Mathematical Models of Blood Coagulation and Platelet Adhesion: Clinical Applications

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Abstract: At present, computer-assisted molecular modeling and virtual screening have become effective and widely-used tools for drug design. However, a prerequisite for design and synthesis of a therapeutic agent is determination of a correct target in the metabolic system, which should be either inhibited or stimulated. Solution of this extremely complicated problem can also be assisted by computational methods. This review discusses the use of mathematical models of blood coagulation and platelet-mediated primary hemostasis and thrombosis as cost-effective and time-saving tools in research, clinical practice, and development of new therapeutic agents and biomaterials. We focus on four aspects of their application: 1) efficient diagnostics, i.e. theoretical interpretation of diagnostic data, including sensitivity of various clotting assays to the changes in the coagulation system; 2) elucidation of mechanisms of coagulation disorders (e.g. hemophilias and thrombophilias); 3) exploration of mechanisms of action of therapeutic agents (e.g. recombinant activated factor VII) and planning rational therapeutic strategy; 4) development of biomaterials with non-thrombogenic properties in the design of artificial organs and implantable devices. Accumulation of experimental knowledge about the blood coagulation system and about platelets, combined with impressive increase of computational power, promises rapid development of this field.

Key Words: Blood coagulation, platelets, mathematical modeling, computer simulation, drug design, biomaterials.

INTRODUCTION

Platelet-mediated primary hemostasis and blood coagulation are two defense mechanisms which prevent bleeding upon vessel wall damage by forming a plug at the site of injury. A disbalance of these delicately regulated systems can result in either hemorrhage or uncontrolled clotting and thrombosis. Moreover, even normal functioning of these systems can result in thrombosis associated with the use of artificial organs or other implantable devices [1,2]. Complexity of hemostatic and thrombotic processes, which involve interactions of numerous plasma proteins with each other and with receptors on blood and vascular cells, makes their control a challenging problem.

The mathematical and computer modeling is an approach to address the complexity of hemostatic and thrombotic processes. In this review, we discuss clinical applications of these methods focusing on four aspects: 1) efficient *diagnostics*, i.e. theoretical interpretation of diagnostic data; 2) understanding the *bases of diseases*, i.e. their mechanisms; 3) planning rational *therapeutic strategy* and selecting targets for *drug design*; 4) development of biomaterials with *non-thrombogenic* properties for the design and clinical use of artificial organs and implantable devices. Molecular modeling in the design of coagulation drugs has been recently reviewed [3-6]. We avoid discussing mathematical details and modeling approaches as our purpose is to evaluate usefulness of the existing models for practical applications and to summarize the most promising findings in this field.

PLATELET-DEPENDENT HEMOSTASIS AND BLOOD COAGULATION

The immediate physiological response to vessel wall damage is formation of the primary platelet plug (for review, see [7,8]), which forms within first minute. Upon adhesion to collagen, platelets become activated that results in a variety of responses, including shape change, adhesion, aggregation, secretion of numerous pro- and anticoagulant substances, shedding of microparticles, and change of the composition of the platelet outer membrane [9]. Platelet aggregation leads to the formation of the plug preventing blood loss. An important positive feedback is secretion of two additional platelet activators - adenosine diphosphate (ADP) and thromboxane A2 - by activated platelets. Platelets also secrete coagulation factors and inhibitors [7,10]. Another link between platelets and coagulation system is mediated by thrombin, the main enzyme of the coagulation cascade and one of the most potent platelet activators.

Coagulation system is a complicated cascade of enzymatic reactions, which acts in concert with platelet-mediated hemostasis [11]. Coagulation cascade is activated by contact of plasma with transmembrane glycoprotein tissue factor

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(TF) present mainly on sub-endothelial cells which become exposed to blood upon vessel wall damage. TF is a cofactor for serine protease FVIIa, present in plasma, and formation of the FVIIa-TF complex (extrinsic tenase) induces activation of FIX and FX by FVIIa and initiates the clotting process. In addition to the TF-dependent pathway, coagulation can be activated by foreign surfaces *via* the contact pathway [12]. In this pathway, FXII binds to a foreign surface and becomes activated. Reciprocal activation of two other proteins, kallikrein and high molecular weight kininogen (HK), results in the explosive formation of FXIIa. FXIIa activates FXI, which in turn activates FIX, and at this stage the contact pathway meets the TF-dependent pathway.

The backbone of coagulation cascade consists of factors XIa, IXa, Xa, and IIa (thrombin) consecutively activating each other. Coagulation cascade includes multiple positive and negative feedbacks, and all activated coagulation factors are regulated by plasma inhibitors, antithrombin III (AT-III) and tissue factor pathway inhibitor (TFPI) being the most important inhibitors. The principal reactions, which support the coagulation process, are activation of FX and prothrombin by intrinsic tenase (FIXa-FVIIIa complex) and prothrombinase (FXa-FVa complex), respectively. They proceed on the membranes of activated platelets, where coagulation factors form calcium-dependent enzymatic complexes. The coagulation process ultimately results in thrombin-catalyzed conversion of fibrinogen into fibrin, which is polymerized and forms a solid gel-like clot at the site of damage strengthening the platelet plug and stopping blood loss.

MATHEMATICAL MODELING IN COAGULATION DIAGNOSTICS

Mathematical modeling is used to address the complexity of hemostatic process in the diagnostics of a disease, which is the first step in clinical practice. The first clinical coagulation test that was computationally modeled was thrombin generation assay [13]. In this assay, the coagulation process in plasma or blood is initiated *via* either the contact or TF-dependent pathway, and thrombin activity is determined as a function of time (for review, see [14]). Fig. (**1**) shows a characteristic bell-shaped curve of thrombin generation, where the lag time and the amount of generated thrombin are considered as indicators of the coagulation system state. In the study by Willems *et al.* [13], thrombin generation in plasma upon activation by TF was simulated using a set of ordinary differential equations which were solved numerically. The model included activation of factors X, V, II, and protein C (PC). Generation of FXa, which initiates the coagulation cascade, was mimicked as a given input *F(t)*:

$$F(t) = Q_{Xa} \cdot \alpha \cdot e^{-\alpha \cdot t}, \quad (1)$$

where Q_{Xa} is total FXa production, and α is the rate of extrinsic tenase activity decay.

The results of model simulations proved to be in good agreement with the experimental data obtained by the same group, Fig. (**1**). This study demonstrated that FV is predominantly activated by thrombin but not by FXa and revealed existence of the threshold FXa production, which, if exceeded, results in explosive activation of prothrombin (for review on activation thresholds in blood coagulation, see [15]). This threshold value (Q_{Xa}=1-10 pM) can be raised by two orders of magnitude by accelerated activation of FX but is not significantly affected by accelerated thrombin inactivation or activation of the PC-dependent negative feedback.

Later, this group developed a mathematical model for estimation of coagulation system parameters from thrombin generation experiments [16]. This model described thrombin generation, which was initiated by FX activator isolated from Russell's viper venom, and contained nine differential equations and sixteen unknown parameters, including initial concentrations of factors X, V, II and reaction constants. The

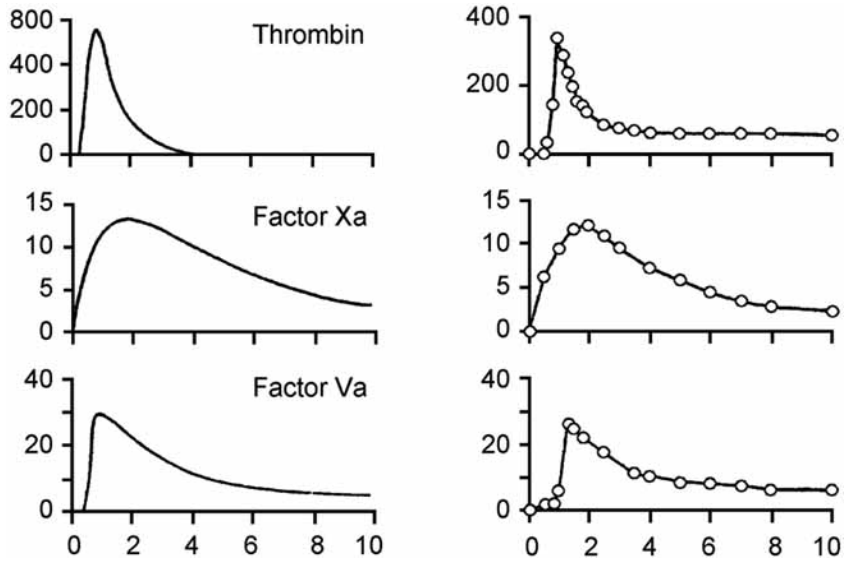

Fig. (1). A computer simulation model of thrombin generation assay. Activity of thrombin, FXa, and FVa is shown as a function of time in platelet-free plasma activated with thromboplastin at 1/30 vol/vol ratio: simulation (left column) and experiments (right column). Reproduced from Willems *et al.* [13].

model satisfactorily described the experimental data; five parameters (or parameter combinations) could be estimated with acceptable accuracy from the singular value decomposition of the covariance matrix of the parameters.

In 1994, a model was developed to describe TF-activated thrombin generation in a reconstituted system containing plasma coagulation proteins without inhibitors and synthetic phospholipids [17]. This model included eighteen differential equations and twenty reaction rate constants, and was thoroughly verified by experiments. In contrast to the previous models, initiation of coagulation was simulated in more detail, starting with the formation of the FVIIa-TF complex. This model has been recently upgraded [18] to include the effect of TFPI and AT-III. The revised version consisted of 34 equations and included 42 reaction constants. Two studies, based on this model, investigated possible roles of circulating small amounts of active coagulation proteases [19] and of circulating TF [20].

A model of thrombin generation activated *via* the contact pathway was suggested by our group [21-23]. By analogy with the earlier study [13], the initiation reactions were not simulated, and the coagulation cascade was modeled beginning with FXIa. Known experimental kinetics of FXIa generation was set as a given input. Recently, our group developed a computer model simulating thrombin generation activated *via* the extrinsic, TF-dependent pathway. Using this model, we revealed that two parameters of the thrombin generation curve provide information about the coagulation system [24,25]. "Lag time" or "time required to reach maximal thrombin concentration" is a parameter to characterize the initiation phase of clotting that occurs on the TF-bearing surface, whereas "maximal thrombin concentration" or "total thrombin potential" (the area under the thrombin generation curve) is a parameter to characterize the propagation phase as it correlated with the rate of spatial clot expansion from TF-bearing surface. Bungay *et al.* proposed a model of TF-activated thrombin generation in a reconstituted system; this model includes detailed description of interaction between coagulation factors and phospholipids [26]. Previous studies assumed that the phospholipid concentration was saturating or constant; and apparent Michaelis parameters were used for the membrane-dependent reactions. In this work, the binding of all factors to phospholipids was included as a reversible reaction:

$$S_F + LBS_S \underset{k_{off,S}}{\overset{k_{on,S}}{\rightleftarrows}} S_L, \qquad (2)$$

where S_F is the free form of factor S, S_L is the lipid-bound form of S, LBS_S are lipid binding sites for S. This work demonstrated existence of a threshold value of phospholipid concentration and also showed that the inhibitory effect of TFPI in the model was maximal at intermediate vesicle concentrations. Two other studies, which explicitly analyzed the roles of activated protein C and platelet activation in the dynamics of thrombin generation, have been recently reported [27,28].

Prothrombin time (PT) test is one of the most widely used clotting assays for assessment of the blood coagulation system. The test is sensitive to changes in the extrinsic pathway of coagulation, such as deficiencies of factors VII, X, or II. In this assay, coagulation is initiated in recalcified plasma by large concentrations of thromboplastin (TF with phospholipids), and the clot formation time is determined [29]. A model for this test has been developed by Pohl *et al.* in 1994 [30] to study the effects of various coagulation disorders on this assay. The authors used χ^2 fit of the experimental data to estimate the values of unknown parameters. The determined values were within the experimentally-determined range thus indicating that the model may be useful in the determination of the coagulation system parameters. Later, Khanin *et al.* proposed another model of PT test [31] to study the time course of activation of coagulation factors.

Activated partial thromboplastin time (APTT) test is another clotting assay often used in combination with the PT and is designed to evaluate the activity of contact activation and intrinsic pathway proteins (factors XII, XI, IX, VIII). In this assay, coagulation is initiated in plasma *via* the contact pathway when a plasma sample is preincubated with negatively charged materials to activate factors XII and XI, and subsequent addition of calcium ions triggers a chain of calcium-dependent enzymatic reactions resulting in fibrinogen clotting [32]. Two models of APTT assay were proposed independently in 2001 [33,34]. Kogan *et al.* [33] investigated the kinetics of coagulation factors' activation and the sensitivity of the assay. Specifically, the mechanism of FXII activation on foreign surfaces in the APTT test, which remained unclear, was studied. It was demonstrated that the predominant mechanism is activation of FXII by trace amounts of FXIIa. Alternative mechanism, i.e. activation due to a conformational change after adsorption of FXII onto a negatively charged surface, plays a minor role. Kramoroff and Nigretto [34] studied FXI activation in the APTT assay. This zymogen can be activated by FXIIa, by positive feedback auto-activation, and by thrombin in a long-range positive feedback loop. By comparing model predictions with experimental data, the authors concluded that auto-activation of FXI plays a major role.

Summarizing, mathematical modeling of coagulation assays provided useful information about sensitivity of assay parameters to various changes in the coagulation system, about their correlation with each other, and about underlying mechanisms. These models also served as the basis for computer studies of drugs in clotting assays, which is discussed in the section "*Drug design and therapeutic strategy planning*". One of the promising areas of model applications is determination of individual factor concentrations and reaction rate constants from general assays [16,30] that can be useful for diagnostics of coagulation disorders and drug evaluation. On the other hand, the existing models include a number of critical assumptions and are not always able to give a quantitative description of experimental results, which limits their wide practical use. Other important coagulation tests, such as thromboelastography, still await development of their computer models.

ANALYSIS OF DISEASE BASIS

Finding a connection between the mechanism and the manifestation of a disease is the next issue following disease identification. This section describes application of mathematical and computer models in the analysis of platelet thrombus formation and mechanisms of coagulation disorders. First mathematical models of platelet thrombus formation were proposed in 1970-ies by several groups [35-38]; for review see [39]. Although these models included a number of simplifying assumptions (such as neglecting time-dependence of the process, saturation of the vessel wall surface by platelet adhesion, constant platelet diffusion, etc.), they enabled analysis of the effects of blood flow, platelet Brownian movement, red blood cells-mediated transport of platelets, and interaction of platelets with the surface, on the adhesion process. More advanced computer-assisted models dismissed these assumptions and proved to be useful for the analysis and predictions in various diseases. One of the most popular applications of platelet thrombus models is stenosis, a partial vessel occlusion by atherosclerotic plaque. Buchanan and Kleinstreuer [40] developed a model of platelet adhesion under flow in a stenotic tube, an axisymmetric artery segment with a partial constriction. According to the model, platelets were concentrated in the recirculation zones and were released upstream, where they formed microemboli, which drifted downstream resulting in secondary stenosis.

A simple model of platelet deposition under two-dimensional flow in axisymmetric stenosis was suggested by Wootton *et al.* [41] based on the species transport model [37]. This model described blood as Newtonian fluid containing a diluted suspension of platelets transported by convection or shear-enhanced diffusion:

$$u\frac{\partial c}{\partial x}+v\frac{\partial c}{\partial y}=\frac{\partial}{\partial y}\left(D_e\frac{\partial c}{\partial x}\right)+\frac{1}{r}\frac{\partial}{\partial y}\left(D_e r\frac{\partial c}{\partial r}\right), \qquad (3)$$

where c is platelet concentration, u and v are axial and radial flow velocities, respectively, and D_e is effective diffusion coefficient calculated as:

$$D_e=\alpha\dot{\gamma}_{max}+D_{th}, \qquad (4)$$

where $\dot{\gamma}_{max}$ is shear rate and D_{th} is coefficient of thermal diffusion. The model assumed that platelet activation took place near the thrombus surface and did not consider specific activators, i.e. the activation and attachment processes were described as a single reaction:

$$\text{Free, intact platelets} \xrightarrow{k} \text{Attached, activated platelets,} \qquad (5)$$

This model provided the first adequate mathematical description of experimentally-registered platelet accumulation in different stenotic regions and was proposed for prediction of stenosis on the basis of angiographic clinical data. In particular, a formula was suggested to estimate thrombotic risk by determining the time to vessel occlusion $T_{occlusion}$:

$$T_{occlusion}=\frac{D_{throat}\,c_{thrombus}}{\phi\, j^{*}_{throat}\, c_{\infty}}+t_d, \qquad (6)$$

where D_{throat} is lumen diameter at the stenosis throat, $c_{thrombus}$ is platelet concentration in thrombus, j^{*}_{throat} is the platelet deposition rate at the throat determined from the model, c_{∞} is free platelet concentration, φ is the coefficient to account for thrombus roughness, and t_d is the onset time of the acute platelet accumulation phase (5-10 minutes). Studies of Sorensen *et al.* [42,43] also modeled platelet adhesion based on shear-enhanced diffusion. Their model was advanced to include into consideration secretion of individual platelet activators ADP and thromboxane A2.

A number of studies used a stagnation point assumption to simulate platelet adhesion both analytically and computationally [44-46]. This type of flow is often present in the circulation, in particular, in the recirculation regions. David *et al.* simulated adhesion of pre-activated platelets as a first-order phenomenon [44]:

$$\text{Free, activated platelets} \xrightarrow{k} \text{Attached, activated platelets} \qquad (7)$$

resulting in a boundary condition

$$\rho D_{pl}\frac{\partial \phi_{pl}}{\partial n}=k\phi_{plwall}, \qquad (8)$$

where D_{pl} is platelet thermal diffusion coefficient (no shear enhancement of diffusion), φ_{pl} is platelet mass fraction, n is a coordinate normal to the wall, and k is the rate of platelet interaction with the surface. In contrast, the model [46], based on the Monte-Carlo simulation method, neglected platelet diffusion and considered the platelet adhesion process in the boundary layer as probabilistic. Adhesion of platelets to the vessel wall was assumed to increase the probability of adhesion/activation for other platelets thus imitating the effect of platelet activators release. These models showed good agreement with experimental data [44-46] and provided an insight into the role of platelet margination by red blood cells. David *et al.* [44] found that the rate of platelet interaction with the vessel wall should depend on the shear rate. The model of Affeld *et al.* [46] can be used in the design of artificial organs to predict the regions of thrombus formation.

While the above models considered a constant flow pattern, a model of platelet thrombus formation by Fogelson and Guy [47] was the first to include a feedback effect of the growing thrombus on the fluid dynamics. This model also described platelet-platelet adhesion and cohesion by introducing existence of elastic bonds between activated platelets and forces between platelets and vessel wall. Consecutive frames in Fig. (**2**) illustrate the embolization process simulated with this model: in response to shear stress, a large platelet aggregate is separated from the vessel wall. While remaining attached to the wall, the aggregate changes its shape due to shear stress that can induce further cycles of embolization and thrombus growth.

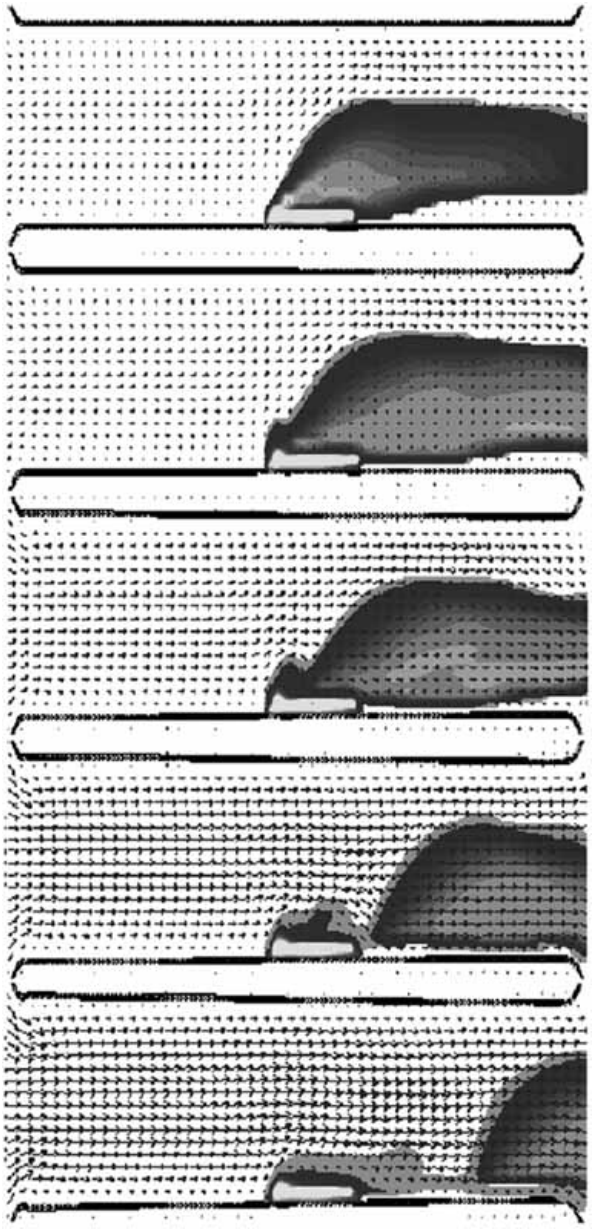

Fig. (2). Embolization of a stenotic vessel. Aggregation intensity (light-gray to dark-gray color reflects the degree of platelet aggregation from low to high) and fluid velocity field **u** (vectors) are shown. Reproduced from Fogelson and Guy [47].

A detailed model to study flow-induced mechanisms of platelet activation in stenosis was proposed by Einav and Bluestein [48]. This model revealed that the cumulative effect of the shear stress level and the duration of the platelet's exposure to it determine whether the platelet is brought beyond its activation threshold. This model allows localization of the regions where activated platelets might be found and aggregate. A discrete element approach was used in [49,50] to study platelet deposition in the regions with non-parallel flow including stagnation, recirculation and reattachment. The feedback effect of platelets and their activation on blood rheology was modeled by Anand and Rajagopal [51].

While platelets represent an important component in thrombus formation process, deficiencies or molecular defects of coagulation factors of the coagulation system lead to disorders associated with uncontrolled bleeding. In hemophilias A and B, factors VIII and IX, respectively, are deficient; a more rare and less severe disorder hemophilia C is associated with FXI deficiency or defect [52]. From a theoretical point of view, it is unclear why insufficient activity of the factors of the intrinsic pathway results in bleeding. Indeed, the intrinsic tenase IXa-VIIIa complex seems redundant as FX can be rapidly activated by the extrinsic tenase VIIa-TF complex. To explore the specific role of the intrinsic tenase, we investigated fibrin clot formation in heterogeneous reaction-diffusion systems using computer simulation models [21,22,53] in combination with an experimental model which enables the real-time monitoring of spatial clot formation in a thin layer of non-stirred recalcified plasma [54]. We revealed that the coagulation process in a reaction-diffusion system proceeds *via* three spatially- and temporally-separated stages: initiation, propagation and termination of clot expansion. In particular, the positive feedback loop of the intrinsic pathway was shown to regulate the second, propagation stage of coagulation [22,54, 55] without any effect on the initiation stage. Thus, hemophilias A and B are not caused by defective onset of clotting but appear to be the diseases of *defective spatial propagation* of the fibrin clot. This specified concept may have important implications for the therapeutic targeting.

Factor Va^{LEIDEN} is FVa mutant resistant to inactivation by activated protein C (APC). This mutation is a very frequent thrombotic disorder afflicting 2-4% of the general population and responsible for ~50% of familial thrombotic cases. A model of the reaction of FVa inhibition by APC is an example of molecular-level kinetic modeling in coagulation [56]. The model includes 29 differential equiations to simulate this single reaction and considers each APC-mediated cleavage in FVa molecule. This model confirmed association of kinetic consequences of elimination of the Arg^{506} cleavage site with thrombotic phenotype of FVa^{LEIDEN} patients.

In summary, the modeling studies provided explanations connecting the changes in the hemostatic system with clinically observed bleeding and thrombotic phenotypes and proposed methods for estimation of thrombotic risk under certain conditions.

DRUG DESIGN AND THERAPEUTIC STRATEGY PLANNING

In this section, we discuss examples of the use of mathematical models in pharmaceutical studies. The mathematical model of TF-initiated thrombin generation in reconstituted systems developed by Jones and Mann [17] was used with modifications by other groups to study the effects of various therapeutic agents and mechanisms of their action. Leipold *et al.* [57] advanced this model to include exogenous serine protease inhibitors with affinity for any or all of coagulation factors VIIa, IXa, Xa, and IIa. Application of the revised model for predicting the affinity profile of the optimal inhibitor and the inhibitory potency of several compounds revealed the following: 1) The predicted thrombin generation times are mostly insensitive to changes in the affinity at the

inhibition constant K_i>1 nM; 2) However, for compounds with high affinity for both FVIIa and FIXa, inhibition of thrombin generation decreases as the affinity for thrombin increases (K_i from 1000 to 5 nM); 3) Inhibitors with affinity for a single serine protease are less potent compared to inhibitors with a spectrum of affinities; 4) Compounds with high affinity for FVIIa, FXa and thrombin are predicted to be highly-potent, regardless of their affinity for FIXa. Predictions of model simulations demonstrated good agreement with the *in vivo* potency of 25 inhibitors evaluated in a rat model of $FeCl_3$-induced carotid artery thrombosis [57].

This model was further expanded to include reactions involving fibrin and AT-III and was applied to investigate the effects of argatroban (thrombin inhibitor) and DX-9065a (specific FXa inhibitor) on TF-initiated thrombin generation in plasma. [58]. The results demonstrated that argatroban was more effective than DX-9065a in reducing endogenous thrombin potential (area under thrombin generation curve), especially when coagulation was activated by high TF concentrations. On the other side, argatroban turned out less potent than DX-9065a in its ability to prolong clot formation time.

Recombinant activated FVII (rVIIa; NovoSeven®, Novo Nordisk, Copenhagen, Denmark) administered at high doses (90-120 mg/kg each 2-3 hours that maintains peak FVIIa concentration of 50 nM in the circulation) has been successfully used for treatment and prophylaxis of bleeding in hemophilia A and B patients who develop inhibitors to FVIII (or FIX) and therefore can not be treated with replacement therapy. Clinical trials demonstrated high efficacy of rVIIa treatment and its safety, as confirmed by a very limited number of documented thrombotic complications [59]. However, there is presently no agreement about the mechanism underlying the therapeutic action of rVIIa [60-62]. As plasma FVIIa is virtually inactive and acquires its ability to activate FIX and FX only in the presence of either TF or phospholipid surface, two hypotheses were proposed to explain the potentiating effect of infused rVIIa on thrombin generation. The first, TF-independent, mechanism was suggested in 1997 [63] and is based on the finding that FVIIa can bind to activated platelets and activate FIX and FX. According to this hypothesis, activation of FX by platelet-bound rVIIa at the site of damage is the predominant mechanism of its therapeutic effect in hemophilia which compensates for the lack of FXa production by intrinsic tenase complex. The second hypothesis [64,65] links the hemostatic effect of infused rVIIa to active formation of complex with TF at the site of vessel wall damage. Formation of the rVIIa-TF complex not only dramatically increases the catalytic activity of rVIIa towards FIX and FX but also overcomes the inhibitory action of zymogen FVII in plasma, which competes with FVIIa for TF, and delays the initiation of thrombin generation.

We used a mathematical model of blood coagulation in platelet-rich plasma to distinguish between these two possibilities [66]. Fig. (**3**) shows computer simulation of thrombin generation in normal and hemophilia A plasmas initiated by either 100 or 10 pM TF (panels A and B, respectively). The model included the terms for TF-dependent and TF-independent activation of FIX and FX and allowed simulating the

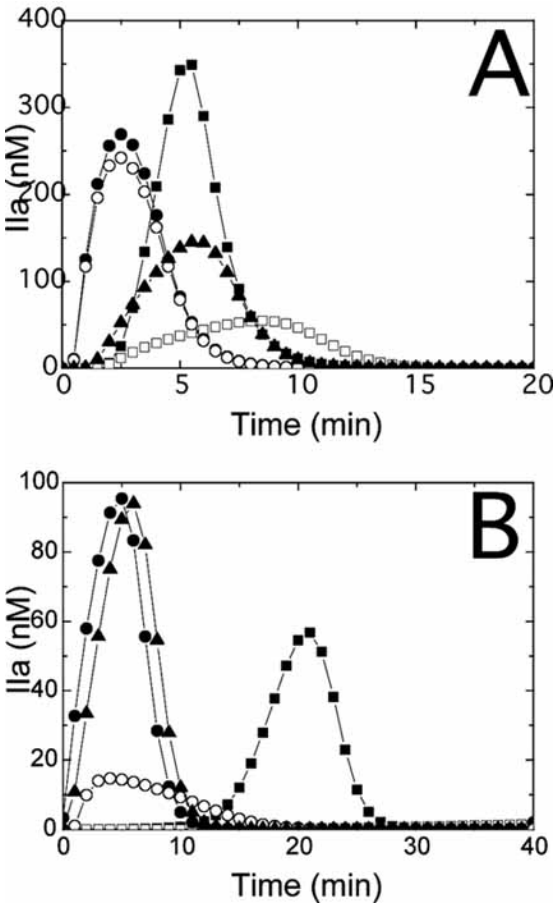

Fig. (3). Potentiation of thrombin generation by recombinant factor VIIa (NovoSeven) in hemophilia: relative contributions of TF-dependent and TF-independent mechanisms. The figure shows computer model simulation of thrombin generation in platelet-rich plasma, where coagulation is initiated by 100 (*panel A*) or 10 (*panel B*) pM of TF. The conditions are: (■), normal plasma; (□), hemophilia A; (●), hemophilia A, 50 nM of rVIIa added and both TF-dependent and TF-independent mechanisms are active; (○), hemophilia A, 50 nM of rVIIa added and only TF-dependent mechanism is active (i.e. rVIIa competes with FVII for TF but does not activate FIX and FX on activated platelets); (▲), hemophilia A, 50 nM of rVIIa added and only TF-independent mechanism is active (i.e. platelet-bound rVIIa activates FIX and FX but does not compete with FVII for TF). TF-dependent mechanism prevails at higher TF concentrations (*panel A*), while TF-independent mechanism is predominant at lower TF concentrations (*panel B*). Reproduced from Panteleev *et al.* [66].

situations when both or only one of two mechanisms is active upon "addition" of rVIIa at therapeutic concentration. The terms for TF-independent reaction had the following form (for FX):

$$\left.\frac{d[Xa]}{dt}\right|_{TF-indep} = k_{eff}^{X,VIIa} \cdot [X] \cdot [VIIa], \qquad (9)$$

where $k_{eff}^{X,VIIa}$ is the effective activation constant estimated from experimental data [63], and [*Xa*], [*X*], and [*VIIa*] are

variable concentrations of factors Xa, X, and VIIa. To simulate the clotting with both mechanisms active, we set: $k_{eff}^{X,VIIa}$=6x10^{-6} min^{-1}nM^{-1} [63], $[VIIa]|_{t=0}$ =50.1 nM. To simulate the clotting when TF-independent mechanism is "switched off", we used: $k_{eff}^{X,VIIa}$=0, $[VIIa]|_{t=0}$ =50.1 nM. To simulate the clotting when TF-dependent mechanism is "switched off", the variable corresponding to FVIIa concentration equation was set at its physiological value (0.1 nM), and added rVIIa was assumed to act only in TF-independent reaction, thus not competing with zymogen. The following substitutions were made:

$$\left.\frac{d[Xa]}{dt}\right|_{TF-indep} = k_{eff}^{X,VIIa} \cdot [X] \cdot 50.1 \quad , \quad k_{eff}^{X,VIIa}=6\cdot 10^{-6} \text{ min}^{-1}\text{nM}^{-1}, \; [VIIa]|_{t=0}=0.1 \text{ nM} \qquad (10)$$

The modeling demonstrated that TF-dependent mechanism functions at higher TF concentrations, while TF-independent mechanism is predominant at lower TF concentrations, Fig. (**3**). As thrombin generation test at low TF (less than 5-10 pM) reflects the *in vivo* situation most adequately [67], our data suggest that TF-independent activation of factors IX and X by rVIIa on activated platelets is a predominant mechanism accounting for the therapeutic effect of rVIIa in hemophilia patients.

TFPI is one of the most important regulators of the coagulation system; it inhibits the FVIIa-TF complex *via* FXa-dependent mechanism [68]. A recombinant form of this protein is now being proposed as a clinical inhibitor, tifacogin [69-71]. Regulation of extrinsic tenase by TFPI was assumed to proceed in a two-step fashion: first, inhibition of FXa and then inhibition of the FVIIa-TF complex. Baugh *et al.* [72] developed a mathematical model to simulate this pathway and demonstrated that the two-step mechanism cannot describe the experimental results on inhibition of FX activation by TFPI as the predicted kinetic constants did not correlate with the directly determined values. It was proposed that the predominant inhibitory pathway may be inhibition of the triple complex FXa-FVIIa-TF by TFPI. A model of the TFPI pathway was later developed by our group [73] and included another assumption of interaction between the FX-FVIIa-TF complex and the FXa-TFPI complex that reconciled the mathematical and experimental data. A recent work by Lu *et al.* studied the regulation of factors IX and X activation by extrinsic tenase in the presence of TFPI and AT-III [74]. Combination of the experiments and model simulations suggested that FIXa is the primary product of the extrinsic tenase in the presence of inhibitors, although factors IXa and X are activated with similar kinetic constants. Thus, mathematical models seem to be especially useful in the analysis of the mechanism of action of TFPI.

The results discussed in this section illustrate applicability of mathematical models in the screening of therapeuticals, in the analysis of mechanisms of their action and in selecting a rational strategy for their clinical use. It is remarkable that models initially developed for basic research [17] have been successfully applied to resolution of practical problems [57,58].

PLATELET ADHESION AND COAGULATION ON BIOMATERIALS

Despite many decades of research and technical efforts, cell adhesion and blood coagulation on biomaterials remain a major obstacle to the successful use of artificial organs and implantable devices. Early theoretical studies of platelet adhesion to foreign materials [35] have identified two important factors: (i) effective diffusion in the flow, which is more important than the Brownian movement, and (ii) the role of red blood cells in transport of platelets to the surface. The process was not adhesion-limited. This model was later modified by Strong *et al.* [39] for a wider range of conditions. The authors concluded that cell adhesion is not diffusion-limited and that effective diffusion is a monotonically increasing function of the shear rate. Subsequent works utilized computer methods for model analysis that extended the range of its applicability.

An essential step was development of a computer model of two-dimensional platelet activation and adhesion to biomaterials by Sorensen *et al.* to predict platelet-mediated thrombosis [42,43]. In addition to platelet deposition, the model considered the roles of thrombin, prothrombin and AT-III, and was able to predict effects of anticoagulants (heparin and PPACK) on the kinetics of platelet thrombus growth on the collagen surface. More detailed models of platelet adhesion, which consider the stagnation point flow, the feedback effect of thrombus on the flow, thrombus shape change under the flow, etc., have been proposed since then [44-47,75] as discussed in the section *"Analysis of disease basis"*, but they were not applied for studies of biomaterials.

First mathematical models of contact pathway-activated coagulation did not simulate contact activation itself but mimicked it by FXIa influx [21-23,76]. A series of studies by Basmajian *et al.* [77-83] systematically analyzed the process of contact activation on a foreign surface in flowing blood and the effects of various factors (flow and mass transport, pulsatile flow, contact pathway, etc.) on thrombus formation. This enabled identification of several principal stages in this process: (i) rapid (<100 s) initial deposition of proteins; (ii) contact pathway reactions (2-3 min); (iii) reactions of the main coagulation cascade. The model predicted that contact activation has no threshold [2,82] and even trace quantities of FXIIa can trigger explosive formation of FXIa and thrombin. From a mathematical point of view, there is single stationary solution in this model, which corresponds to non-zero concentrations of activated factors (activated state). In contrast, the main reactions of the coagulation cascade (from FXIa to thrombin) do have thresholds. This is in agreement with the early analysis of Khanin and Semenov [76] who demonstrated that non-zero concentrations of FXIa did not necessarily result in a burst of thrombin generation. Basmajian *et al.* concluded that initiation of contact activation cannot be prevented; however, it might be possible to block thrombus formation. They hypothesized that development of coagulation-inert biomaterials is not feasible [2], and

that the research should be rather focused on development of biomaterials, which are actively antithrombogenic.

In contrast, another theoretical study [84] suggested that contact activation does have a threshold. Concentrations of activated factors depended on the activation signal value in a hysteretic manner. Fig. (**4**) shows the amount of kallikrein produced (a dimensionless variable *y*) as a function of concentration of FXII binding sites on the activating surface (a dimensionless variable *s*). In the region of relatively small signals, two stable states (stationary solutions) coexist: activated and non-activated. According to the model, coagulation is not triggered until the system reaches the upper threshold; to become non-activated, it has to return to the activation values *below* the lower threshold, i.e. much lower than the initial signal. It was suggested that blood drawing can change the excitation level to exceed the upper threshold, and the system cannot return to the normal level until the excitation drops to levels below those in the circulation. This may be the reason why non-thrombogenic biomaterials activate the clotting *in vitro*, and not because they are more thrombogenic than blood cells.

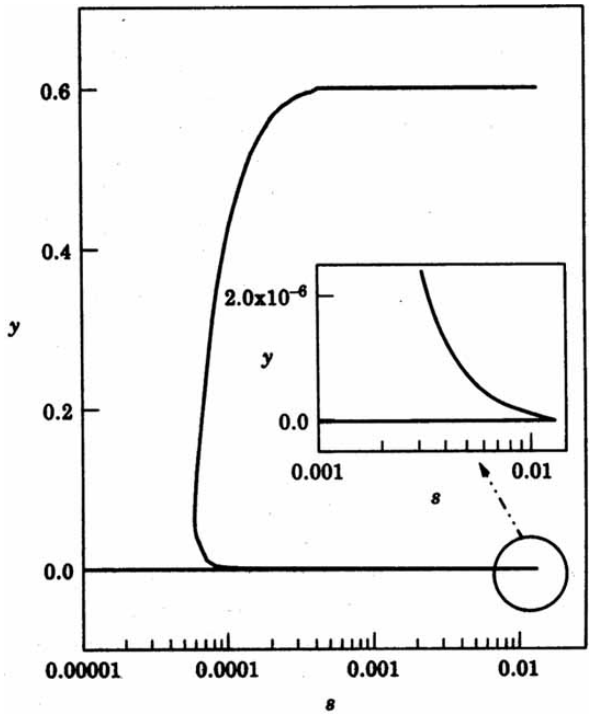

Fig. (4). Hysteresis and trigger properties of the contact activation system. Steady-state concentration of kallikrein (*y*) as a function of the binding sites concentration (*s*) in the mathematical model of Pokhilko and Ataullakhanov [84]. The variables are dimensionless. The inset shows the encircled region on an enlarged scale. Reproduced from Pokhilko and Ataullakhanov [84].

The contradiction between the conclusions of these model studies is most likely due to the difference in model assumptions. The model of Basmajian *et al.* [2,82] assumes that all HK is present in the active form and HKa is not inhibited (as the kinetics of activation was not known, the authors assumed that it was rapid). In contrast, Pokhilko and Ataullakhanov [84] were able to estimate the rates of HK activation by kallikrein and FXIIa from the experimental data. As no information on the inhibition of HKa was available, the authors assumed that it was inhibited in a first-order reaction by analogy with majority of coagulation factors with a rate constant of 0.002 sec^{-1} (the arbitrary value chosen by analogy with other factors). Thus, the equation for HKa had the form:

$$\frac{d[HKa]}{dt} = k_1 \cdot [XIIa] + k_2 \cdot [kallikrein] - k_3 \cdot [HKa], \quad (11)$$

where k_1-k_3 are effective constants.

As noted above [76], a first-order inhibition of system components implies existence of the activation threshold in a system with positive feedback loops. Thus, the model of coagulation on biomaterials by Basmajian *et al.* assumes that HKa is infinitely rapidly activated and is not inhibited [82] and predicts no threshold, while the model of Pokhilko and Ataullakhanov describes HK activation correctly but uses the arbitrary reaction to describe its inhibition [84] which predicts existence of a threshold. Experimental verification of kinetics of HK activation and inhibition will be required to conclude whether or not there is a threshold in the contact activation pathway.

CONCLUSIONS

In this review, we attempted to summarize the accumulated information on application of mathematical modeling and computer simulation in solving the problems of clinical practice, and pharmacology and in developing rational therapy strategy. Development of mathematical models of various coagulation assays, such as thrombin generation assay, prothrombin time test and activated partial thromboplastin time test, provided useful information about sensitivity of assay parameters to the changes in the coagulation system and enabled determination of individual factor concentrations and reaction rate constants, which can be useful for the diagnostics of coagulation disorders and drug evaluation.

Computer simulation models proved helpful in the analysis of the mechanisms underlying thrombosis-associated and bleeding-associated diseases. Specifically, models of platelet thrombus formation can be used for prediction of stenosis in clinical situations on the basis of angiographic data; for the estimation of thrombotic risk under certain conditions; and in the design of artificial organs to predict thrombosis-prone regions. Advanced mathematical models of thrombus formation include feedback effects of platelets and of the growing thrombus on blood rheology. Development of computer simulation models describing spatial formation of fibrin clot has contributed to the elaboration of the role of intrinsic tenase in sustained and far-ranging propagation of the clotting process from TF-bearing initiator cells, which is impaired in bleeding disorders hemophilias A and B.

Mathematical models were used to study the mechanisms of the effects of various therapeutic agents, including argatroban and factor rVIIa, and to select an optimal strategy of their use. Specifically, it was elucidated that efficiency of

rVIIa (NovoSeven®) in facilitating thrombin generation at low (physiological) TF concentrations is mainly attributed to TF-independent activation of factors IX and X by VIIa bound to activated platelets.

Several mathematical models have been developed to systematically analyze the process of contact activation on foreign surfaces in flowing blood and the effects of various factors on thrombus formation. These studies can substantially assist in solving the major problems – cell adhesion and blood coagulation on biomaterials – in the design and clinical use of artificial organs and implantable devices.

The general limitation for a wide practical use of the existing mathematical models is the fact that they include a number of critical assumptions and in certain cases there remain contradictions between predictions of different simulation models. Therefore, there is a strong need for a further verification of mathematical models as well as for the experimental testing of the conclusions of these theoretical studies. Existing mathematical models describe platelet adhesion and blood coagulation separately. Development of models which integrate both systems of hemostasis will most probably be the next stage in the theoretical research of hemostasis and thrombosis. Accumulation of the detailed experimental knowledge about the blood coagulation system and about platelets, combined with impressive increase of computational power, promises rapid development of this field.

ABBREVIATIONS

FV, FVII, FVIII, FIX, FX, FXI, FXII and FVa, FVIIa, FVIIIa, FIXa, FXa, FXIa, FXIIa, coagulation factors V, VII, VIII, IX, X, XI, XII and their activated forms

ADP	=	Adenosine diphosphate
TF	=	Tissue factor
TFPI	=	Tissue factor pathway inhibitor
AT-III	=	Antithrombin III
PT	=	Prothrombin time
APTT	=	Activated partial thromboplastin time
HK	=	High molecular weight kininogen
PC	=	Protein C
APC	=	Activated protein C

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