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Exploring potential anticoagulant drug formulations using thrombin generation test

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SUMMARY

Many anticoagulant drugs inhibiting proteins of the coagulation cascade have been developed. The main targets of anticoagulant drugs are thrombin and factor Xa; inhibiting these factors delays thrombus growth, thus preventing thrombosis while increasing bleeding risk. A balance between thrombosis and bleeding is ensured in the 'therapeutic window' of the anticoagulant drug concentration range. Novel anticoagulant drugs and combinations thereof are being developed. We rank coagulation factors as potential anticoagulant drug targets in combination with thrombin inhibitors, aptamer HD1 and bivalirudin, providing a background for several promising dual target treatment strategies.

The thrombin generation test was used to assess the whole coagulation cascade in normal and factor-deficient human blood plasma. Potential therapeutic windows were estimated for coagulation factors, ranking them as targets for anticoagulant drugs. Thrombin and factor Xa have been revealed as the most promising targets, which fully agrees with the current drug development strategy. Inhibitors of factors Va and VIIa are expected to have narrow therapeutic windows. Inhibitors of factors VIIIa and IXa are expected to have a moderate anticoagulant effect. Factors XI and XII are poor targets for anticoagulant drugs. Compared with plasma that is deficient in factor II, the thrombin inhibitors bivalirudin and aptamer HD1 had increased activity. Both inhibitors were tested in deficient plasma providing a model of potential drug combination. The most promising combinations were anti-thrombin with anti-V/Va and also anti-thrombin with anti-IX/IXa. Each combination had an incremental dose-effect dependence that is promising from the standpoint of the therapeutic window.

1. Introduction

The coagulation cascade together with platelets provide thrombus formation in several seconds after a vessel injury. The coagulation cascade yields fibrin fibers that tighten the plug formed from aggregating platelets. Both processes are tightly regulated by several feedback mechanisms (accelerating as well as inhibitory) to ensure a controlled, prompt response. Genetic alterations, inflammation, obesity, cancer, immobilization, and other causes lead to conditions of excessive thrombus formation [1–4]. Oral anticoagulant drugs and platelet inhibitors have been developed to prevent thrombotic events in high-risk patients. Parenteral anticoagulant drugs have been developed for surgical procedures [3,5,6]. The causes of thrombosis are manifold and treatment thus requires adapted approaches employing distinct medications and combinations thereof. Therapeutic combinations of several platelet inhibitors and also combinations of platelet inhibitors with an anticoagulant drug have currently passed clinical trials [3,7–9]. Other combinations still have to be explored providing safer and more efficient thrombosis treatment.

The coagulation cascade is triggered with the tissue factor that is exposed upon vessel damage. The tissue factor triggers factor VII activation to factor VIIa, which in turn catalyzes the activation of factor X to factor Xa, which catalyzes the conversion of prothrombin into thrombin. Thrombin and also factors VIIa and Xa catalyze several positive regulatory loops that significantly accelerate thrombin generation (see Fig. 1). The main function of thrombin *in vivo* is to catalyze the conversion of fibrinogen into fibrin, which spontaneously associates into a net of fibrin fibers. Excessive thrombin is mainly neutralized by antithrombin III, a serpin-family protein acting like a suicide substrate. A set of inhibitors is responsible for disabling the cascade after a proper fibrin fiber net is formed; among them are heparin cofactor II inhibiting thrombin, activated protein C with protein S inhibiting factors Va and VIIIa, as well as TFPI inhibiting the factor Xa-dependent activation of factor VII [11–13].

The thrombin generation test (TGT) is a sophisticated technique

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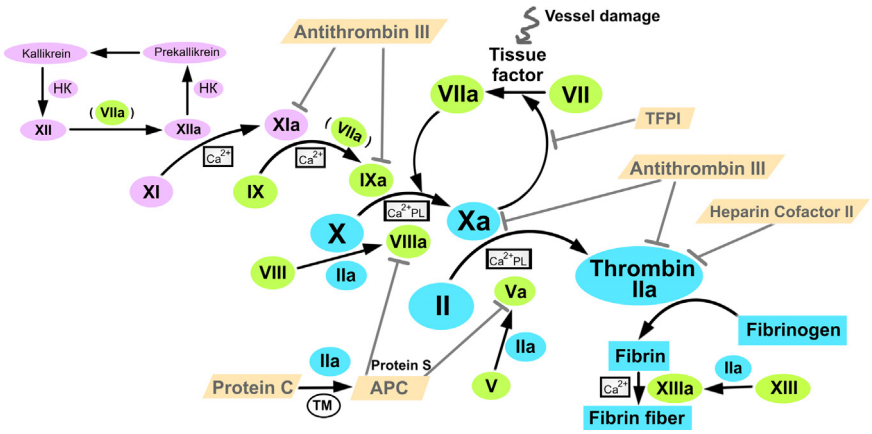

Fig. 1. The coagulation cascade is a sophisticated regulatory network controlling the formation of fibrin fiber. Tissue factor triggers coagulation under vessel damage, whereas factor XII induces supplementary contact activation intrinsic pathway. HK – high molecular weight kininogen, PL – phospholipid surface, TM – thrombomodulin, APC – activated protein C, TFPI – tissue factor pathway inhibitor. The scheme is derived from a diagram from Enzyme Research Laboratories [10].

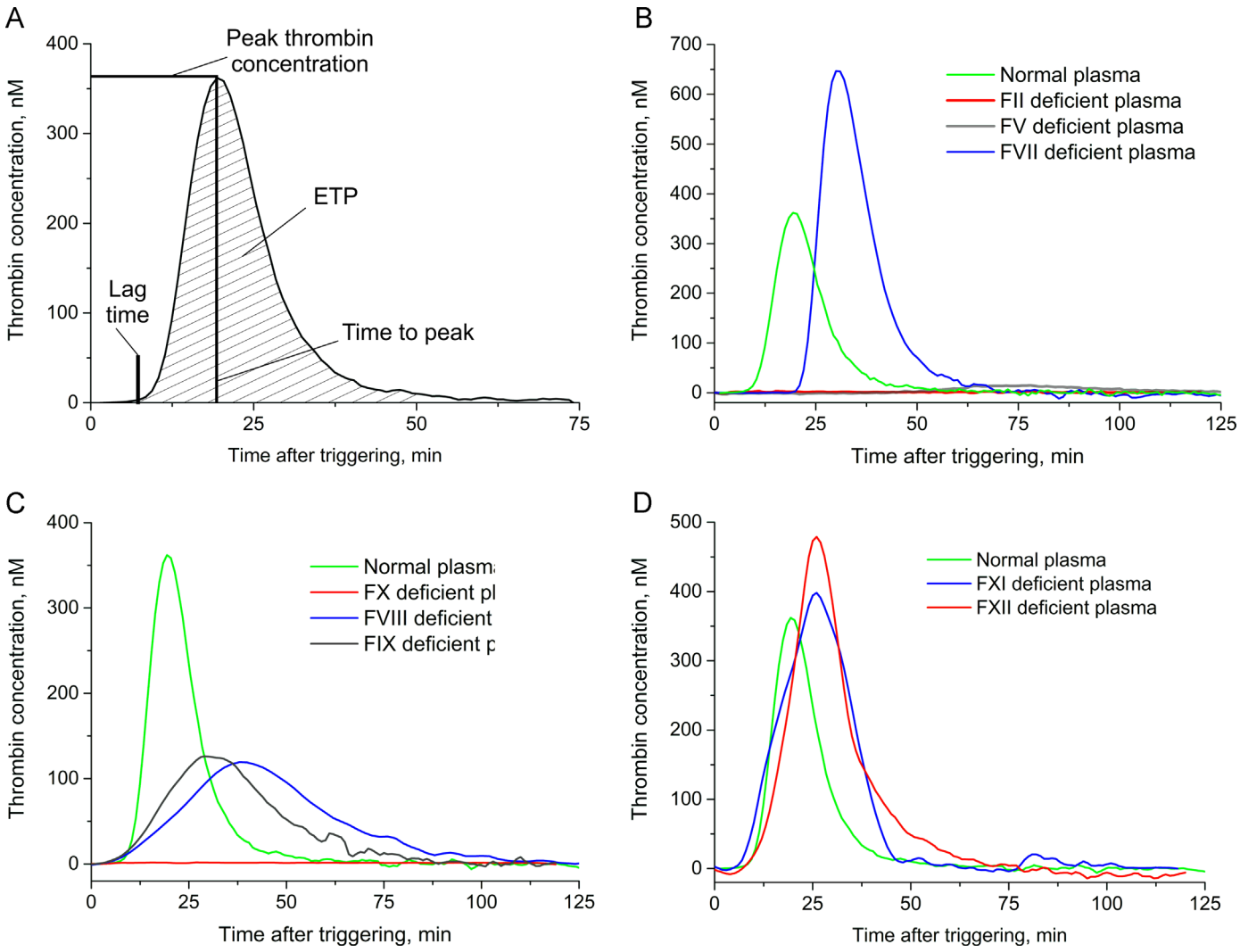

Fig. 2. Thrombin generation in normal and deficient plasma. A – TGT parameters; B – thrombin generation in normal, factor II-, factor V-, and factor VII-deficient plasma; C – thrombin generation in normal, factor VIII-, factor IX-, and factor X-deficient plasma; D – thrombin generation in normal, factor XI-, and factor XII-deficient plasma.

to study the coagulation cascade in detail. The TGT allows real-time tracing of thrombin produced in a blood plasma sample. The technique traces thrombin generation and its subsequent inactivation using a fluorogenic substrate. The thrombin generation profile is sensitive to anticoagulant drugs and hemophilia-related disorders and is a sharp tool for diagnostics and for research issues [14–19]. For example, the TGT has recently been used to test therapeutic combinations of edoxaban, a factor Xa inhibitor, with the platelet inhibitors clopidogrel and ticagrelor [18].

A wide variety of thrombin inhibitors have been developed,