

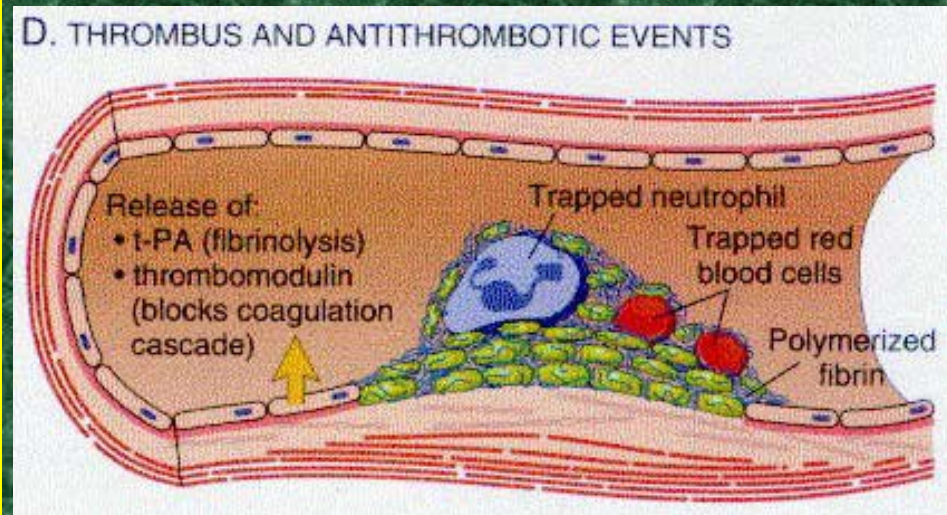
UNDERSTANDING THROMBOLYSIS

THE ALPHABET SOUP OF THROMBOLYTICS

Thrombus/Antithrombotic mechanisms

Platelet adhesion, activation, thrombus, aggregation

- Formation of a permanent plug.
- Antithrombotic mechanisms are activated to restrict the hemostatic plug to the site.

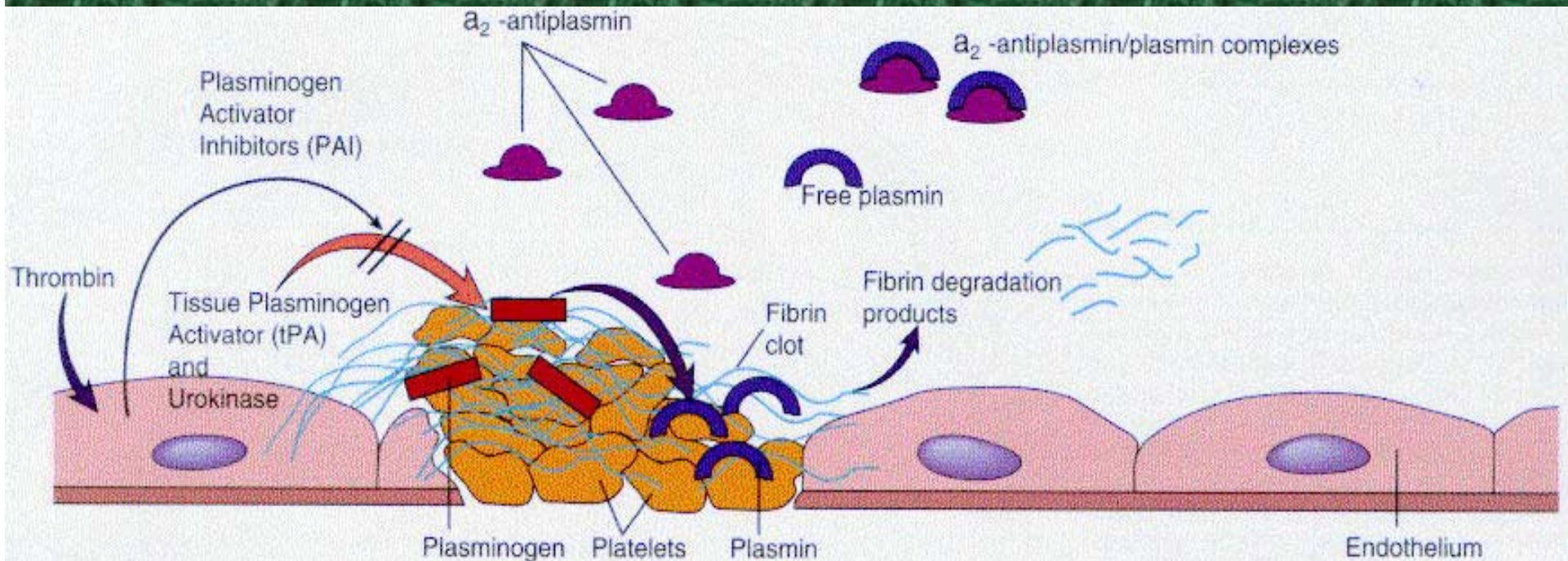


Clotting Regulation

- 3 types of natural anticoagulants
 1. *Antithrombins* (antithrombin III) inhibit thrombin activity and other serine proteases (factors IXa, Xa, XIa, and XIIa). Antithrombin III activated by binding to heparin-like molecules on endothelial cells.
 2. *Proteins C and S*: Vitamin K-dependent proteins able to inactivate factors Va and VIIIa via activation by thrombomodulin.
 3. *Plasmin*, activated from plasminogen, degrades fibrin and interferes with fibrin polymerization. Fibrin split products can also act as weak anticoagulants.

Plasminogen

- Cleaved to plasmin by factor XII-dependent pathway or by 2 distinct groups of plasminogen activators.



INCREASED RISK OF CLOTTING INCREASED PAI-1 LEVELS

Correlates with post-op DVT

Paramo JA, Thromb and Haem 1985, 54:713-6

Smoking (not in Type 1 diabetics)

Simpson AJ, Diab Med 1998, 15:683-7

Surgery and trauma

D'Angelo A, Eur J Clin Invest 1985, 15:308-12

Hip surgery

Increase PAI and $\alpha 2$ antiplasmin, decrease t-PA

Paramo JA, Thromb and Haem 1985, 54:713-6

INCREASED RISK OF CLOTTING DEPRESSED t-PA LEVELS

GYN malignancy esp ovarian

16% incidence of DVT

Doman KA, Gyn Onc 1986, 23:141-8

Other malignancy

Smoking (>10 cig/d) and BC pills (esp > 5 yrs)

Kjaelgaard A, Act Obs Gyn Scand 1986, 65:219-22

Thrombolytic Drugs

SK -- Streptokinase

Streptokinase plus

Indirect plasminogen activator

UK -- Urokinase - UK is BACK

Urokinase plus

Direct plasminogen activator

tPA family -- Tissue Plasminogen Activators

Direct plasminogen activators

rPA, rtPA, TNK

ACTIVATION OF PLASMINOGEN

UK linear effects and min change from clot to plasma

All tPA molecules enhanced by the presence of fibrin, rtPA x 400-1000

(Bachmann F, Semin Thromb Hemost, 84: 6-17)

(Ranby M, Biochem Biophys Acta, 82: 461-9)

All tPA molecules lower affinity for circulating plasminogen

(Collen D, Circ, 83: 462-5)

All tPA molecules activated markedly less by fibrinogen

(Verheijen JH, Thromb Res 1982, 27:277)

THE PA MOLECULE

The protease domain

Similar in all

PAI binding -- TNK modified to be less

Finger -- High affinity fibrin binding - t,TNK

EGF -- High affinity liver binding - t,TNK

K1 -- Multiple receptor binding - u,t,TNK_m

K2 -- FSP and low affinity fibrin binding - u,r,t,TNK

Carbohydrates -- Mediate plasma clearance - t, TNK_m

t-PA BIOCHEMISTRY

Cleared by RE cells

Neutralized by α -2 antiplasmin

less by α -2 macroglobulin

(Plasmin neutralized by α -2 antiplasmin)

rtPA KINETICS

2 healthy volunteers

Dose 40-60 mg/90 min then 5 mg/hr x 6 hrs

Max plasma levels 1.08-1.56 $\mu\text{g/ml}$

Stable levels x 6 hrs at 0.25 $\mu\text{g/ml}$

T 1/2 5.7 and 78 minutes

At 90 minutes labs as % of baseline

Fibrinogen 74% and 57%

Plasminogen 55% and 48%

α 2-antiplasmin 28% and 18%

No sig change over next 6 hrs

Verstraete M, *Thromb Heme* 1986, 56:1-5

FIBRIN AFFINITY

FIBRIN SPECIFICITY

Affinity: Binds to fibrin

UK < rPA < TNK < rtPA

Enzymatic activity with fibrin rtPA = TNK

Stewart RJ, J Biol Chem 2000, 275:10112-20

Specificity: Lyses fibrin > fibrinogen

UK = rPA < rtPA < TNK

HALF-LIFE STICKING TO THE LIVER

Short is good (rt-PA 5-7 minutes)

No unwanted side effects

Rapid clearance for next Rx

Long is good (UK 16, rPA 15-19, TNK 18-22 minutes)

Bolus administration

Prolonged high serum level

FIBRIN AFFINITY PERCOLATE vs STICK

High affinity is good

Pulse spray works better and faster with rtPA

True in rabbits

Bookstein, JVIR 2000

True in patients

Yusuf, Eur J Vasc Endo Surg 1995

Chang (NIH) DVT technique

Low affinity is good

PE lyses faster with rPA

Tebbe, Am H J, 1999

Lysis speed increases with dose of rPA (and UK)

Fisher, Fibrinolysis Proteolysis, 1997

SYSTEMIC FIBRINOLYTIC STATE

Decreased levels of

Fibrin

Fibrinogen

α 2-antiplasmin

Factors V and VIII

Increased levels of

Fibrin(ogen) split products

The danger seems to arise with DDE
binding TNK < tPA, rPA and U prob
also lower but free plasmin levels are
equal for tPA and rPA

Fisher S, Fibrin and Prot 1997, 11:129-35

HEPARIN EFFECT

Activation vs enhancement?

Dog model

rt-PA lysis 27%

rt-PA plus heparin 79%

rt-PA plus ancrod pre (fibrinogen depletor) 68%

Cercek B, *Circulation* 1986; 74:583-7

rt-PA DOSE and BLEEDING

Experiment in rabbits IV therapeutic dose

Given over	15 min	30 min	1 hr	4 hrs
Lysis	96%	88%	87%	36%
Bleeding	No	No	Min	Sig

Double dose over 4 hrs

Lysis	96%
Bleeding	Fatal

rt-PA FOR PAO DOSE COMPARISON

	0.10 mg/kg/hr	0.05 mg/kg/hr
Efficacy	95%	100%
Speed	4-8 hrs	4-8 hrs
α -2 antiplasmin depletion	< 20% 4 hrs	< 20% 4 hrs

rt-PA FOR PAO DOSE COMPARISON

0.10 mg/kg/hr

0.05 mg/kg/hr

Fibrinogen > 75%

of baseline

48%

63%

Both doses retained normal coags throughout the infusion

Risius B, Radiol 87: 465-68

FIBRINOGEN DEPLETION WITH HIGH-DOSE rt-PA

Infusion at 0.1 mg/kg/hr

PAO avg infusion time 3.6 hrs

Longer clot > shorter

Old clot = new

Plasminogen (Clauss) fell to < 50% baseline

Overall 52%

1/11 infused < 3 hrs

9/14 infused > 3 hrs

α 2 antiplasmin depletion to < 50% baseline

0/6 infused < 2 hrs

16/19 infused > 2 hrs

(Risius B, Radiol 86: 183-88)

rt-PA PROTOCOL

BIG CHANGES TO SMALLER DOSES

PAO and DVT

0.02 mg/kg/hr goes to 0.005 mg/kg/hr

Average 1/4 to 1/2 mg/hr = 1-2 cc/hr

Catheter infusion

0.001-0.002 mg/kg/hr goes to 0.0005 mg/kg/hr

Average 0.1-0.2 cc/hr not tenable so

dilute 1/10 and run at 1-2 cc/hr

Catheter “lock” -- No change

FUTURE ADDITIONS TO THE ALPHABET SOUP

P = plasmin

Delivered IN CLOT

Potentially very low risk as long as
 α 2-antiplasmin levels are adequate

χ t-PA

Will increasing fibrin affinity help

AS = antiselectins

Platelet effects are both immediate and longer
term mediated through other interactions

INFLAMMATION AND THROMBOSIS

P (also E and L) selectins

Expressed by hematopoietic cells

Binding of activated platelets and neutrophils

Neutrophil penetration of vessel wall

Release of inflammation mediators

Cytokines, proteases, eicosanoids

Free radicals

Possibly inhibited by heparin and Reopro

Wakefield TW, *Thromb Haemost* 1993, 69:164-72

INFLAMMATION AND THROMBOSIS

P-selectin monoclonal Ab

Primate model; electrolytic fem art injury

Post-injury; heparin and lysis

Platelet-leukocyte tissue factors decrease

Fibrin deposition decreases

Faster and easier lysis

Toombs CF, *J Pharm Exp Ther* 1995, 275:941-9

rP-selectin glycoprotein ligand-Ig

Porcine model; iliac artery thrombus

Accelerate lysis 61/70% at 500/250 mcg/kg

Prevention of rethrombosis

Kumar A, *Circulation* 1999, 99:1369-9

ALPHABET SOUP THE BOTTOM LINE

It likely depends more on dose and delivery

Many observations have no rationale

Practical matters are important

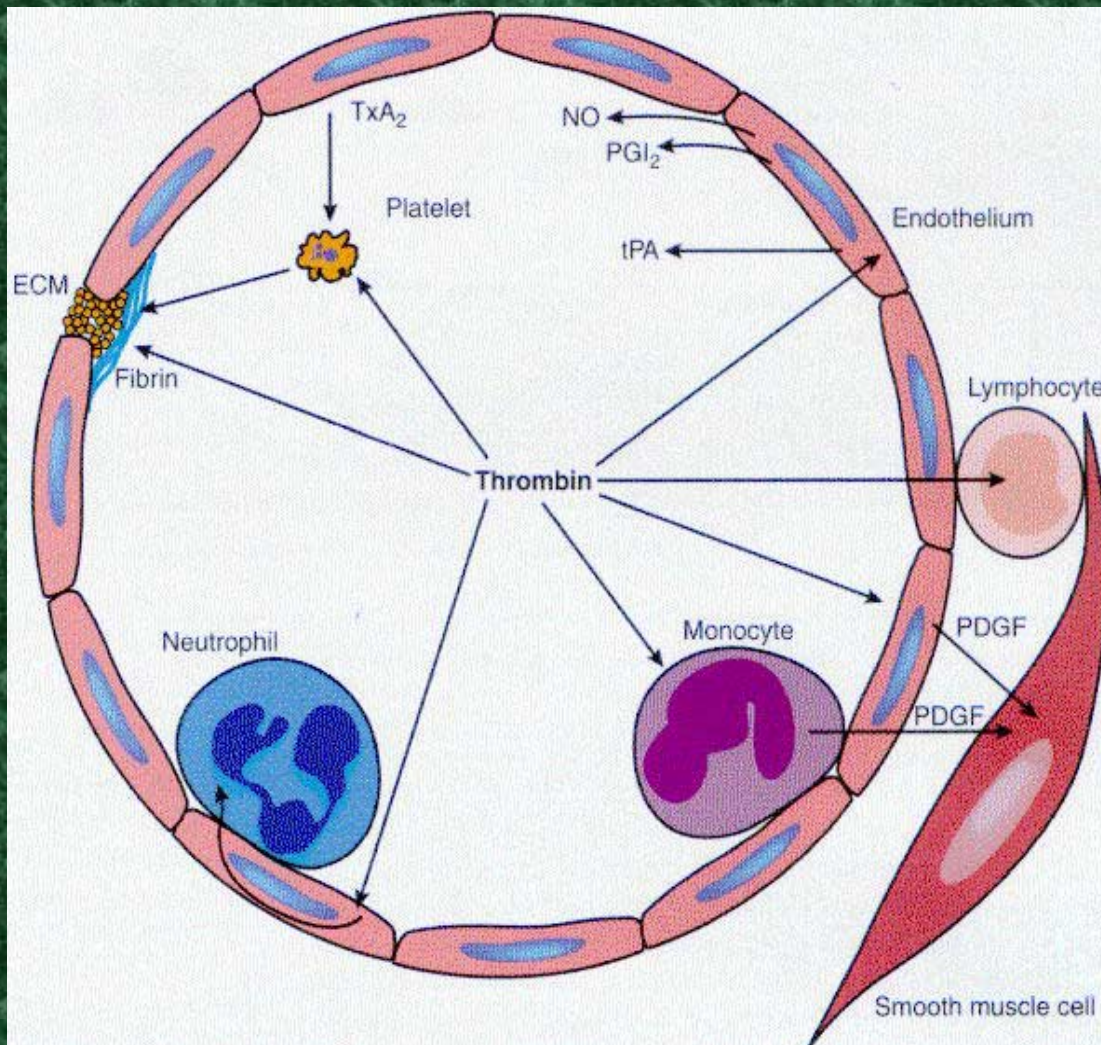
Rep availability

Support for research

Dose vial availability

PRICE

Central Role of Thrombin



- Generates cross-linked fibrin
- Directly induces platelet aggregation and secretion
- Activates endothelium to generate leukocyte adhesion molecules and various mediators.
- Mononuclear cells may be activated by thrombin

Plasmin Regulation

Plasminogen activity usually restricted to sites of thrombosis by combination of 3 mechanisms:

1. t-PA activates plasminogen best when bound to fibrin meshwork
2. Any free plasmin in circulation is rapidly bound and neutralized by alpha 2-antiplasmin
3. t-PA activity restricted by PAIs.

Plasminogen Activators

1. Urokinase-like PA (u-PA) capable of activating plasminogen in the fluid phase. Plasmin can convert the inactive pro-urokinase precursor to active u-PA, creating an amplification loop.
2. Tissue-type PA (t-PA), synthesized by endothelial cells and most active when attached to fibrin which targets the fibrinolytic activity to sites of recent clotting.
3. Plasminogen can also be activated by the bacterial product streptokinase, which may have some significance in bacterial infections.

rt-PA KINETICS

45 pts with acute MI

Dose 0.24-0.50 mg/kg/hr IV

Plasma levels 0.52-1.4 $\mu\text{g/ml}$

Dose < 0.3 mg/kg/hr (20 mg/hr) min success

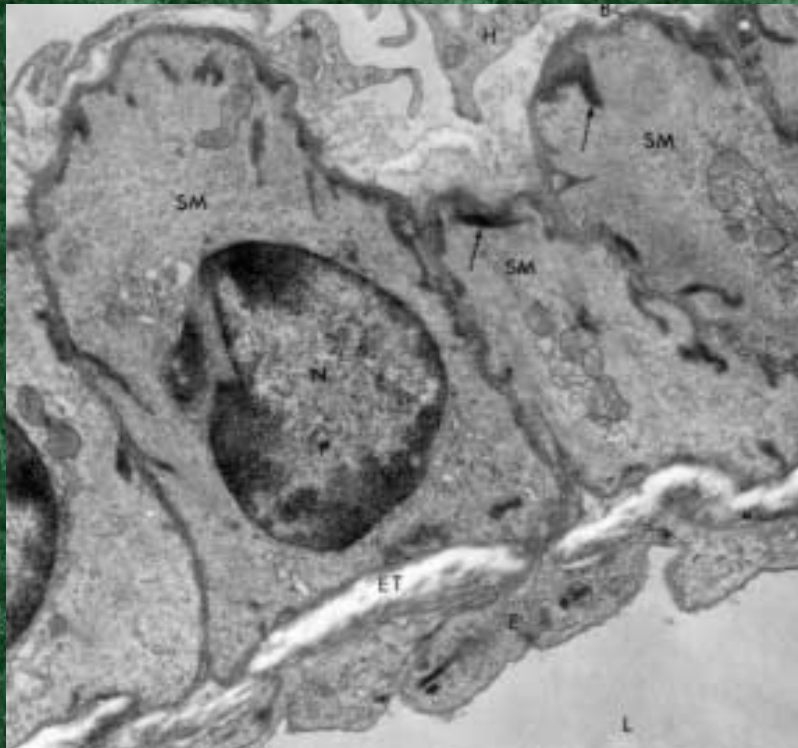
Linear relationship dose : plasma level

Each pt different slope (distrib vol)

$t_{1/2}$ of 5.6 and 46 min

Garabedian HD, Am J Card 1986, 58:673-9

Plasminogen Activator Inhibitors



- Endothelial cells modulate hemostasis by releasing PAIs that block fibrinolysis.
- PAIs are increased by thrombin as well as certain cytokines and likely play a role in the intravascular thrombosis that accompanies severe inflammation.