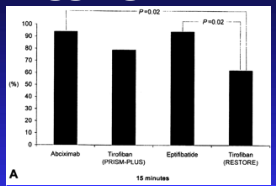
CP983778-11

GP IIb/IIIa Receptor



COMPARE Study – early platelet aggregation





Proportion of patients (%), in whom >80% inhibition of 20 µmol/L ADP-induced PA was achieved

73 ACS pts planned for PCI
Samples drawn in PPACK
Batchelor WB et al Circulation 2002

Platelet Glycoprotein IIb/IIIa Inhibitors

	Abciximab	Eptifibatide	Tirofiban
Treatment of bleeding	Platelet transfusion (8-10 units)*	Fibrinogen	Fibrinogen
		Cryopre-cipitate (8-10 units) or fresh frozen plasma (16-20 units)	Cryopre-cipitate (8-10 units)or fresh frozen plasma (16-20 units)
		Platelets (16-20 units)*	Platelets (16-20 units)*

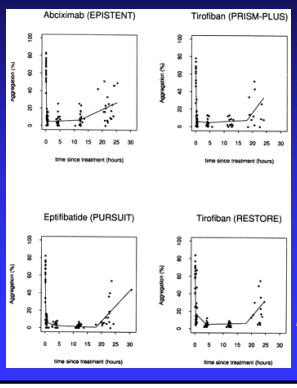
*Random donor

CP983778-12

Why is Monitoring of GP IIb/IIIa Inhibitor Therapy Necessary ?

1. Significant variability in the individual response to GP IIb/IIIa inhibitors
2. Clinical effect dependent on factors such as renal function (for the small molecule inhibitors) and platelet count (mainly for abciximab)
3. Need to evaluate platelet function after the GP IIb/IIIa is withdrawn, for instance before CABG

COMPARE Study – late platelet aggregation



Batchelor WB et al Circulation 2002

GOLD Study

- 485 patients undergoing a PCI with planned use of 1 of the 3 approved GP IIb/IIIa inhibitors
- Platelet function evaluated at various time points by RPFA correlated to clinical endpoints – MACE at 30 days
- Platelet inhibition at 10 min and MACE:
  ≥ 95% inhibition ⇒ 6.4% MACE
  < 95% inhibition ⇒ 14.4% MACE (p=0.006)
- Platelet inhibition at 8 hrs and MACE:
  ≥ 70% inhibition ⇒ 8.1% MACE
  < 70% inhibition ⇒ 25% MACE (p=0.009)

Steinhubl et al, Circulation 2001; 103: 2572-2578

Monitoring of GP IIb/IIIa Inhibitor Therapy – cont.

4. Narrow therapeutic window – low dosages result in higher rates of ischemic complications (e.g. IMPACT II), overdosage increases risk of bleeding

Moderate levels of platelet inhibition (especially for prolonged periods) may also induce prothrombotic + proinflammatory effects!

At high levels of GP IIb/IIIa receptor occupancy → inhibitory effect on inflammatory markers, whereas at low levels increase in platelet-monocyte complexes and CD40L (*Li et al ATVB 2000, Nannizzi-Alaimo et al Circ. 2001)*

Optical Platelet Aggregation

- Most common assay, employs platelet rich plasma (PRP), or less frequently whole blood
- The assay measures light passing through a sample of PRP after stimulation with a platelet agonist
- Advantages: wide use, correlates highly with bleeding time and with clinical efficacy of anti-platelet agents
- Disadvantages: time consuming, requires technical proficiency, high degree of intra-test and inter-laboratory variation, not sensitive at receptor occupancy levels of <30% and >80%

Monitoring of GP IIb/IIIa Therapy

Platelet function assessment:

standard laboratory methods

vs. bedside rapid assays

Perfusion Chamber

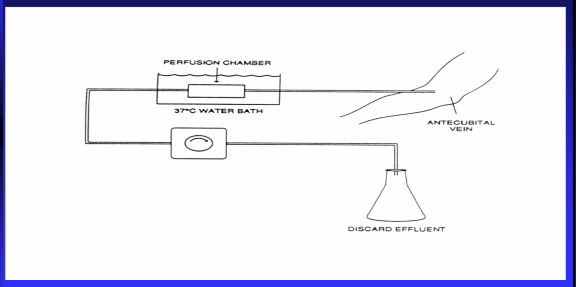
- Evaluates total blood thrombogenicity, not just platelet function.
- Venous blood is pumped directly from the patient into the chamber which contains 3 cylindrical flow channels with thrombogenic surfaces
- Porcine aortic tunica media prepared by peeling off the intima serves as a model of severe arterial injury *(Badimon J, ATVB 1991)*
- Rheologic conditions mimic those typical of a patent artery and mild-moderate coronary stenosis

Flow Cytometry

- Becoming the gold standard for platelet function eval.
- FACS measures the specific characteristics of a large number of cells after fluorescent labeling (typically fluorescent conjugated MoAbs)
- Mainly used for evaluation of platelet activation, for instance with FITC-labeled anti-fibrinogen MoAb (measures fibrinogen binding), PAC-1, P-Selectin or platelet-monocyte complexes
- Can also be used for evaluation of GP IIb/IIIa receptor occupancy (MoAbs for the binding sites)
- Advantages: accurate, measures many aspects of platelet function , low variability in results
- Disadvantages: Time consuming, requires very high technical proficiency, expensive

Perfusion Chamber

Perfusion Chamber



Perfusion time:	**5 minutes**
Blood flow:	**10 ml/min**
Shear rate:	**1690/sec**

Accumetrics' *Ultegra*® System

- Ultegra Rapid Platelet Function Assay (RPFA) is a cartridge-based, automated rapid assay that is based on the interaction between platelet GP IIb/IIIa receptors and fibrinogen-coated beads leading to the agglutination of the beads.
- The assay incorporates anticoagulated whole blood, fibrinogen beads, buffers and modified TRAP. Results expressed as percentage of baseline.
- High correlation to aggregation and FACS (r>0.8)
- Disadvantage: price, requires baseline sample

Smith et al, Circulation 1999; 99:620-5

Monitoring of GP IIb/IIIa Therapy

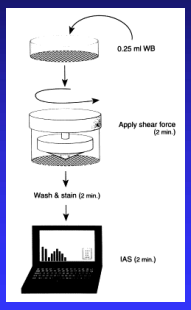
Platelet function assessment:

standard laboratory methods

vs. bedside rapid assays

Cone and Plate(let) Analyzer CPA

- Measures platelet deposition under high shear rate flow conditions.
- Whole blood is placed on wells. Shear force is applied, using a rotating cone (1300 sec^{-1}, for 2 min). Samples washed and stained.
- Surface platelet deposition is evaluated using an image analyzer.
- 4 samples can be analyzed during less then 10 min. A fully automatic version of the CPA is currently being developed
- High correlation to aggregation and FACS (r>0.8)



Varon et al, Thromb Res 1997;85:283-294

Accumetrics' *Ultegra*® System

Insert cartilage

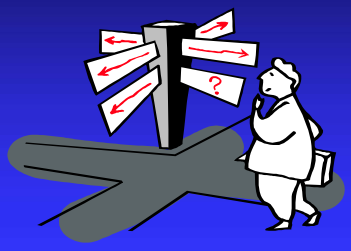
Insert whole blood sample

Results within 60 sec

CLOPIDOGREL

Pharmacokinetics, Response and Variability

Oral IIb/IIIa inhibitors

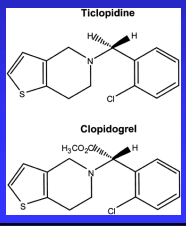


Pharmacokinetic properties

- Requires metabolism by the hepatic cytochrome P450-1A enzyme system to acquire activity
- Peak plasma concentrations of the main circulating metabolite, an inactive carboxylic acid derivative occur at 1 hour.
- Active metabolite identified in vitro by incubation of human liver microsomes *(Pereillo JM et al Drug Metabol and Disp 2002)*
- Contrary to ticlopidine, its bioavailability is unaffected by food

CLOPIGOGREL

- A thienopyridine , inhibits ADP induced platelet aggregation
- The specific target of inhibition appears to be the $P2Y_{12}$ receptor
- Fewer side effects than ticlopidine



P2Y Receptors

- The effect of ADP on platelets is mediated by two P2Y receptors - $P2Y_1$ and $P2Y_{12}$
- Activation of the $P2Y_1$ receptor leads to platelet shape changes and a rapid reversible wave of platelet aggr.
- Activation of the G-coupled $P2Y_{12}$ receptor leads to a progressive and sustained wave of platelet aggr. (mediated by inhibition of adenylate cyclase) as well as activation of GP IIb/IIIa by another pathway
- The $P2Y_{12}$ receptor is the target of clopidogrel (metabolite)
- Clopidogrel also inhibits platelet aggregation induced by other agonists, by inhibiting the effects of ADP released from platelet dense granules (in activated platelets)

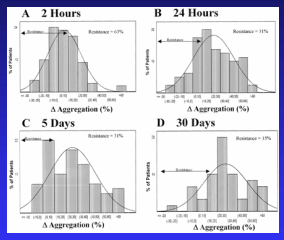
Pharmacdynamic properties

- Dose dependent inhibition of platelet aggregation can be seen 2 hours after a single dose
- Inhibition of ADP induced platelet aggregation reaches a maximum of 40% to 60% after 3 to 5 days. Similarly, recovery of platelet function is delayed after discontinuation, occurring slowly over 3 to 5 days

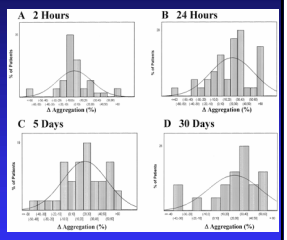
Response to Clopidogrel

- Marked interindividual variability in response to clopidogrel as measured by inhibition of aggregation.
- "Resistance", defined as baseline aggregation minus post-treatment aggregation ≤10% by 5 µmol/L ADP, was present in 31% and 15% of patients at 5 and 30 days. *Gurbel P et al Circulation 2003*
- 5-11% non responders (≤10% delta in ADP aggr.) and 9-26% semi-responders (10-29% delta) in the study of *Muller et al Throm Haemost 2003*

Response to Clopidogrel



5 µmol/L ADP aggregation



20 µmol/L ADP aggregation

96 patients undergoing elective PCI

Gurbel P et al Circulation 2003; 1107: 2908-13

Clopidogrel – Statin Interaction

- "Atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation: a new drug-drug interaction" *Law W et al Circulation 2003; 107: 32-7*
  Atorvastatin, but not pravastatin, attenuated the antiplatelet activity of clopidogrel in a dose-dep. manner
- Pro – "Lipophilic statins interfere with the inhibitory effects of clopidogrel on platelet function – a flow cytometry study". *Neubauer et al EHJ 2003; 24: 1744-9*
- Con – "Effect of statins on platelet inhibition by a loading dose of clopidogrel" *Muller et al Circ. 2003; 108: 2195-7*

Possible Reasons for Low Response

- Mutations in the ADP receptor $P2Y_{12}$
  5 frequent polymorphisms identified in the gene for the P2Y12 receptor. Among healthy volunteers, two groups of subjects with low and high responsiveness to ADP aggr. were identified and associated to one of the polymorphysims. *Fontana et al Circulation 2003; 108: 989-95*
- Interaction with other drugs – mainly drugs metabolized by cytochrome P450-1A (CYP3A4)
- Differences in the rate of conversion of clopidogrel to its active metabolite

ISAR-REACT Trial

2,159 low-risk patients undergoing elective stenting, excluding patients with:

- Acute coronary syndrome
- Acute MI with 14 days
- ST-segment depression
- Positive biomarkers
- Insulin-dependent diabetes
- Chronic total occlusions
- EF <=30%
- Thrombus presence
- Lesions in bypass grafts

Clopidogrel
(600 mg loading dose 2 x 75 mg/d through discharge, 75 mg/d for 4 weeks)

Abciximab (n = 1,079)	Placebo (n = 1,080)

Endpoints:

- Primary – 30 day death / MI / urgent target vessel revascularization
- Secondary –30 day bleeding complications

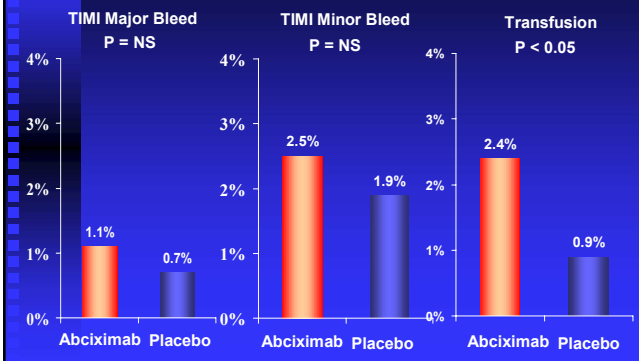
ACC 2003, Late Breaking Trials

Cardiovascular events according to antiplatelet effect of clopidogrel

Quartile according to % reduction of ADP-induced platelet aggregation	Platelet aggregation at day 6 (as % of baseline)	% of patients with a CV event at 6 months
1	103	40
2	69	6.7
3	58	0
4	33	0

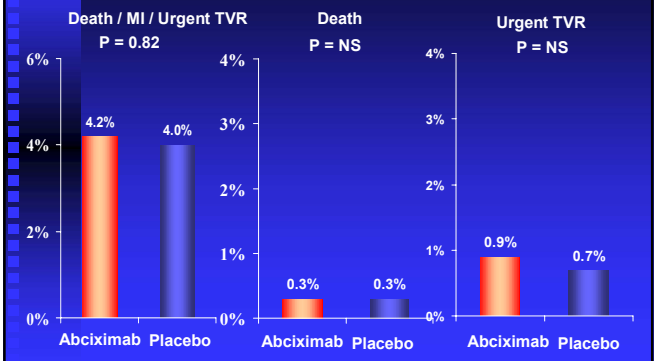
(Matetzky S et al. *Circulation 2004*

ISAR-REACT Trial: Bleeding Results



ACC 2003, Late Breaking Trials

ISAR-REACT Trial: 30 Day Endpoints



ACC 2003, Late Breaking Trials

Activation and inhibition of coagulatiion

Coagulation pathways		Anti-coagulants
Initiation	Tissue factor/factor VIIa	**Tissue factor pathway inhibitors** NAPc2, VIIai
Thrombin generation	Factor X, Factor IX, Factor IXa, Factor VIIIa, Factor Xa, Factor Va	**IXa inhibitors** IXai, IXa antibody **Protein C activators** Protein C, thrombomodulin **Xa inhibitors** TAP, pentasaccaride, DX9065
Thrombin activity	IIa (thrombin)	**Thrombin inhibitors** Hirudin, bivalirudin, argatroban

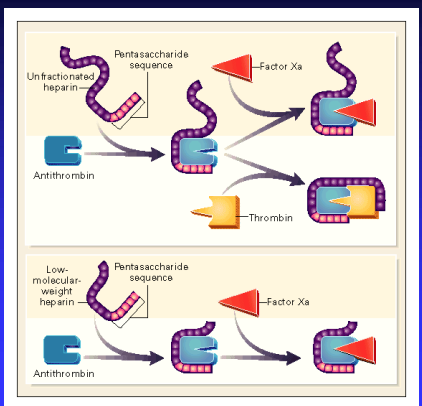
Anticoagulants

TABLE 1. COMPARISON OF LOW-MOLECULAR-WEIGHT HEPARIN PREPARATIONS.

PREPARATION	METHOD OF PREPARATION	MEAN MOLECULAR WEIGHT	ANTI-Xa: ANTI-IIa RATIO*
Ardeparin (Normiflo)	Peroxidative depolymerization	6000	1.9
Dalteparin (Fragmin)	Nitrous acid depolymerization	6000	2.7
Enoxaparin (Lovenox)	Benzylation and alkaline depolymerization	4200	3.8
Nadroparin (Fraxiparine)	Nitrous acid depolymerization	4500	3.6
Reviparin (Clivarine)	Nitrous acid depolymerization, chromatographic purification	4000	3.5
Tinzaparin (Innohep)	Heparinase digestion	4500	1.9

*The ratios were calculated by dividing the anti–factor Xa (anti-Xa) activity by the antithrombin (anti-IIa) activity. The ratios are based on information provided by the manufacturers.

Catalysis of Antithrombin-Mediated Inactivation of Thrombin or Factor Xa by Unfractionated Heparin or Low-Molecular-Weight Heparins



Thrombotic Process – Pathophysiology
Thrombin

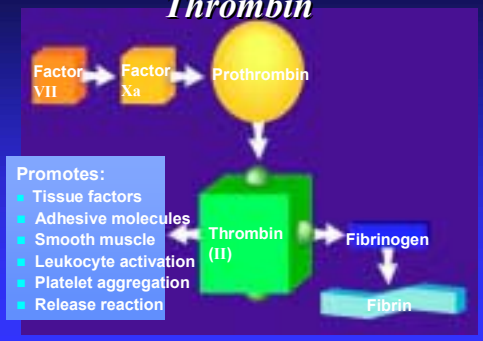
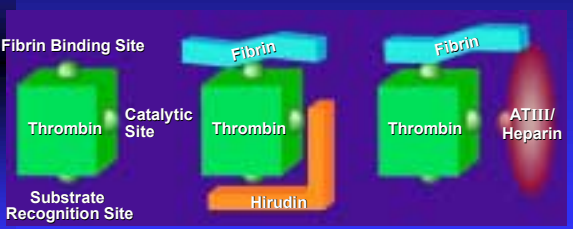


TABLE 2. MECHANISMS RESPONSIBLE FOR THE PHARMACOKINETIC ADVANTAGES OF LOW-MOLECULAR-WEIGHT HEPARINS OVER UNFRACTIONATED HEPARIN.

ADVANTAGE	MECHANISM
More predictable anticoagulant response	Less binding to plasma proteins and to proteins released from activated platelets and endothelial cells
Better bioavailability at low doses	Less binding to endothelium
Dose-independent clearance mechanism	Less binding to macrophages
Longer half-life	Less binding to macrophages

Anticoagulants
Direct Thrombin Inhibitors
Hirudin-Thrombin Binding
Mechanisms of Thrombin Inhibition



Rihal, Flather, Hirsh, Yusuf, 1995

Anticoagulants
Direct Thrombin Inhibitors
Hirudin

- 65 amino acid protein
- Originally identified in saliva of medicinal leech (*Hirudo medicinalis*)
- Now available through recombinant DNA technology (lepirudin and desirudin)

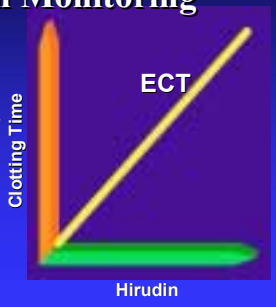
Anticoagulants
Direct Thrombin Inhibitors
Hirulog

- Synthetic antithrombin agent
- Not as potent as hirudin in binding to thrombin
- Investigated in an angioplasty population
- Has shown modest benefits compared to heparin, with lower bleeding risks
- Under investigation for acute MI

Anticoagulants
Direct Thrombin Inhibitors
Hirudin Monitoring

Ecarin clotting time (ECT)

- Sensitive, rapid
- Linear correlation – hirudin/time
- No heparin/warfarin interaction



Trial Design

REPLACE-2

Bivalirudin vs Heparin + GP IIb/IIIa During PCI

N = 6010 Patients: Urgent or Elective PCI

Randomization - double blind, triple dummy

Heparin	target ACT ≥ 225 sec	Bivalirudin
65 U/kg initial bolus		0.75 mg/kg initial bolus, 1.75 mg/kg-hr during PCI
Planned GP IIb/IIIa (abciximab or eptifibatide)		Provisional GP IIb/IIIa (abciximab or eptifibatide)

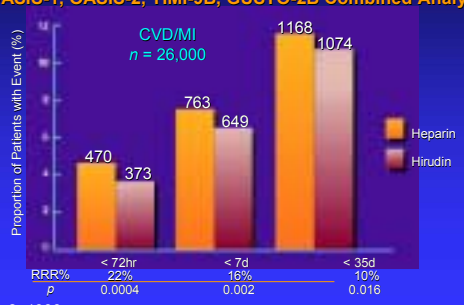
abciximab: 0.25 mg/kg bolus, 0.125 µg/kg-min (max 10 µg/min) x 12 hrs

eptifibatide: 180 µg/kg double bolus, 2.0 µg/kg-min x 18-24 hrs

• "Quadruple Endpoint" at 30 Days •

Clinical RCTs
Hirudin vs UFH

OASIS-1, OASIS-2, TIMI-9B, GUSTO-2B Combined Analysis



OASIS-2, 1999

REPLACE-2

Statistical Methods - 1

Imputed Comparison

O.R.(Biv-Hep)

Heparin	Heparin + GP IIb/IIIa	Bivalirudin
EPISTENT and ESPRIT O.R.(Hep-GP) = 0.68 (0.55 - 0.84)		REPLACE - 2 O.R.(Biv-GP)

1st Hypothesis: Bivalirudin superior to Heparin:

O.R.(Biv-Hep) = O.R.(Biv-GP) x O.R.(Hep-GP)

2nd Hypothesis: Bivalirudin not inferior to GP IIb/IIIa + Heparin:

O.R.(Biv-Hep) preserves half benefit of O.R.(Hep-GP)

REPLACE-2

Study Flow

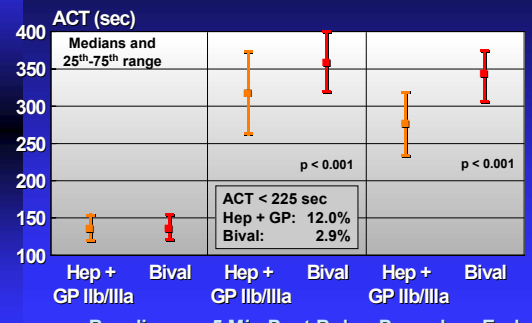
Screen and consent

Interventionalist's choice of abciximab vs eptifibatide

Telephone randomization

Bivalirudin	Aspirin Clopidogrel pre-RX recommended	Heparin + GP IIb/IIIa
Bivalirudin Heparin placebo GP IIb/IIIa placebo	Blinded ACT and 2nd bolus (active drug if ACT < 225s)	Bivalirudin placebo Heparin GP IIb/IIIa
If provisional GP IIb/IIIa: GP IIb/IIIa	PCI	If provisional GP IIb/IIIa: GP IIb/IIIa placebo

REPLACE-2 Activated Clotting Times

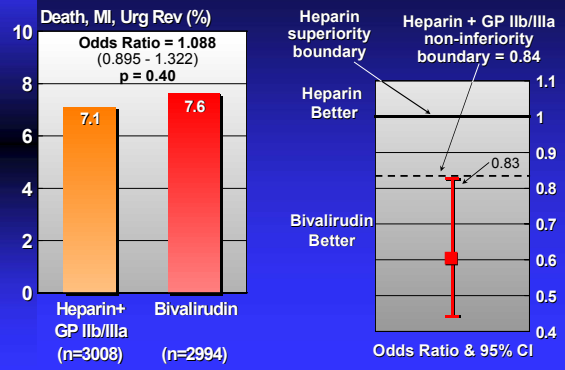


REPLACE-2 IIb/IIIa Inhibitors

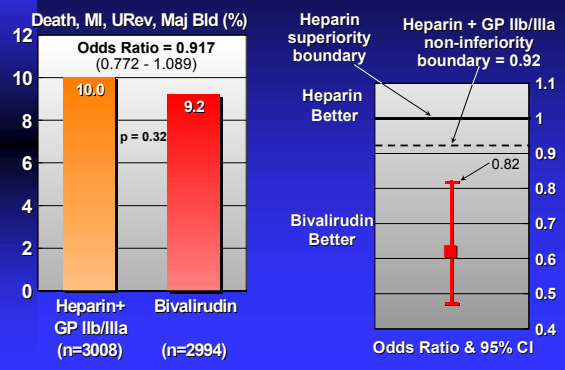
	Bivalirudin (N = 2994)	Heparin + GP IIb/IIIa (N = 3008)
Planned GP IIb/IIIa (%)	**0.07**	**96.3**
abciximab (%)	0	42.9
eptifibatide (%)	0.07	53.4
Provisional GP IIb/IIIa (%)	**7.2 ***	**5.2 ***
abciximab (%)	3.5	0
eptifibatide (%)	3.7	0
placebo (%)	0	5.2

*p = 0.002

REPLACE-2 Triple Ischemic Endpoint

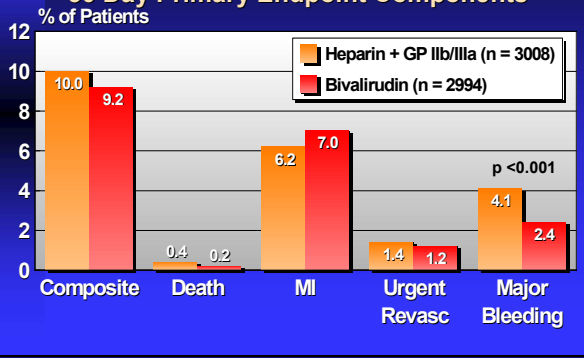


REPLACE-2 Primary Quadruple Endpoint



Oral Direct Thrombin Inhibitors

REPLACE-2 Quadruple Endpoint



ESTEEM: Post-hoc analysis

End point	Placebo (%)	Combined ximelagatran groups (%)	Hazard ratio (95% CI)
All-cause mortality, nonfatal MI, nonfatal stroke	11.1	7.4	0.66 (0.48-0.90)

ESTEEM: Primary end point

End point	Placebo (%)	Combined ximelagatran groups (%)	Hazard ratio (95% CI)
All-cause mortality, nonfatal MI, severe recurrent ischemia	16.3	12.7	0.76 (0.59-0.98)

SPORTIV III/V combined analysis

Outcome	Warfarin	Ximelagatran	p
Thromboembolic events (n)	93	91	0.94
Primary event rates (all strokes plus SEE) (%/yr)	1.7	1.6	0.941
Secondary event rates (%/yr)	3.3	2.8	0.625
Major bleeding rates (%/yr)	2.5	1.9	0.054
Combined minor and major bleeding rates (%/yr)	39	32	<0.0001
ALAT levels >3 times normal (%)	0.8	6.1	HS*

SEE=systemic embolic events
*Highly signficant

ESTEEM: Bleeding complications

End point	Placebo (%)	Combined ximelagatran groups (%)	Hazard ratio (95% CI)
Major bleeding	1	2	1.97 (0.80-4.84)
Total bleeding (major or minor)	13	22	1.76 (1.38-2.25)