Circulatory Concentrations of Fibrinolytic Species During Thrombolytic Therapy Estimated by Stirred-Tank Reactor Analysis

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Purpose. A model for calculating the circulatory concentrations of fibrinolytic species is proposed and the findings are compared to reported values where available.

Methods. The model uses a CSTR analysis with fourth order Runge-Kutta solution of the differential equations to determine concentration profiles of key fibrinolytic species as a function of time during fibrinolytic therapy. Concentrations of the species are also determined for various dosage regimens of streptokinase and plasminogen.

Results. Data calculated by the model is in agreement with general experimental trends determined in vitro and in vivo.

Conclusions. The proposed model can be used to predict concentration profiles of key fibrinolytic species during administration of streptokinase.

KEY WORDS: streptokinase; fibrinolytic therapy; fibrinolytic agents.

INTRODUCTION

When the quasi-equilibrium between the demand for oxygen by the myocardium and the supply of oxygen by the blood to the muscle is disturbed by substantial decrease in blood flow, ischemic damage to the myocardium ensues, resulting in an infarcted zone. Acute Myocardial Infarction (AMI) is a leading cause of death in many countries. In the United States alone, about 1 million cases of AMI had been reported annually during the eighties with 350,000 resulting in death (1). Even with increased attention to exercise and diet, it remains a major cause of death in this country.

In conjunction with evidence concerning the critical event of AMI, thrombolytic therapy has come into increasing use. During the 70's the underlying cause of diminished oxygen supply to the heart muscle was the subject of debate; the decrease in blood flow was thought to result from either vasoconstriction of the coronary arteries supplying the muscle or from the presence of a thrombus occluding the lumen of the coronary arteries with a mass of blood cells and fibrin polymer. Studies by DeWood (2) provided cogent evidence of the role of the thrombus in AMI. A thrombus which blocks blood flow may be formed at the site, or arrive at the location and lodge there after breaking off from some other location. In either case, the result is a diminished oxygen supply to the myocardium with the possibility that it will be severely and irreparably damaged unless flow is soon restored.

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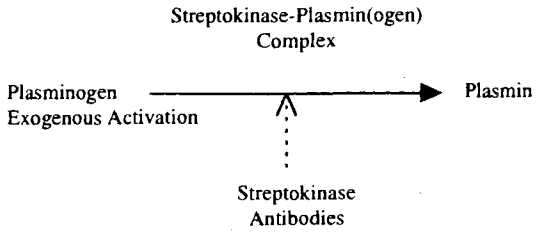

Fig. 1. Diagram showing activation path of the fibrinolytic system.

Thrombolytic therapy has found increasing use in treating coronary heart disease and several large scale trials have been conducted with the so-called "clot-busting" agents (3–6). These medications promote reactions that lyse all or part of the thrombus, thereby recanalizing the artery and permitting the reflow of blood to the myocardium. If this is accomplished soon after the onset of AMI, the chances of permanent damage to the muscle are diminished. However, the underlying coronary stenosis is not decreased by thrombolytic therapy and further treatment is generally necessary such as coronary bypass surgery, or balloon dilation to reduce the risk of rethrombosis (7–8).

All thrombolytic agents which are currently in use lyse the thrombus by activating components of the fibrinolytic system inherently present in mammalian blood (9,10). More explicitly, activation occurs as the thrombolytic system converts the proenzyme plasminogen, which is in the circulation as well as bound within any thrombus, into plasmin, a proteolytic enzyme which acts to lyse the clot but has poor specificity. Figure 1 shows the pathway by which this system is activated (9,11). The thrombolytic agents most associated with such treatments are streptokinase (SK), urokinase (UK), and tissue plasminogen activator (t-PA) or genetically engineered or chemically modified variants (e.g. APSAC).

The present work attempts to analyze quantitatively, by means of a mathematical model, some of the kinetic factors that influence thrombolytic therapy and also seeks to assess their relative importance. To this end, the focus is on the systemic concentration profiles of key fibrinolytic species with time for various infusion rates.

This problem is approached by studying the kinetics of the multiple reactions involved from the standpoint of chemical reactor dynamics. The chemical mechanism of clot lysis by the action of streptokinase on the fibrinolytic system is very complex, though the essential pathways of the mechanism are known, and kinetic data on some aspects have been collected (11–15). Data on diffusion within the dynamic structure of the clot and on the plasmin/fibrin or plasmin/fibrinogen reaction in the clot are sketchy, so only homogenous reactions have been presently considered. The local transport and kinetic events are also important and are under investigation by others (16,17).

METHODS

The circulatory system has been modeled previously as a well mixed vessel for pharmacokinetics (18). To simulate the reactions in the case of intravenous administrations of a fibrinolytic agent, the circulatory system is represented as a well mixed reactor within which multiple reactions are taking place. Reactor analysis using standard techniques is then applied, and the

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resulting equations for a Continuous Stirred Tank Reactor (CSTR) under transient conditions are solved numerically (19). In the system assumed the feed represents the vascular infusion and the exit stream represents normal means of fluid loss (e.g. kidneys).

The usual assumptions for a CSTR are made in addition to the following:

a) the effect of antibodies to streptokinase, present in some individuals, is not considered;

b) the system is considered to be adequately described by the reaction scheme given below, and the rate constants determined in vitro are considered to apply here;

c) streptokinase participates only in the reaction scheme shown; it is not metabolized or significantly removed from the circulation by any organ system over the time period considered, nor is it degraded in any other reaction, and partitioning of streptokinase and other reaction species onto cell surfaces has been neglected;

d) although more than one form or species of plasminogen and streptokinase are known, the model treats plasminogen as a single circulating species, and streptokinase is also treated as existing in one form;

e) the volume of the circulatory fluid is taken as constant because of continuous removal of fluid and because the volume infused is small compared to the volume of circulating fluid.

The reactions are:

$$A + S1 \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} AS1$$

$$AS1 + S1 \underset{k_{-2}}{\overset{k_2}{\leftrightarrow}} AS1.S1 \overset{k_3}{\rightarrow} AS1 + P1$$

$$P1 + I \underset{k_{-4}}{\overset{k_4}{\leftrightarrow}} (P1.I) \overset{k_5}{\rightarrow} P1.I^*$$

$$P1 + S2 \underset{k_{-6}}{\overset{k_6}{\leftrightarrow}} (P1.S2) \overset{k_7}{\rightarrow} P1 + P2$$

$$P1 \overset{k_8}{\rightarrow} D$$

where the species are streptokinase (A), plasminogen (S1), streptokinase/plasminogen complex (AS1), plasmin (P1), α_2-antiplasmin (I), inert plasmin/antiplasmin complex (P1.I*), fibrinogen (S2), fibrinogen degradation products (P2) and degraded plasmin (D). Intermediates are transitory molecules or complexes.

Rate constants employed in the analysis were obtained from the literature (11–15), except the rate constant for the last reaction. In this case an estimate was obtained by using the half-life recorded for plasmin and modeling the reaction as unimolecular first order. The rate constants are as follows: $k_1 = 1\ s^{-1}$, $k_{-1} = 3.0e\text{-}5\ s^{-1}$, $k_2 = .367\ s^{-1}$, $k_{-2} = 1.73*k_2\ s^{-1}$, $k_3 = 6.71\ s^{-1}$, $k_4 = 280.0\ s^{-1}$, $k_{-4} = 5.6e\text{-}3\ s^{-1}$, $k_5 = 4.0\ e\text{-}3\ s^{-1}$, $k_6 = 10.0\ s^{-1}$, $k_{-6} = 300.0\ s^{-1}$, $k_7 = 25.0\ s^{-1}$, $k_8 = 6.93\ s^{-1}$.

The overall rate of formation, r_i of each species i, was then obtained in terms of concentrations of other species. For example,

Plasmin (P1):

$$r_{P1} = k_3[AS1.S1] + k_{-4}[P1.I] + (k_{-6} + k_7) \times [P1.S2] - k_4[P1][I] - k_6[P1][S2] - k_8[P1]$$

Streptokinase/plasminogen complex (AS1):

$$r_{AS1} = k_1[A][S1] - k_{-1}[AS1] + (k_2 + k_3) \times [AS1.S1] - k_2[AS1][S1]$$

Fibrogen (s2):

$$r_{s2} = k_{-6}[P1.S2] - k_6[P1][S2]$$

Similar equations are derived for the other 9 components.

Data on specific rate constants for these systems are limited and difficult to obtain. A concern of this study must be the reliability and compatibility of rate constants drawn from a number of different laboratories and, in some cases, for the fibrinolytic system in different species. In spite of these concerns it is expected that results will provide insight into the dynamics of the fibrinolytic system and findings will be evaluated against experimental measurement.

With the assumptions made above, the basic equation for a CSTR is a species balance for component i:

$$\frac{d[VC_i^o]}{dt} = q\,C_i^f - q\,C_i^o + V\,r_i$$

where C_i^f and C_i^o are concentrations of the feed and the reactor (or the outlet stream) respectively, q represents the flow rate of the feed stream and the outlet stream, while V represents the volume of the system considered. In this case, q is the volumetric infusion rate and V is the circulatory volume, assumed constant. One such equation is written for each species or intermediate component in the reaction scheme, describing its rate of change of concentrations completely. For the model described here, 12 separate chemical entities are seen to exist, so 12 equations of the form above are needed. Simultaneous solution of all the coupled differential equations then provides the calculated concentrations for each species as a function of time. A fourth order Runge-Kutta method has been used in this work to solve the equations (20).

Input rates into the system, modeled as "feed" rates, were needed for some of the chemical species in the reaction scheme since these are being continually produced and released into the circulatory (reactor) system. For example, estimates of the numerical values for the feed rates of plasminogen, α_2-antiplasmin and fibrinogen were made from their half-lives and baseline concentrations (21). Typical streptokinase feed rates were obtained from the physician's reference sheet supplied with the drug (22–23).

In addition to trying to understand the dynamics of the fibrinolytic system with streptokinase administration, another goal of this study was to consider partial optimization of the therapeutic potential of the thrombolytic agent. In order to accomplish this, different regimes of the streptokinase concentration were examined by varying the infusion rate over four orders of magnitude, keeping all other parameters constant. Also, plasminogen rates into the reactor were increased by factors of as much as 10,000 over that ordinarily generated within the bloodstream, to examine the effects of co-administration of this

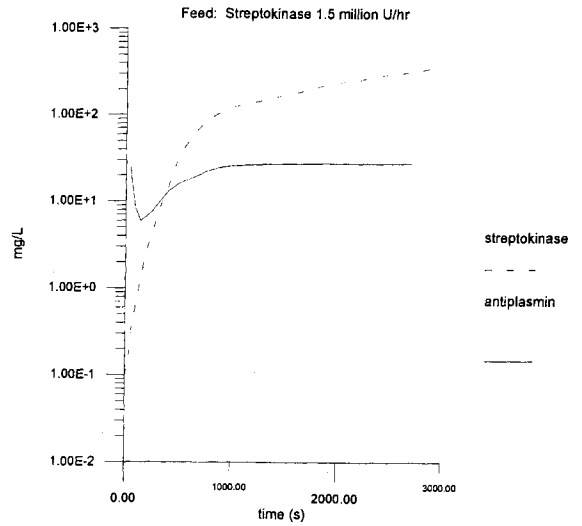

Fig. 2. Concentration profile of streptokinase and α2-antiplasmin.

species. Transient concentration responses were obtained for each of these cases.

RESULTS

Clinically, the time period of administration of streptokinase is about one hour for intravenous infusion; concentrations as a function of time were obtained for the entire course of administration. Figures 2 through 4 show typical responses of the model for several stable species and complexes involved in the reactions.

Upon infusion of streptokinase at 1.5 million IU/h, the following effects are seen in the model of the circulatory fluid volume: streptokinase concentrations rise very rapidly in the initial 1000 s, with a slower rate of increase subsequently. After 1 h, its concentration in the blood stream is lower than that in the feed by a factor of about 30. Plasminogen levels drop very rapidly in the first 200 s, and more slowly after that, with a final concentration about 6 orders of magnitude lower than the initial value. Streptokinase/plasminogen complex is considered to be one of the species responsible for eventual clot dissolution. In circulation it is expected from the model to attain a steady value of 3.9 mg/L very rapidly.

Although the basic enzyme responsible for proteolysis in thrombolytic reactions is plasmin and especially clot-bound plasmin, the concentration of the streptokinase/plasminogen complex in circulation is seen to be about 5 orders of magnitude higher than that of plasmin after 45 minutes, a time period over which clots typically dissolve. Corresponding to the initial sharp rise in plasmin concentration, levels of α_2-antiplasmin drop suddenly but are replenished rapidly in the first 500 s to attain values of about 40% of their baseline levels. The inhibition of plasmin by α_2-antiplasmin is reflected in the levels of the stable plasmin/antiplasmin complex. These grow rapidly in the first 300 s after infusion and then reach a steady value close to .6μM. The effect of a short half-life for plasmin is evidenced in the very rapid rise in the concentration of degraded plasmin within the first 400 s, followed by a leveling off of this species at about 1 μM.

The level of fibrinogen predicted by this model is higher than that observed in clinical practice, and must therefore be regarded with some skepticism. Computer simulation shows the drop in fibrinogen concentration to be about 20% of its initial baseline value, but in clinical practice even a 30 minute infusion with 250,000 IU of streptokinase causes a drop in fibrinogen levels of about 80% to 50% of pre-infusion values (10). This discrepancy can probably be attributed to limitations and approximations required in obtaining the kinetic rate constants from the literature. In this case, the values were obtained from the apparent K_m for the bovine fibrinogen and plasmin interaction. Species differences in observed depletion of fibrinogen have been attributed to differing effectiveness of inhibitors (24). Additionally it was also assumed that the rate constant for formation of the plasmin-fibrinogen intermediate k_6 was limited by the diffusion rate of the two proteins in solution (25).

Figure 5 shows the variations in the systemic concentration for 6 key species when the calculations are repeated for different

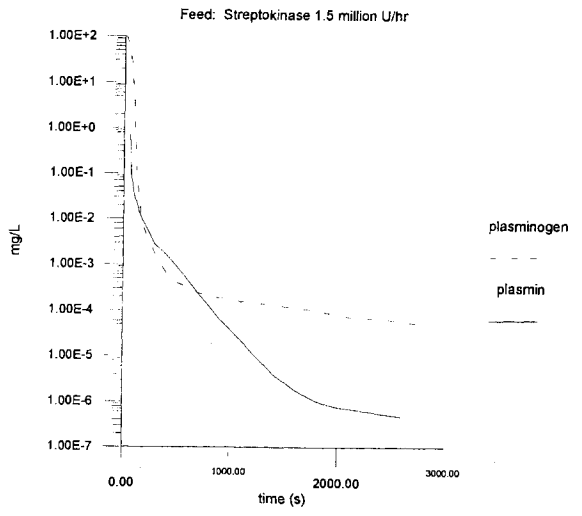

Fig. 3. Concentration profile of plasmin and plasminogen.

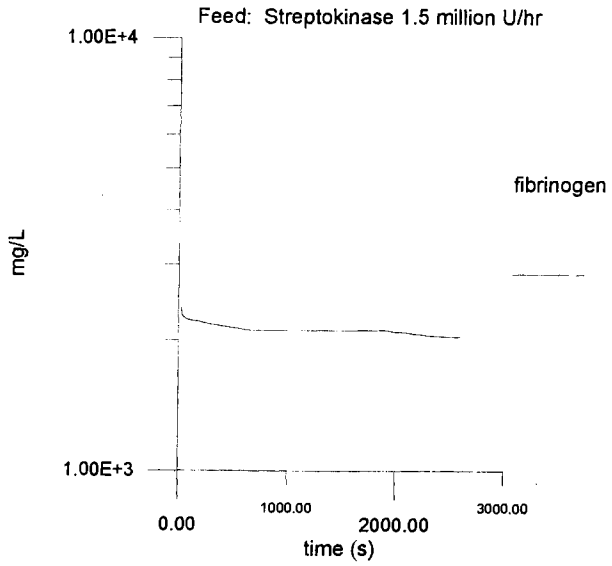

Fig. 4. Concentration profile of fibrinogen.

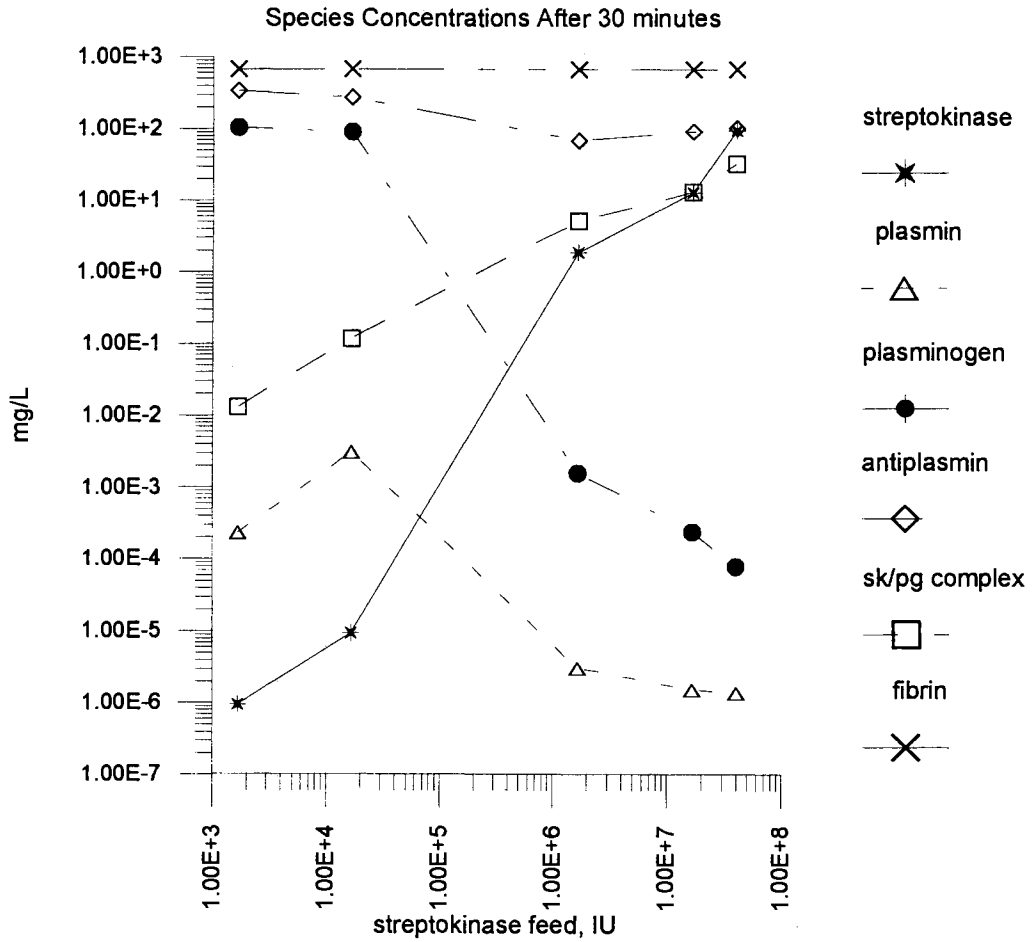

Fig. 5. Variations in systemic species concentrations after 30 minutes for differing dosage regimens of streptokinase.

infusion rates of streptokinase. Results were obtained at 30 minutes from the start of infusion. The 6 species plotted are fibrinogen, plasminogen, α_2-antiplasmin, streptokinase/plasminogen complex, plasmin, and free streptokinase.

It was seen that a decrease in streptokinase feed from 1.5 million IU to 15,000 IU produced a corresponding increase in circulating plasmin level (see Figure 5). For the same decrease in streptokinase infusion rate, circulating streptokinase/plasminogen complex levels dropped by a factor of about 100. It was also noted that, circulating concentrations of some species are more sensitive to changes in the infusion rate. For example, circulating streptokinase levels dropped by about 6 orders of magnitude while circulating plasminogen levels increased by 5 orders of magnitude. These are profound changes in the profile of fibrinolytic species, reflecting chemical amplification. The differences in concentration between plasmin and streptokinase/plasminogen complex at different feed concentrations of streptokinase may be significant in determining the nature of the mechanism for clot dissolution which would prevail for varying infusion rates or from the presence of antibodies (See Discussion).

Figure 6 demonstrates the effect of plasminogen co-administration when various concentrations are fed into the "reactor" with the standard streptokinase infusion rate. This figure includes the resulting concentrations for 6 species at 30 minutes from the start of the infusion. Altogether, infused concentrations of streptokinase were decreased over 4 orders of magnitude, and those of plasminogen were increased over 4 orders of magnitude. Increasing plasminogen feed levels by four orders of magnitude while maintaining the streptokinase infusion level at 1.5 million IU/h gave an interesting result. The computer simulation for fixed time intervals after infusion, namely 30 minutes (values at 60 minutes post-infusion are substantially similar to those at 30 minutes), showed that plasmin concentration levels responded with an increase by a factor of only 10, while circulating plasminogen levels showed an increase by a factor of about 100. Streptokinase levels were virtually unaffected.

In general when comparing the responses of this mathematical model for two different inputs, namely, reduction in streptokinase feed concentrations or an increase in plasminogen feed concentrations, it was seen that fibrinogen and α_2 -antiplasmin were the least affected in both instances. The impact on plasminogen complex was more pronounced when streptokinase feed concentrations were decreased compared to the case when plasminogen feed concentrations were increased.

DISCUSSION

In pharmacokinetic modeling of drug disposition, the circulatory system has often been treated as a one-compartment

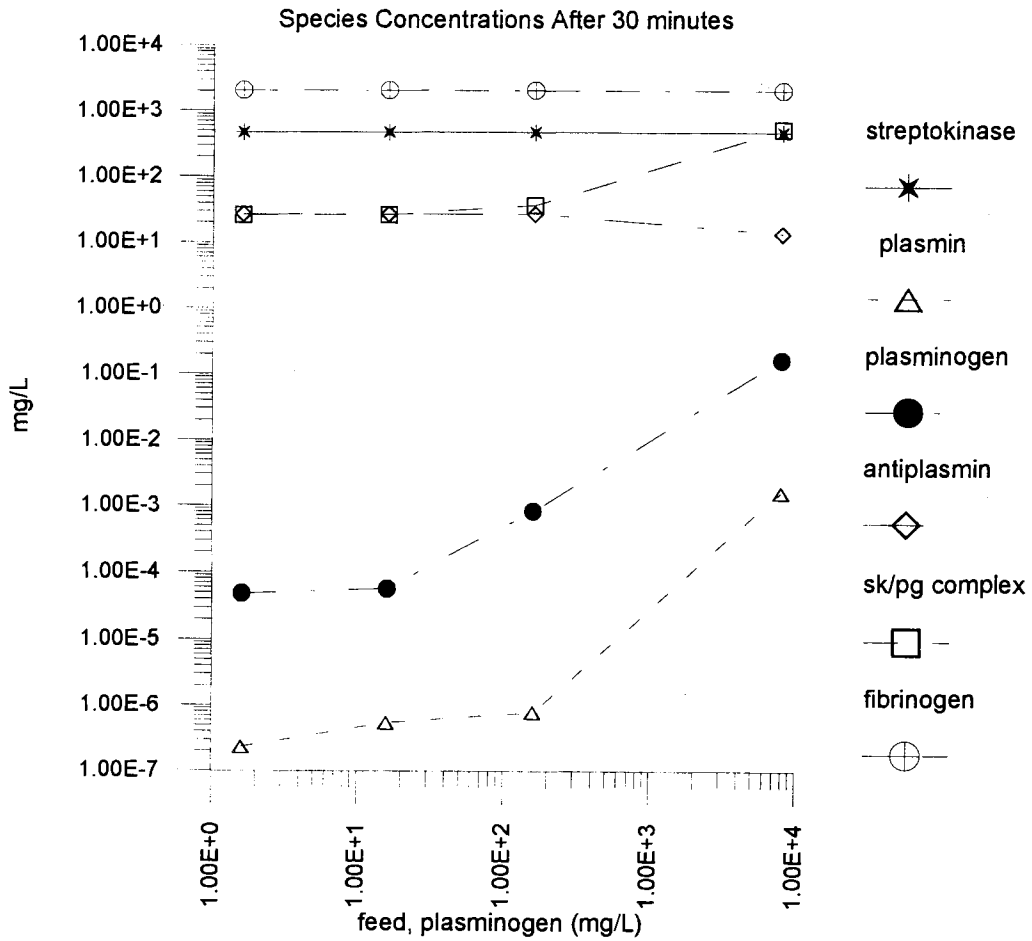

Fig. 6. Systemic species concentrations after 30 minutes for differing levels of plasminogen co-administration with standard dosage of streptokinase.

model in which the physiological system is treated as a single unit (18). Analysis of the homogenous fibrinolytic reaction scheme was carried out and concentration profiles of various species were obtained as a function of time after the onset of infusion, by solving the systems of differential equations as described above. CSTR calculations provide meaningful results with manageable computational effort in spite of the complexity of the reaction system.

Transient results (Figures 2 through 4) were obtained by applying the design equations for the CSTR and the model reaction system described. Key reaction species selected for discussion here are those for which some comparison can be made with clinical or other data and which can provide meaningful interpretation of the results.

A species of particular interest was plasmin because of its proteolytic activity in general, and its possible effect upon the clot. Also important was the streptokinase/plasminogen complex, because of its suggested role as activator brought about by its diffusing into the clot, reacting with bound plasminogen and producing plasmin in situ. Fibrinogen levels were a concern because a decrease in fibrinogen is associated with systemic bleeding problems, even though decreased fibrinogen might also act against rethrombosis.

A standard dosage of streptokinase that has been adopted is 1.5 million international units (I.U.) administered in 100 ml of saline solution intravenously over a period of one hour. Numerical analysis of the transient behavior of the model reaction system shows that for an ideal system which is well mixed, the concentrations of many important species at the end of one hour are very different from baseline concentrations. Under the administration protocol usually adopted (23), the results of computer simulation show that there is a very large difference between concentrations of the streptokinase/plasminogen complex and concentrations of plasmin. The concentration of the complex is almost 7 orders of magnitude larger than that of free plasmin. It is noted that the affinity of plasmin and of plasminogen for fibrin are virtually similar, therefore it is possible that the lower concentration of plasmin in the systemic circulation might not offset the higher streptokinase/plasminogen complex concentration even if the complex had a lower possible affinity for the clot (26). Since the streptokinase/plasminogen complex is present in circulation in a much larger concentration than plasmin, and since the affinity for the clot of each of these species is apparently similar, it is likely that in conventional therapy the streptokinase/plasminogen complex plays a greater role in dissolution of the clot.

Generally, the enzyme that performs proteolysis is plasmin; in light of this fact, the higher concentration of circulating streptokinase/plasminogen complex compared to circulating plasmin is consistent with the view that, in conventional therapy, it may not be the agent directly performing thrombolysis. More likely, thrombolysis is occurring because of plasmin formed at the site of the clot from interaction between plasminogen bound to the clot and the streptokinase/plasminogen activating complex. This complex is present at the clot as a combined effect of diffusing into the clot from the circulation and also as a product of reaction between clot-bound plasminogen and circulating streptokinase. Our computer simulations for different ranges of streptokinase feed concentration suggest that this need not always be the case, because the balance between circulatory concentrations of plasmin and streptokinase/plasminogen complex can be altered. Findings for this model predict that interesting differences occur when a dose four orders of magnitude lower than that which is used in current practice is administered intravenously over an hour. Under these circumstances, levels in circulation of plasmin are seen to increase greatly, while those of the complex show a substantial drop, although not in quite as marked manner (Figure 5). Consequently, their concentrations would be much closer and differ by only a factor of 15. Under these circumstances, the circulating plasmin level will be much greater, and the fibrinolytic potential may increase. These trends in concentrations have been confirmed clinically by Onundarson (27). The transition region 10^5–10^6 IU also corresponds roughly to different effectiveness in dosages observed by Six *et al.* (28).

Results for varying infusion rates of streptokinase as well as for concomitant infusion of plasminogen indicate that the amounts of plasmin and of streptokinase/plasminogen complex can be changed to a profound degree. As it appears possible to alter both concentrations of these descendant species by orders of magnitude, consideration of other dosage regimens should be evaluated. Though it is generally recognized that clot-bound plasminogen is especially effective in thrombolysis, the optimal therapeutic approach may be a combination of activation of bound plasminogen and lysis from free plasmin. The relative importance of this avenue compared to the case where plasmin is formed in situ in the clot by action between streptokinase/plasminogen complex and bound plasminogen is an important feature that merits further study. However, evaluation of these possibilities indicates the need for the development of heterogeneous models. Ideally, future models will take into account many factors which have been excluded from this mathematical treatment because of their complexity. Additional insight into the dynamics of the CSTR representation could be gained from parameter sensitivity analysis.

A computer model has its limitations because of the inability to predict complications that may arise in a clinical setting. To the extent that they can be compared, experimental studies do support the computer model predictions. For example, the concentration profile of streptokinase follows the in vivo results of Gemmill *et al.* (29) with a monotonic increase of more than 2.5 orders of magnitude over 60 minutes. The concentration profile of plasminogen and plasmin both show good agreement in the trends determined in vivo by Onundarson *et al.* (27). In this study, plasminogen drops rapidly to 45% at 10 minutes, and down to 25% at 20 minutes. Moreover, the profile obtained for antiplasmin exhibits the same decrease and recovery found by Onundarson although the results do not indicate the same degree of recovery. High experimental variance from rapidly changing concentrations reflects the difficulty in characterizing the system dynamics by venipuncture and chemical assay and attests to the potential value of a computational model.

The chief benefit of the computer model for reaction dynamics developed here is exploring dosage regimens that can be gained from mathematical simulation of the thrombolytic system. It is versatile enough to accommodate computer simulations with various feed concentrations of species and, given appropriate kinetic data and a viable reaction pathway, the dynamics for any plasminogen activator can be studied. The findings also suggest some potentially important means of therapy in the future where a regulation of the dose of the thrombolytic agent administered causes different regimes in the expression of the fibrinolytic system with differing levels of efficacy on the rate of clot lysis.

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