Engineering Approaches for Thrombolytic Therapy

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Introduction

A major cause of myocardial infarction (MI) is an end-stage thrombotic event triggered by highly thrombogenic fissures of atherosclerotic plaque on a diseased and narrowed coronary artery. Several thrombolytic agents, such as tissue plasminogen activator (tPA), urokinase (uPA), and streptokinase (SK), have been developed to dissolve blood clots. Thrombolytic therapy for the treatment of acute myocardial infarction includes intravenous infusions of a thrombolytic agent, and more recently intracoronary and intrathrombic catheter delivery. The pharmacokinetic analysis of this therapy represents a challenging problem in cardiovascular drug delivery because the binding target (the clot) is changing constantly as a result of the therapy. To understand the relation between dose and outcome as well as the rate-limiting steps of thrombolytic therapy requires the biomedical engineering tools of fluid mechanics, transport phenomena, and reaction engineering. An integrative approach that includes molecular level information (binding affinities, catalytic rates) along with mass transport theory for an evolving biphasic medium provides a framework for understanding and improving the emergency thrombolytic treatment of heart attack victims. To quantify rate processes, particular attention must be placed on the rates of penetration, driven by arterial pressures, of plasma constituents into thrombi. Protease transport is uniquely complicated in that these proteins bind dynamically to the solid fibrin fiber network. As the bound plasmin dissolves the fibrin, these proteins are then solubilized back into the interstitial fluid of the clot. In this paper, analytic approaches are outlined that relate the pharmacokinetics of a given thrombolytic therapy (for any dosage regimen) to reperfusion time and flow rate.

Biophysical Considerations in Thrombosis and Thrombolysis

During platelet activation in a damaged vessel, prothrombin is activated to thrombin, which then cleaves the negatively charged fibrinopeptides A and B from the N terminal of α and β chains of fibrinogen, yielding a fibrin monomer (see Figure 1). This monomer rapidly polymerizes longitudinally, forming protofibrils by electrostatic interactions *(1, 2)*. The protofibrils form fibers which further associate into fiber bundles with diameters up to about 500 nm. Entrapped platelets can retract the fibrin gel (through the transmembrane glycoprotein receptor GP IIb-IIIa) to about a tenth of the original gel volume by squeezing serum from the clot. Often, retracted clots are resistant to fibrinolytic degradation *(3)*. Also, thrombin can activate Factor XIII which covalently crosslinks fibrin. Under physiological conditions, the crosslinking of γ-chains of the fibrin monomers occurs at a much faster rate than the crosslinking of α-chains. Late stage α-α crosslinks are associated with poor lytic susceptibility *(4)*.

Fibrin gel structure is very sensitive to polymerization conditions *(2, 5)*. The fiber diameter, D_{fiber}, can be thick (200-500 nm) in coarse gels formed at physiologic ionic strength, whereas fine gels formed at high ionic strength have a typical fiber diameter of 20 to 50 nm. The effective pore diameter in fibrin gels can vary from 100 nm in fine gels to 5,000 nm in coarse gels. The effective diffusion coefficient of bovine serum albumin (MW 67 kDa) in fibrin has been found to be 5×10^{-7} cm^2/sec *(6)*, a value quite similar to that in water, consistent with the fact that proteins have a hydrodynamic radius much smaller than the lattice spacing of the gel. The specific permeability, k, is much higher in coarse clots ($k \sim 10^{-8}$ cm^2) than in fine gels ($k \sim 10^{-10}$ cm^2). Retracted whole blood clots possess a permeability on the order of 10^{-11} cm^2 *(7)*. Clots formed *in vivo* under flow conditions are likely more dense, with permeabilities as low as 10^{-13} cm^2 due to flow compaction and repolymerization of fresh fibrinogen by clot-bound thrombin.

Mathematical expressions for the specific permeability of a fibrous medium are often given as variations of the Ergun equation, which correlates specific permeability with the porosity (ε) and a length scale of the microstructure such as the fiber diameter. The specific permeability of fibrin is suitably correlated by the Davies equation:

$$k = D_{fiber}^2 / \left(70(1-\varepsilon)^{3/2}\{1+52(1-\varepsilon)^{3/2}\}\right). \quad (1)$$

Permeation of plasma through clots may be minuscule compared to nominal blood flow rates through open vessels; nevertheless, this permeation can dictate the thrombolytic rate. Some permeation must occur because radiolabeled fibrinogen injected into dogs with existing occlusive coronary clots is found to be distributed along the length of the clot in under an hour *(8)*. We

Keywords: hemodynamics, plasminogen, fibrin, diffusion

estimate the permeation velocities across coronary thrombi to be about 0.001 cm/sec *(9, 10)*. To obtain reperfusion within 45 to 90 minutes, some permeation of the lytic agent into the clot must occur because protein diffusion is such a slow process over length scales of a centimeter, a typical length of an arterial thrombus.

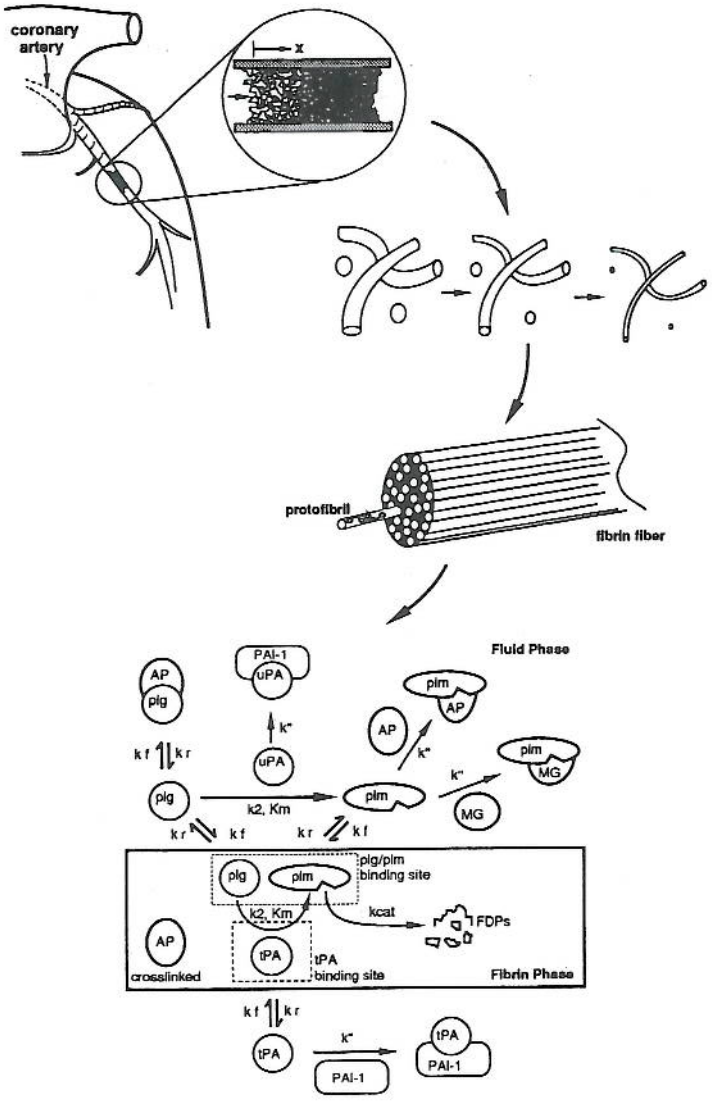

Figure 1. Summary of thrombolytic therapy. An occlusive thrombus resides in a coronary artery and causes myocardial infarction. The thrombus is held together by a protein polymer meshwork called fibrin. The fibrin fibers are actually bundles of protofibrils formed from polymerized fibrinogen. For the purpose of modeling, these fibers are assumed to dissolve by reduction of their diameter at constant length. Plasminogen (plg) is activated to plasmin (plm) in the fluid phase by urokinase (uPA), while fibrin bound plasminogen is activated by tissue plasminogen activator (tPA) in the fibrin phase. The free phase plasmin is inhibited by the antiplasmin (AP) and macroglobulin (MG), while the bound phase plasmin is protected from inhibition. The uPA and tPA are inhibited in the free phase by plasminogen activator inhibitor type 1 (PAI-1). Soluble species can reversibly adsorb and desorb with fibrin under kinetically controlled conditions. Plasminogen and plasmin compete for the same sites on the intact fibrin monomer, while tPA binds a unique site. Adsorption/desorption rates are estimated from K_d in the literature and from the estimated time needed to reach equilibrium. Glu to Lys plasmin(ogen) forms, single-chain/two-chain forms, and carboxyl terminal lysines of nicked fibrin are not shown. Rate constants are taken from the literature as described in *(21)*.

The molecular biology and biochemistry of fibrinolysis are well established (see Figure 1). Urokinase can activate plasminogen to plasmin in the fluid phase whereas tPA, which serves as a cofactor for plasminogen activation, is most active when bound to fibrin. Distinct degradation products are produced during plasmin digestion of fibrin. In non-crosslinked gels, the intermediate X and Y fragments can be degraded further to E and D fragments, whereas in factor XIIIa-crosslinked gels, large fragments contain significant quantities of D-D dimers and DD/E fragments *(11)*. In the early stages of lysis, plasmin cleaves several sites in the C-terminus of the α chains of fibrin to yield fragment X polymer. Fragment X polymer generation in fibrin is considered to be the critical stage for enhanced tPA binding to partially degraded fibrin, and this augmented tPA binding requires carboxyl-terminal lysine residues of nicked fibrin. Plasminogen also displays augmented binding to partially degraded fibrin. Plasminogen can compete with tPA for the carboxyl-terminal lysine residues in digested fibrin.

Specific structural aspects of clots have received attention in terms of lytic susceptibility and penetration of lytic agents *(3, 9, 12, 13)*. For example, coronary artery thrombi formed during unstable angina are grayish-white, while those present during acute MI are reddish in appearance as determined by angioscopy *(14)*. These grayish-white coronary thrombi tend to be resistant to thrombolytic therapy; they are particularly rich in platelets, are older, have a very tight fibrin structure *(15)* and are believed to be formed under flow conditions. The reddish thrombi of acute MI are rich in fibrin and RBC, tend to be younger, and form under conditions of stasis.

It is possible that tight fibrin networks do not allow sufficient penetration of lytic agents. Platelet retraction is known to alter the lytic susceptibility of fibrin fibers via a GP IIb-IIIa-dependent mechanism *(16)*. Also, ultra-fine fibrin clots formed from plasma of patients with Dusart's Syndrome, a disease characterized by the existence of a mutant form of fibrinogen, are resistant to fibrinolysis due to architectures that are reported to impair access of lytic agents.

Arterial thrombi within the coronary artery are about 1 cm in length and the intracoronary pressure will drive permeation across these structures. Clinical evidence indicates an important role for permeation and its impact on the rate at which reperfusion occurs. The role of permeation is apparent in the observation that patients with hypotension or cardiogenic shock are poor candidates for successful thrombolytic treatment of myocardial infarction *(17, 18)*. The impact of pressure-driven permeation of lytic agents into clot structures was demonstrated in a canine model of left anterior descending (LAD) coronary thrombosis under conditions of severe hypotension with systolic pressure of 75 mmHg *(18)*. LAD catheter administration of recombinant tPA (re-TPA) to the clot was significantly less effective under conditions of hypotension than at systolic pressures of 130 mmHg *(18)*, although the delivery of the agent to the proximal face of the clot was the same (by intracoronary catheter); the driving pressures for permeation, however, differed markedly. Similar transport mechanisms have been shown for the lysis of pulmonary emboli.

In human cardiogenic shock and MI, the success of thrombolytic therapy was enhanced with administration of inotropic agents with

consequent increase of systolic pressure from 64 to 102 mmHg *(17)*. In a canine model of cardiogenic shock with LAD thrombosis where systolic pressure had been reduced to 75 mmHg by phlebotomy, intraaortic balloon counter-pulsation to increase coronary pressure also resulted in enhanced thrombolysis using intravenous re-tPA. Studies using ultrasound also demonstrate the importance of transport processes. Francis et al. *(19)* have shown that 1-MHz ultrasound at intensities up to 8 W/cm^2 significantly enhanced the rate of lysis of a plasma clot submerged in solutions of re-tPA (1 μg/ml) as well as uPA. Such levels of ultrasound do not cause fragmentation or heating of the thrombus; instead, micromixing and transport facilitation induced by ultrasound was the cause of enhanced lysis. Through a cavitation-dependent mechanism, ultrasound was shown to facilitate transport of reactants without impairment of enzymatic activity. When ultrasound is applied to fibrin clots that are maintained under a hydrostatic pressure head, permeability also increases through a cavitation-dependent mechanism that alters fibrin fiber structure. Enhancement in permeability by ultrasound can lead to increased permeation of lytic agents into the whole blood clot with consequent reduction of reperfusion times *(20)*.

Biomedical Engineering Analysis of Thrombolytic Therapy

At the upstream face of the clot, thrombolytic therapy is a process characterized by the dispersive and convective penetration of proteolytic enzymes that bind the erodible fibrin fibers that compose a blood clot *(9, 21, 22)*. Over the cardiac cycle, the time-averaged permeation velocity depends on the time-averaged pressure proximal and distal to the clot. The upstream pressure is essentially the time-averaged aortic pressure of about 90 mmHg while the downstream pressure depends on the extent of collateralization of the myocardium. In the absence of collateralization, the time-averaged downstream pressure may be only slightly higher than the mean right atrial pressure of about 5-10 mmHg. With collateralization, which is less common, higher mean pressures of 40 to 50 mmHg may occur distal to the clot. For a 1 cm-long clot, the pressure drop across an occlusive arterial thrombus may range from about 70-80 mmHg/cm to as low as 40 mmHg/cm with collateralization. Flow pulsatility is expected to enhance dispersion but not net permeation.

To describe the biochemical changes in the circulation, the plasma of the circulation can be treated as a single, well-mixed compartment that can receive a user-specified infusion regimen for any biochemical species. Each species may be eliminated at a given rate by hepatic or tissue clearance. The concentration of each species can then be determined by solving a set of coupled ordinary differential equations describing the reaction network shown in Figure 1, in which fibrin is replaced by a nonspecific soluble tPA cofactor such as fibrinogen *(21)*. Reaction rate expressions may be of any form, typically Michaelis-Menten or second-order association kinetics. For example, the circulatory half lives of tPA and uPA (two-chain) are 5 and 60 min, respectively. The calculated dynamic levels of species within the circulation can then serve as inlet conditions for simulation of local clot lysis dynamics.

At the clot, fibrinolysis consists of a multicomponent reaction network with fluid phase reactions as well as heterogeneous reactions catalyzed by species bound in the solid fibrin fiber phase (Figure 1) *(21, 22)*. For transport of species in eroding fibrin, the instantaneous concentration of a soluble species at some position in the fluid phase of the fibrin gel is described by Eq. 2. The rate of change of the local volume-averaged concentration of the ith species in the fluid phase (c_i) depends on the net transfer rate of that species to the fibrin fibers ($\partial N_i^T/\partial t$), dispersion (D), permeation (v), homogeneous reaction in the free phase between the l and k species (R_{lk}), and the effects due to change of volume of the solid phase ($\partial\varepsilon/\partial t$) as species are released during solubilization of the solid phase and free phase species are diluted to a small extent in the lost fibrin fiber volume. The rate of change of a fibrin-bound concentration of the ith species (s_i) depends on the heterogeneous rate of reaction between the l and k species in the solid phase ($^sR_{lk}$) and the rates of adsorption and desorption where the jth species may compete for the rth binding site (θ_r) as described in Eqs. (3) and (4). ($\xi_{rj} = 1$: interaction; $\xi_{rj} = 0$: no interaction)

$$\varepsilon\frac{\partial c_i}{\partial t} = (s_i - c_i)\frac{\partial \varepsilon}{\partial t} - (1-\varepsilon)\frac{\partial N_i^T}{\partial t} + \nabla\bullet(\mathbf{D}\bullet\nabla(\varepsilon c_i)) \qquad (2)$$

rate of change of c_i | gain due to lysis | net transfer to fibrin | diffusion/dispersion

$$-\nabla\bullet(\varepsilon c_i \mathbf{v}) + \varepsilon\sum_l\sum_k R_{lk}^i$$

permeation | homogeneous reaction

$$\frac{\partial N_i^T}{\partial t} = k_{r,i}^r c_i(\theta_r - \sum_j \xi_{rj}s_j) - k_{r,i}^r(s_i) \qquad (3)$$

net transfer to fibrin | adsorption | desorption

$$\frac{\partial s_i}{\partial t} = \frac{\partial N_i^T}{\partial t} + \sum_l\sum_k {}^sR_{lk}^i \qquad (4)$$

rate of change of s_i | rate of transfer | heterogeneous reaction

The superficial permeation velocity $\bar{v}$ through the porous fibrin matrix of the blood clot is given by Darcy's law,

$$\bar{v} = -\mathbf{K}\nabla P \qquad (5)$$

where $\mathbf{K} = k(x,y,t)/\mu$ for plasma of viscosity μ permeating through fibrin with a specific permeability k. For two-dimensional calculations of $\bar{v}_x$ and $\bar{v}_y$ at every position and time, the Poisson equation of the form

$$\nabla\bullet\bar{v} = \nabla\bullet(\mathbf{K}(x,y,t)\nabla P) = 0 \qquad (6)$$

is solved with the complication that the hydraulic permeability ($\mathbf{K}$) is varying in space and time due to lysis. The specific permeability depends directly on the local fiber diameter $\mathbf{D}_{\text{fiber}}(x,y,t)$ and fibrin gel porosity $\varepsilon(x,y,t)$.

Coupled Transport and Reaction Processes in Dissolving Clots

We have measured the effects of diffusive and convective

transport on fibrinolysis *(10)*. Using a constant pressure drop ($\Delta P/L$) from 0 to 3.7 mmHg/cm clot to drive fluid permeation, various regimens of lytic agents were delivered into fine and coarse fibrin gels (3 mg/ml) and whole blood clots. Using plasmin (1 μM) delivered into pure fibrin or urokinase (1 μM) delivered into glu-plasminogen (2.2 μM)-laden fibrin, the velocity at which a lysis front moved across fibrin was greatly enhanced by increasing $\Delta P/L$. Lysis of fine and coarse fibrin clots by 1 μM plasmin at $\Delta P/L$ of 3.67 and 1.835 mmHg/cm clot, respectively, led to a twelve- and sixteen-fold enhancement of the lysis front velocity compared to lysis without pressure-driven permeation. For uPA-mediated lysis of coarse fibrin at $\Delta P/L = 3.67$ mmHg/cm clot, the velocity of the lysis front was twenty-five times greater than the lysis front velocity measured in the absence of permeation. A similar permeation-enhanced phenomenon was seen for the lysis of whole blood clots. Without permeation, the placement of a lytic agent adjacent to a clot boundary led to a reaction front that moved at a velocity dependent on the concentration of plasmin or uPA used. Overall, transport phenomena within the clot can play a major role in determining the time needed for reperfusion during fibrinolysis. The large ten- to twentyfold enhancements in lysis rates when very small pressure drops (< 5 mmHg/cm-clot) were applied to fibrin or whole blood clots indicated that pressure-driven permeation alleviated severe diffusion limitations.

Numerical simulations provided quantitative predictions of experimental data obtained with purified fibrin gels or blood clots dissolved under conditions of diffusive or convective delivery of fibrinolytic mediators. At physiological or clinical enzyme concentrations of lys_{77}-plasmin, uPA, or tPA (less than 1 μM), the experimentally measured lysis front position also propagated in a manner nearly proportional to $t^{1/2}$ as expected for diffusional processes and was accurately simulated by Eqs. 2-4 *(22)*. We have also found that placement of lytic solutions adjacent to plasma gels caused sharp lysis fronts to move across the gels. Sharp fronts proceeded under conditions of diffusion for several hours when human glu_1-plasmin or lys_{77}-plasmin were placed adjacent to coarse fibrin or when uPA or tPA were placed adjacent to coarse fibrin copolymerized with glu-plasminogen. This was consistent with computer simulations of fibrinolysis in which very sharp lysis fronts were predicted. At high supraphysiological levels of plasmin, uPA or tPA (> 1 μM), fronts moved unusually fast in a manner linearly proportional to time due to a rheological mechanism involving fiber retraction under rapid lysis conditions *(22)*. This type of front movement has been encountered in other dissolution systems *(23)*.

The "Plasminogen Steal" Paradox

The effects of transport and biochemistry are sometimes difficult to deconvolute. The observation that thrombolysis is dramatically slower when high concentrations of uPA or tPA are used *(24)* is only partially understood. This paradoxical observation was first described as "plasminogen steal" and was postulated to be due to depletion of extrinsic plasminogen and an associated "leaching" of clot-bound plasminogen out of the clot and into the plasminogen-depleted plasma.

Given the relatively long time required by diffusional processes to transport fibrin-binding proteins over distances of millimeters, we hypothesized that the dramatically decreased lytic rate observed at high tPA concentration was a direct biochemical effect and did not require a "steal" of plasminogen from the clot. That the lysis rate of thrombi can display a "bell-shaped dose response curve" *(25)* at increasingly high tPA concentrations is suggestive of competition between species for limited number of binding or cleavage sites. The diffusion-mediated elution (steal) of inner-clot plasminogen by a reduction of plasminogen levels in the extrinsic phase is a very slow process. We have conducted numerous simulations indicating that diffusional processes such as plasminogen leakage would take many hours for clots on the order of a few millimeters in diameter. We have found that a maximum in lysis rates is observed for tPA-mediated lysis, but that this observed maximum does not require the presence of α_2-antiplasmin and is independent of intrinsic plasminogen leakage *(26)*. Using fibrin gel lysis assays which have an extrinsic phase and assays using well-mixed fibrin fiber suspensions which have no extrinsic phase, we have found that tPA can directly reduce the activity of plasmin on fibrin. These findings suggest a mechanism that establishes an optimal dosage during thrombolytic therapy via tPA administration. Continually increasing the level of tPA at the site of the thrombus will eventually lead to reduced efficacy.

Because plasma can permeate through interstitial regions of blood clots and fibrin, driven by arterial pressures, the continual binding and rebinding of tPA that is released during lysis can create unusually high concentrations of tPA at a lysis front. In clinical situations, depletion of circulating plasminogen will result in the permeation of plasminogen-poor plasma into the front of the clot with a subsequent efflux out the back of the clot of fluid containing constituents of the coagulation milieu. Also, clot retraction may exude plasminogen-rich fluid from the clot. Under these conditions of low prevailing inner-clot plasminogen concentrations and low plasmin generation, the interference of plasmin action on fibrin by accumulating tPA may be pronounced. The protein engineering of novel plasminogen activators should seek to optimize the full reaction network under realistic transport conditions without interference of plasmin.

Simulation of Intravenous Thrombolytic Therapy in Humans

Using kinetic data for the reaction network (Figure 1) and *ex vivo* arterial thrombi properties and initial plasma composition, we have simulated an accelerated therapeutic regimen for intravenous tPA therapy for coronary thrombolysis *(21)* involving a 15-mg bolus followed by infusion of 85 mg over 90 min. In the simulation, this regimen provided a relatively constant circulating level of tPA of about 0.04 μmol/L during the first hour of therapy. Circulating plasminogen dropped to less than half of its original level with concomitant consumption of antiplasmin and macroglobulin and appearance of inhibited plasmin complexes in the circulation after one hour of therapy.

In the simulation of a 1-cm coronary thrombus, the lysis front advanced at a nearly constant velocity of about 1 mm every 10 min for the first 40 min of the therapy (Figure 2A), after which lysis proceeded more rapidly, with the remaining 6 mm of the thrombus dissolving in the next 10 min. This acceleration toward reperfusion between 40 and 50 min after initiation of the therapy was due to

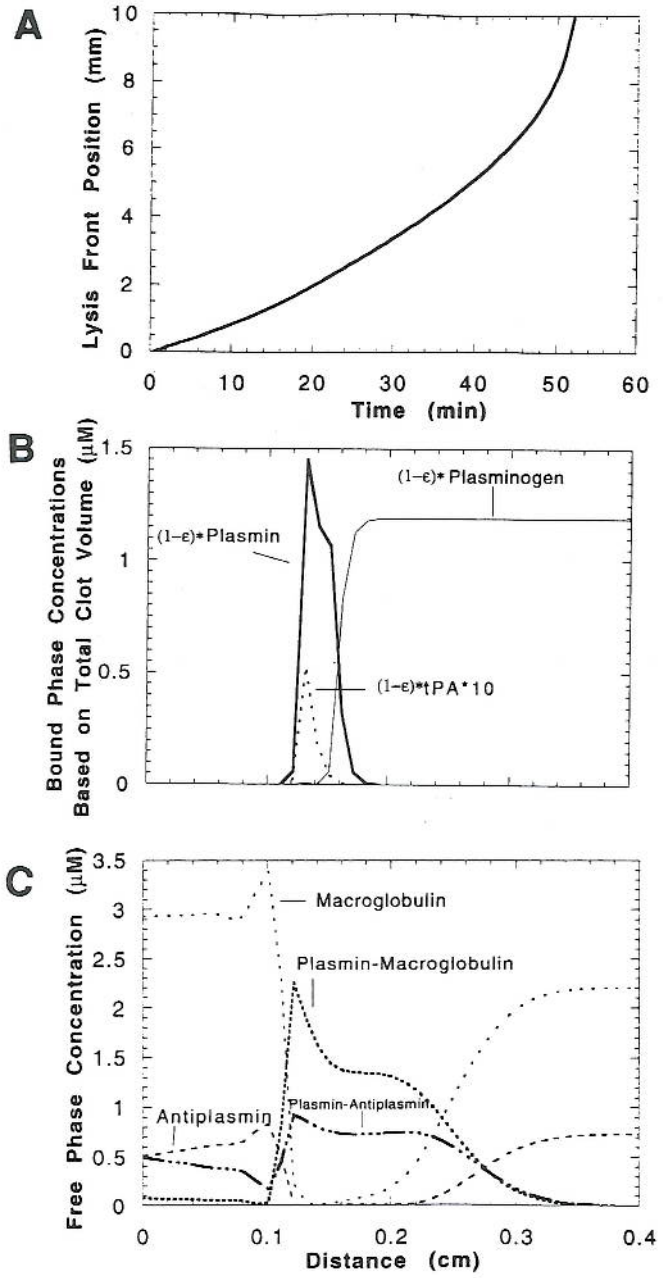

Figure 2. Simulation of lysis of an arterial thrombus by intravenous tPA thrombolytic therapy for an intravenous therapy initiated by a 15 mg tPA bolus followed by infusion of 85 mg tPA over 90 min. Initial clot length is 1 cm and initial pressure drop was set at 50 mmHg/cm. Complete reperfusion is achieved at 52 min (A). The concentration profiles of free and bound species (B,C) across the clot are shown after 10 min of therapy.

the shortening of the clot at constant proximal pressure head (increasing pressure drop and permeation) and accumulation of reaction species at the lysis front. The prediction of clot lysis (with no adjustable parameters) is consistent with observed reperfusion times between 45 and 90 min. Snapshots of the initial 1-cm clot domain after 10 min of therapy are shown in Figures 2B and 2C. At 10 min, the lysis front had advanced over 1.2 mm into the clot and the most active lysis zone was seen to be a region of about 0.25 mm thickness. In this thin reaction zone, dramatic changes in reactant concentrations occurred. Some fibrin-bound plasminogen was released by solubilization and this plasminogen readsorbed and accumulated at the lysis front to levels in excess of prevailing plasma levels. After permeation for 10 min in the simulation, the continual binding of tPA at the lysis front resulted in tPA levels that were two- to threefold higher than circulating levels. Interestingly, antiplasmin and macroglobulin were predicted to have become depleted in the "spent" fluid that was moving with the lysis front, allowing plasmin to exist as a long-lived species. Inhibited complexes do not bind fibrin and penetrated about 1 mm further ahead of the lysis front after 10 min of lysis. The lysis front was moving at a velocity slower than the permeation velocity, but much faster than would be expected in the absence of pressure-driven permeation. The overall thrombolytic rate (i.e., lysis front velocity) achieved as a result of the dosing scheme for this clot structure and composition can be improved only marginally up to the point where the lysis front proceeds at a velocity comparable to the permeation velocity.

In a one-dimensional simulation, mixing eddies proximal to the clot cannot be simulated because the reacting fluid can only move in the forward direction. Rather, changes in the systemic concentrations of species are immediately manifest only at the inlet of the initial reaction domain, not at the lysis front. The one-dimensional model corresponds to anatomical situations where the thrombi are a substantial distance from a well-mixed region of the circulation. If red blood cells accumulate on the proximal face of the clot due to thrombin leakage, blood stagnation, and/or plasma infiltration into the thrombus, this loose structure would offer little resistance to permeation, but would damp fluid mixing proximal to the clot. The hemodynamics of this situation are captured in a one-dimensional model. In this approach, the biochemistry of the plasma that reaches the proximal face of the clot in the first minutes of the therapy has the most impact on subsequent dynamics. This is consistent with the motivation for front-loaded therapies, in which a large bolus of drug is given to initiate treatment *(27)*. Accelerated delivery of tPA can offer clinical benefits in some cases.

Lysis Front Instability and Clot Cannulation

During thrombolytic therapy, reperfusion is typically graded as flow through the clot increases. Clot cannulation often occurs, resulting in a residual, annular thrombus that is highly thrombogenic and prone to reocclusion. This annulus may be slow to lyse because there is little radial pressure drop across the annular clot toward the vessel wall. Radially-directed delivery of plasminogen activator into the thrombi is predominantly by diffusion, although a high level of lytic agent is maintained at the inner surface of the clot annulus due to blood flow. A variety of mechanisms may cause the advancing lysis front to be unstable and undergo a transition from a stable planar front to an unstable front with dissolution fingering (clot cannulation). The mechanisms that cause lysis front instability and clot cannulation may include initial positional variations in clot permeability or in biochemistry, permeation causing drag-induced deformation of the clot with consequent nonuniform pressure gradients, or reaction conditions that lead to the growth of microscopic front instabilities *(28)*.

Our simulations of clot-dissolving therapy based on the convection-diffusion of plasmin into two-dimensional domains of

fibrin indicate that fibrinolysis proceeds by the progression of a sharply defined lysis front with steep spatial gradients of species concentration and material properties. We have conducted numerical studies to understand potential mechanisms by which clot cannulation occurs. The orientation and density of fibers in the fibrin and consequently the clot porosity can vary considerably within a real clot. In order to simulate this effect, a porous bed was defined by randomly generating the fibrin density at each cell in the domain subject to a random fluctuation about a mean value. This local fiber density was then used to calculate the initial porosity and permeability at each location in the domain. The spatial variation of the permeability was dependent on the random generation of the density and hence on the seed used to generate the random numbers for each cell. The fine structure of the velocity field obtained was dependent directly on the nature of the permeability field and hence a given seed resulted in a specific velocity field.

We increased the magnitude of the maximum variation of the initial fibrin density without changing the seed of the random number generator. Increasing the variability of the initial fibrin density resulted in increasing the size of lytic fingers. In Figure 3, we show the lysis front at various times for maximum fibrin density variations between 4% and 25%. In Figure 3, the mean variation in fibrin density ranged from 2 to 13% and the mean variation in permeability ranged from 7.2 to 49.65%. The depth of penetration of the dissolution fingers increased dramatically with increase in the variability of the permeability field. Small spatial variations in the initial density of the fibrin can thus dictate the pattern of lysis and resulting lytic breakthrough with consequent early but low-grade reperfusion. Permeation transport mechanisms that enhance the rate of lysis (compared to diffusional transport) can also increase the risk for clot cannulation with consequent low-grade reperfusion and enhanced reocclusion probabilities. Considerable work remains to design therapies that result in rapid but uniform lysis of clots with low rates of reocclusion.

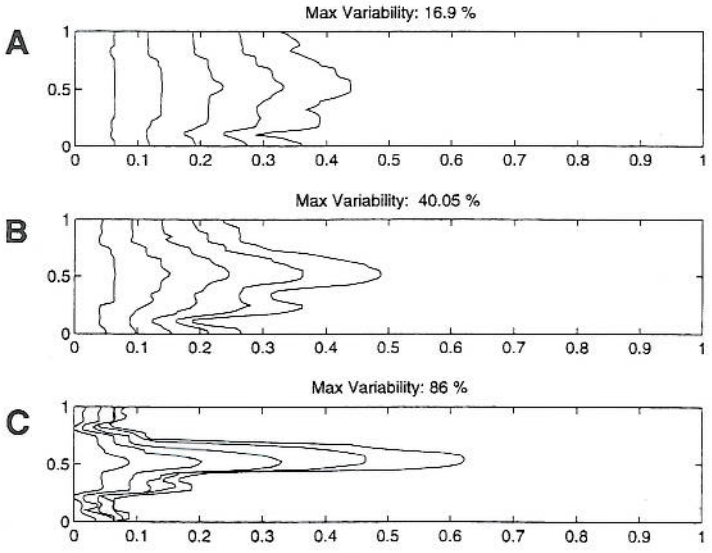

Figure 3. Lysis front progression at 10-min intervals ranging from 20 to 60 min for fibrin beds of increasing variability in local fibrin density and permeability. Plasmin (1 μM) is permeating left to right. The initial permeability field for all simulations had random variations in permeability around a baseline value of 3.16×10^{-11} cm^2. For fibrin with maximum variation in permeability of 16.9% (mean variation, 7.2%) associated with a 4% maximum variation in fibrin density (2% mean variation), lysis front positions are given (A). For fibrin with maximum variation in permeability of 40.05% (mean variation, 21.25%) associated with a 15% maximum variation of fibrin density (7% mean variation), lysis fronts are shown (B). For fibrin with maximum variation in permeability of 86% (mean variation, 49.65%) associated with a 25% maximum variation of fibrin density (13% mean variation), lysis fronts are given (C). $\Delta P/L$ = 50 mmHg/cm clot.

Summary and Future Challenges

Biomedical engineering approaches provide a framework to understand the complex biochemical pathways important to blood clot-dissolving therapy. The utilization of *in vitro* testing methods that control the transport of agents into the clot can improve the analysis and comparison of clot-dissolving studies, particularly when several agents are used simultaneously. Improved *in vitro* tests coupled with computer simulation of lytic reactions may help resolve existing discrepancies between various animal models and clinical trials. Because fibrinolysis is a biphasic reaction process that occurs under nonequilibrium conditions, the determination of binding dynamics in dissolving fibrin is critical to the quantification of the lytic process. The quantitative evaluation of fibrinolytic reactions in clot structures provides rational design criteria for future recombinant proteins as well as guidelines for administration regimens for novel lytic agents. Transport limits are particularly difficult to overcome through protein engineering and represent an important rate limit.

Lytic therapy is an established treatment in the U.S. for acute MI as well as for peripheral arterial and venous thrombosis. However, the therapy is yet to be fully accepted or optimized. Large-scale clinical trials must be conducted to evaluate adequately the safety and efficacy of thrombolytic agents and administration regimens. Several new plasminogen activators (bat-tPA, TNK-tPA, staphylkinase, and numerous deletion mutants and chimerics) have been identified within the last five years. Computer simulation of the thrombolytic process can link *in mechanistic terms* clinical outcomes to pharmacokinetics to simulate intravenous, intracoronary, or intrathrombus delivery of a combination of lytic agents to a given clot/structure composition for thrombolysis. Such simulations give valuable, otherwise unattainable insight into the efficacy of the process itself. Information thus obtained augments efficacy information gained from clinical trials, thereby lessening the chance of post-trial problems such as those experienced with other drugs whose mechanisms were incompletely understood prior to marketing.

During thrombolytic therapy, clot cannulation can occur due to lysis front instability and non-uniformity of dissolution. This appears to be a very common occurrence as indicated by angiography of reperfused vessels. More studies are needed to help understand the therapeutic conditions that lead to the development of clot cannulation as opposed to uniform lysis. Prevailing hemodynamics, clot structure, and the administration regimens of the lytic agent all contribute to clot cannulation. Laboratory experimentation and numerical simulation may help rank the various physicochemical and pharmacological factors that lead to cannulation.

Biomedical engineering methodologies may provide significant tools to define when and why clot cannulation occurs, since these structures are potentially very thrombogenic and may promote low rates of sustained patency over the 24-hr period following therapy. Nonuniform lysis may also be associated with increased potential for embolism and rethrombosis. Moreover, a number of unresolved issues still exist regarding arterial thrombolytic therapy. For instance, it is not clear why reperfusion rates decrease so dramatically if initiation of therapy is delayed until 4 to 6 hr after onset of MI symptoms. Changes in biochemistry, clot structure, and transport phenomena may play a role. Additionally, it is not clear why some clot structures and some patient subsets are poor candidates for thrombolytic therapy.

With the advent of intracoronary and intrathrombus delivery of plasminogen activators, issues involving transport and reaction in clots become very significant. Thrombolytic treatment of peripheral femoral arterial thrombosis (in diseased vessels or grafts) by intrathrombus delivery via multiport or porous catheters is often accompanied by emboli that accumulate in the feet. While these emboli can be tolerated during femoral artery thrombolysis, the risk of embolism is quite dangerous during thrombolytic treatment of stroke victims. In the treatment of stroke, the improvement of catheters, choice of agent(s) and dosing regimen to maximize rapid but uniform thrombolysis represents a challenging, coupled device and pharmaceutics problem. Biomedical engineering principles can help guide the development of these new therapeutic approaches.

Acknowledgments

This work was supported by National American Heart Association Grant-in-Aid 96-667 and National Institutes of Health R01 HL56621. The author would like to thank Dr. Jung-He Wu and Dr. Sriram Anand for their important contributions to this work.

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