Journal of Theoretical Biology 253 (2008) 725–738

Contents lists available at ScienceDirect

Journal of Theoretical Biology

journal homepage: www.elsevier.com/locate/yjtbi

A model for the formation, growth, and lysis of clots in quiescent plasma. A comparison between the effects of antithrombin III deficiency and protein C deficiency

M. Anand [a,1], K. Rajagopal [b], K.R. Rajagopal [a,*]

[a] Department of Mechanical Engineering, Texas A&M University, College Station, TX 77843, USA
[b] Department of Surgery, Duke University Medical Center, Durham, NC 27710, USA

ARTICLE INFO

Article history:
Received 19 November 2007
Received in revised form
18 March 2008
Accepted 9 April 2008
Available online 25 April 2008

Keywords:
Hemostasis
Mathematical model
Hypercoagulable state
Antithrombin III
Protein C

ABSTRACT

A mathematical model comprised of 23 reaction–diffusion equations is used to simulate the biochemical changes and transport of various reactants involved in coagulation and fibrinolysis in quiescent plasma. The growth and lysis of a thrombus, as portrayed by the model equations, is governed by boundary conditions that include the surface concentration of TF–VIIa, the generation of XIa by contact activation (in vitro), and the secretion of tPA due to endothelial activation. We apply the model to two clinically relevant hypercoagulable states, caused by deficiency of either antithrombin III or protein C. These predictions are compared with published experimental data which validate the utility of the developed model under the special case of static conditions. The incorporation of varying hemodynamic conditions in to the current fluid static model remains to be performed.

1. Introduction

The accurate and precise determination of clinically relevant risk factors in patients presenting with thrombotic and hemorrhagic disorders is of great importance. Collectively, thrombotic/thromboembolic diseases due to acquired and/or inherited etiologies are substantial contributors to patient morbidity and mortality. Evaluation of patients usually involves diagnostic laboratory testing, which also serves the purpose of guiding treatment. Unfortunately, most routine laboratory testing of hemostatic system function is complicated by at least two problems: (1) testing is conducted under static conditions with respect to flow, whereas thrombus formation generally occurs in vivo in the setting of local blood flow; and (2) individual tests only assess specific aspects of hemostatic system function. For these reasons, the results of laboratory tests of hemostatic system function fail to consistently correlate with clinical thrombotic or hemorrhagic states.

A mathematical model of hemostatic system function would provide a theoretical underpinning for the validity (or lack thereof) of specific laboratory tests. More importantly, such a model may have the capacity to make predictions about the effects of specific perturbations in the hemostatic system that laboratory tests cannot currently assess, and may spur the development of more clinically useful assays (see (Hemker and Ataullakhanov, 2005) for an overview on the role of mathematical modeling in understanding the hemostatic mechanism). Although many mathematical models have been posited for various sets of reactions in the enzymatic coagulation cascade, none has been developed that incorporates the entire sequence of events from thrombus formation through lysis (i.e. both coagulation *and* fibrinolysis). The model presented in this paper addresses this deficit, and is a step forward in building models that can be used to make clear clinical correlations. The proposed model, which deals with the biochemistry surrounding clot formation and lysis, is meant to build upon an earlier model that we developed (Anand et al., 2003, 2005). The proposed model's effectiveness is assessed by comparing predictions for the special cases of two hypercoagulable states, antithrombin-III (ATIII) deficiency and protein C (PC) deficiency, with clinical data for the same.

We formulate the problem in the next section and survey the literature on models for clot formation and lysis under static conditions with respect to blood flow. We then highlight the salient features of our model and the criteria governing the formation, growth, and lysis of the clot in Section 3: the details pertaining to the reaction–diffusion equations in the model and the parameters for these equations are given in Appendices A and B. In Section 4, the model predictions are compared with

* Corresponding author. Tel.: +1 979 862 4552; fax: +1 979 845 3081.
E-mail addresses: anandm.phd@gmail.com (M. Anand), rajag004@notes.duke.edu (K. Rajagopal), krajagopal@tamu.edu (K.R. Rajagopal).
[1] Present address: CGGVeritas, 10300 Town Park Drive, Houston, TX 77072, USA.

0022-5193/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jtbi.2008.04.015

experimental data: first, for the coagulation reactions in synthetic plasma with normal, and deficient concentrations of ATIII and PC, and second, for the lysis of a formed clot. In Section 5, the model predictions for the formation, growth, and lysis of a clot are documented, and the model is also used to predict the consequences of ATIII deficiency and PC deficiency on parameters that indicate the rapidity and extent of clot formation, growth, and lysis. The results are discussed in Section 6, and this includes a subsection on the limitations and extensions to the model.

2. Problem formulation

The fluid nature of blood is held in a state of delicate balance by multiple interacting factors and processes that promote or inhibit thrombus generation and lysis, and underlie thrombus generation, maintenance, and lysis. Thrombus generation and maintenance occurs due to an imbalance in favor of prothrombotic factors, and clot maintenance is determined by stimuli such as vessel wall injury, endothelial dysfunction, high endothelial and platelet surface shear stresses, and flow recirculation and stasis. Clot lysis follows the removal of this imbalance. Clot generation, maintenance, and lysis in flowing human blood is a complex process, and involves various biochemical, physiological, and rheological influences (Colman et al., 2001). In this paper, we posit a model, consisting in a set of reaction–diffusion equations and a set of criteria, to simulate thrombus formation, growth, and thrombolysis in a quiescent pool of plasma exposed to a thrombogenic surface. The biochemical schematic for this special case, as collated from the various experimental and numerical studies that we have examined, is elaborated upon below.

We consider the case when the intima of a blood vessel is subjected to a surface injury exposing the subendothelial layer, rich in membrane-bound tissue factor (TF), to a quiescent pool of plasma. Two interacting processes—platelet activation followed by adhesion and aggregation, and TF-initiated coagulation—are thereby initiated, culminating in clot formation. Platelets adhere to collagen and various glycoproteins (importantly, von Willebrand Factor) found in the subendothelium, and undergo a set of morphologic and biochemical changes (platelet activation) in conjunction with the coagulation reactions. The activated platelets bind each other, and also fibrin, thereby forming aggregates.

TF-initiated blood coagulation occurs in three stages—initiation, propagation, and termination—that follow the progress of reactions in the extravascular space exposed by the surface injury, those in the region enclosed by and including the growing clot, and those that occur in the intact intravascular compartment surrounding the clot that actively inhibits the procoagulant reactants (Orfeo et al., 2005; Butenas and Mann, 2002; Mann et al., 2006). This picture of TF-initiated coagulation is based on data from three models: in-vitro experiments using whole blood drawn from healthy donors, and those with coagulation abnormalities (Brummel et al., 2002; Butenas and Mann, 2002); experiments using highly purified natural and recombinant coagulation proteins (the "synthetic plasma" model) (Lawson et al., 1994; van't Veer and Mann, 1997; van't Veer et al., 1997; Butenas et al., 1997); and numerical simulations (Jones and Mann, 1994; Hockin et al., 2002).

In the initiation stage, TF in the subendothelial layer binds to VIIa in plasma forming the TF–VIIa complex that activates IX and X to IXa and Xa, respectively. Xa activates prothrombin (II) to thrombin (IIa); IIa thus produced is found in picomolar amounts and is produced very slowly. IIa partially activates platelets, and activates V and VIII to Va and VIIIa, respectively. These reactions occur in the extravascular space exposed by injury. The duration of the initiation stage is governed in large part by the concentrations of TF–VIIa and tissue-factor pathway inhibitor (TFPI): TFPI inhibits TF–VIIa in a Xa-dependent manner (and this dependence prevents the premature neutralization of the thrombogenic stimulus), and also Xa itself. TFPI is present in plasma, as well as bound to the endothelial cells on the intact intravascular surface.

In the propagation stage, platelet-bound intrinsic factor tenase (VIIIa–IXa) activates X 50–100 times faster than TF–VIIa (Butenas and Mann, 2002), while prothrombinase (platelet-bound Xa–Va) converts prothrombin to thrombin at a very high rate in the extravascular compartment; thrombin appears in nanomolar concentrations in the propagation stage whereas it is generated in picomolar concentrations during the initiation stage. Fibrin (Ia) strands appear and bind to activated platelets: clot formation begins at the inception of the propagation stage. Platelet activation and fibrin production proceed rapidly, alongside the rapid production of thrombin, and fill the extravascular space above the injury.

The rapid formation of the clot results in a barrier (composed of activated platelets laden with procoagulant complexes and enmeshed in a fibrin network) that makes it increasingly difficult for plasma, and therefore a fresh supply of procoagulant zymogens, to reach the extravascular compartment. In the termination stage of coagulation, the increasingly impermeable barrier sequesters zymogens and active enzymes within, leading to the cessation of thrombin generation as all reactants are consumed. Any thrombin that escapes is actively inhibited on the intravascular surface of the clot. Data suggest that the tenase and prothrombinase complexes are well insulated from the inhibitory processes found in the intravascular compartment (Orfeo et al., 2005): even if the barrier is disrupted before healing is complete, thrombin generation would resume until platelet activation and fibrin generation seal the leak.

Procoagulant enzymes escaping from the clot are inhibited in the adjacent intravascular compartment principally by two mechanisms: the antithrombin III—(endothelial cell produced) heparan sulfate complex (HS–ATIII), and activated protein C (APC), which is formed by a conformational change when PC complexes with thrombin bound to endothelial thrombomodulin. The HS–ATIII complex inactivates IIa, Xa, and IXa, preventing thrombus extension to sites distant from the locus of vascular injury. APC inactivates Va and VIIIa, thereby suppressing the formation of prothombinase and tenase, and consequently the production of thrombin and fibrin. Unlike the HS–ATIII mechanism, the APC mechanism, becomes effective only after a substantial amount of the clot is formed and IIa reaches the intravascular surface to complex with endothelial thrombomodulin. Intravascular thrombin generation should thus be suppressed, whereas the already substantial thrombogenesis should lead to sequestration of the thrombin activating potential in the extravascular compartment distant from APC.

Fibrinolysis, the process that follows the formation and (attenuation of) growth of the clot, proceeds in tandem with the healing of the surface injury beneath the thrombus. Fibrinolysis also occurs in three stages—initiation, propagation, and termination—that follow the progress of reactions in the intravascular compartment, clot surface, and extravascular compartment (Kolev et al., 2005; Sakharov and Rijken, 1995; Medved and Nieuwenhuizen, 2003; Kolev and Machovich, 2003; Booth, 1999).

In the initiation stage of fibrinolysis, IIa and, to a lesser extent, Ia induce the endothelial cells on the intravascular surface to release tissue-plasminogen activator (tPA). tPA binds to plasminogen (PLS) in plasma and Ia (on the clot surface) to form a ternary complex wherein plasminogen is activated to plasmin (PLA). PLA degrades bound Ia into smaller products initiating fibrinolysis.