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Intravascular Anticoagulation; Increasing the Disease-specificity of Antithrombotic Therapy

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Introduction

The prognosis of vital blood vessel thrombosis is very poor. Vascular occlusions play central role in the pathogenesis of high mortality diseases, including ischemic stroke, heart attack, severe systemic inflammatory response syndrome (SIRS), pulmonary embolism, and several others. Treatment options are limited in part because the most potent antithrombotic agents also paralyze hemostasis when dosed to full efficacy. Thrombosis thus remains the leading cause of human mortality in the U.S. CDC statistics indicate that thrombotic diseases account for more deaths than all other causes combined (Anderson & Smith; 2002). Better understanding of the differences between thrombosis and hemostasis may help us develop safe thrombosis-specific antithrombotic therapies.

Differences and Similarities between Thrombosis and Hemostasis

Thrombosis and hemostasis share most of their attributes—the key to pharmacological targeting of thrombosis only lies in a few but substantial differences. Pathologically significant vaso-occlusive thrombi can form under flow conditions at virtually all locations in the cardiovascular system. Arterial thrombosis accounts for the majority of fatal diseases, including heart attack and stroke. Thrombotic occlusion of veins and small vessels also accounts for significant morbidity or mortality.

Normally, blood remains in solution inside blood vessels despite continuous thrombogenic stimuli and injuries (homeostasis). Blood clots when it is passes through injured vessel wall and gets in contact with the extraluminal environment (hemostasis). Achieving hemostasis requires the generation of the essential enzyme, thrombin (factor IIa). Thrombin converts fibrinogen into insoluble fibrin (FN) and activates platelets that provide further surface and anchor for additional FN generation. Thrombin generation can be initiated by contact activation of the coagulation enzyme, factor XII (FXII), or association of activated factor VII (FVIIa) to tissue factor (TF), a transmembrane protein that is abundant in the subendothelial and extravascular space. Upon small injuries, the enzymatic process leads to rapid and massive thrombin generation and the formation of hemostatic plugs. Cessation of bleeding (i.e. hemostasis) thus prevents further exsanguination and maintains the integrity of the circulation. This very effective hemostatic thrombin generation is almost exclusively dependent on the exposure of blood to extraluminal TF. Exposure of circulating blood to almost any “foreign” material that does not normally occur in significant quantities in circulating blood triggers the enzymatic cascade of thrombin generation inside the vessel lumen as well. The circulation is well isolated from the rest of the body and the exposure of blood to foreign matter (e.g., TF, bacteria, viruses, etc.) is limited. Thrombin that is normally generated inside the blood vessel is effectively inhibited and its further formation is down-regulated by essential natural anticoagulant systems in the micro-environment of the intraluminal boundary layer of blood flow (Figure 1). Intraluminal thrombin is effectively inhibited by protease inhibitors in the presence of vessel wall-bound catalyzers as glycosaminoglycans (GAG). Remaining free thrombin is captured and extracted from circulating blood by the endothelial membrane-associated molecule, thrombomodulin. Thrombomodulin-bound thrombin gains new substrate specificity and activates protein C. Activated protein C (APC) prevents further thrombin generation by inactivating cofactors of the coagulation cascade. Disturbance of the intravascular homeostasis, however, can lead to excessive intraluminal thrombin generation, blood coagulation, and thrombosis. When the initially vessel wall-dependent process expands towards the center of blood flow, the endothelium-dependent control-mechanisms are not spatially available any more. Other, control mechanism might still be at work, e.g. flow or shear-dependent processes and endogenous fibrinolysis. When all controls fail or are overwhelmed, the thrombus reaches the opposite vessel wall and the flow of blood is blocked. The intravascular antithrombotic regulatory mechanisms of the coagulation cascade eventually fail to control the intraluminal runaway generation of thrombin in over 50% of people, ultimately leading to their death.

Thrombi, hemostatic plugs, and blood clots bear certain similarities (Figure 2). Thrombi are pathological intravascular bodies with the consistency of solid tissues and comprise selectively enriched and transformed components of normal blood. The critical building component of thrombi is a dense fibrin network that holds together a significant mass of
activated platelets and leukocytes. Since erythrocytes are also entrapped during thrombus formation, fresh thrombi have an intense red color upon autopsy. Transvascular hemostatic plugs closely resemble the composition of thrombi, however, only part of the plug is exposed to circulating blood flow in the vessel lumen. The most notable difference between thrombi and hemostatic plugs is that the majority of the hemostatic plug is positioned in the extravascular space and exposed to an abundance of various thrombogenic material (thus TF, collagen, etc.) and high shear blood flow from the wound. There is an increased concentration of fibrin(ogen) and platelets in thrombi and hemostatic plugs, because the surface specifically extracts these components from the blood that passes by. Static blood can also coagulate and eventually form clots in blood vessels. Blood clots might form in vessels that have already been occluded and the ischemic condition already exists due to primary thrombi or other causes of stasis. Blood clots are different from thrombi, predominantly because they contain cells and other native or transformed proteins (e.g., fibrin(ogen)) in proportion to their occurrence in whole blood. Blood clots might cause vaso-occlusive diseases, but their clinical significance, if any, has not been clearly documented. They are discussed here predominantly because clots have long been employed, sometimes justifiably, in research as surrogates for thrombi.

Currently Available Pharmacological Antithrombotic Treatments are not Specific for Thrombosis

The most often used antithrombotic therapies include anticoagulants, platelet inhibitors, and pro-fibrinolytic agents. Unfortunately, available antithrombotic therapies target those components of the blood coagulation process or platelet activity that are also necessary for normal hemostasis. Accordingly, antithrombotic agents in current use are not specific for thrombosis. They also impair hemostasis and invariably cause bleeding at their most efficacious doses. A reasonable approach to using such nonspecific treatments to a disease is to find a dose with a favorable balance between antithrombotic benefits and antihemostatic hazards. Similar to the practice of pharmacological cancer therapies, antithrombotic drugs are therefore not dosed to full efficacy.

Possible solution to this fundamental problem is to continue research, and identify and explore antithrombotic molecular and cellular mechanisms that are not essential for or do not interfere with normal hemostasis. The results of this research should help identify new molecular targets for pharmacological interventions. Once these targets are identified, we need to design and develop pharmaceutically acceptable agents that are specific for the new molecular targets. Thrombosis-specific antithrombotic agents should target blood coagulation predominantly inside blood vessels. These agents

Figure 1. Thrombosis and hemostasis: commonalities and differences. Activation of the coagulation cascade via factor XII (FXII) or factor VII (FVII) + tissue factor (TF) leads to the generation of thrombin (factor IIa). Inside the blood vessels (top panel), further thrombin generation and extension of the process towards the lumen is inhibited at the vessel wall by the endothelial surface-associated antithrombotic mechanisms. These include, among others, the glycosaminoglycan (GAG)-catalyzed antithrombin (AT) system that directly inhibits several coagulation enzymes, and the thrombomodulin (TM) and endothelial protein C receptor (EPCR)-dependent protein C system that generates the directly anticoagulant enzyme, activated protein C (APC). Since most extravascular tissues are poor in GAG and essentially lack both TM and EPCR, thrombin generation in blood that exist the blood vessel is not regulated effectively (bottom panel). Accordingly, thrombin will clot fibrinogen, activate platelets, and effectively contributes to hemostasis.

Figure 2. Comparison of a fresh blood clot to a fresh arterial thrombus. Native blood clotted in a glass dish without mixing (left panel, HE stain) and allowed to retract for 60 min consists of proteins and cells proportional to their content in unclotted native blood. In sharp contrast, a thrombus that formed for 60 min in a 4 mm ID vascular graft in a baboon (right panel) contains approximately 10-fold more fibrin(ogen), 50-fold more platelets, and about 100-fold more white blood cells than the same volume of retracted blood clot.
should not fundamentally interfere with the extraluminal process of normal hemostasis and should therefore be safe. Once such the molecular or cellular targets are identified and appropriate agents are obtained, we need to explore the safety and efficacy of the new pharmacological treatments in comparative studies using animal models or thrombosis and hemostasis.

The Safety and Efficacy of Targeting the Contact Phase of Blood Coagulation and Inflammation

One of the best rational approaches to identifying targets for safe antithrombotic therapy is to explore genetic abnormalities that affect non-essential proteins of blood coagulation or platelet function. For example, inherited deficiencies of the contact activation complex (factors XI and XII, prekallikrein [PK], and high molecular weight kininogen [HMWK]) have limited (FXI) or no known effects (PK, FXII, HMWK) on normal hemostasis. Accordingly, inhibitors of the contact phase should not induce very significant adverse antihemostatic effects characteristic of existing anticoagulants, antiplatelet drugs, and fibrinolytic agents. The question that needs to be answered however whether inhibition of the contact phase has antithrombotic effects? Several enzymes and cofactors participate in the intraluminal contact activation of blood coagulation and inflammation, and generate two key enzyme products, activated FXI (FXIa) and kallikrein (KK) (Figure 3).

Using a model of hemostasis and acute vascular graft thrombosis, we found indeed that near-complete inhibition of factor XI by an antibody was indeed as antithrombotic as high dose heparin but without hemostasis impairment in large primates (Gruber & Hanson, 2003). These findings were recently reinforced in a rabbit model of thrombosis (Yamashita et al. 2006). Another group of investigators has recently found that inhibition of FXII activity was also antithrombotic and did not impair the primary hemostasis. Using a small molecule inhibitor of FXIIa (PCK), they demonstrated safe antithrombotic effects in various disease models in mice. (Renne et al. 2006). PCK infusion prolonged the partial thromboplastin time (aPTT) but hemostasis was not affected in a tail amputation assay. In contrast, heparin infusion at 200 U/kg prolonged the aPTT to an equivalent degree and markedly prolonged the bleeding time, demonstrating it's well documented and substantial risk for hemorrhage. Moreover, they showed that inhibition of FXIIa inhibited cerebrovascular thrombus formation and improved the outcome in a mouse model of ischemic stroke (Kleinschnitz et al. 2006).

Potential indications for prospective contact phase inhibitors include all chronic uses that include, among others, atrial fibrillation, peripheral arterial disease, cerebrovascular disease, cardiovascular disease, diabetes, organ transplants, thrombophilias, etc. Potential acute uses include ischemic stroke, myocardial infarction, interventional cardiology, embolism and thrombosis, major surgeries and trauma, thrombogenic infections (e.g., SIRS), DIC, etc.

The Safety and Efficacy of Using Protein C Activators Targeted to the Intraluminal Boundary Layer of Blood Flow

Activated protein C (APC) is a potent natural anticoagulant enzyme that inhibits the coagulation cascade by proteolytic cleavage of activated coagulation co-factors V (FVa) and VIII (FVIIIa). APC also has direct antiapoptotic and anti-inflammatory effects by interacting with protease activated receptors (PAR) on various cells. Natural protein C activation is confined to the intravascular boundary layer of blood flow by its dependency on the endothelial transmembrane cofactors, thrombomodulin (TM) and endothelial protein C receptor (EPCR). Native APC is therefore an intrinsically safe antithrombotic molecule that does not cause systemic impair-

Figure 3. Molecular targets of the intravascular contact phase for antithrombotic drug discovery. Auto-activation of FXII to activated FXII (FXIIa) is followed by activation of PK and FXI to the potent enzymes (KK, FXIa) in the presence of HMWK. KK generates the potent inflammatory peptide, bradykinin (BK), while FXIa activates the coagulation cascade via factor IX (FIX), leading to explosive generation of thrombin (T). Since in the extraluminal space, "intrinsic" coagulation cascade can be bypassed by the coagulation enzyme factor FVIIa + TF at the level of FIX, inhibition of the contact phase should be more specific for thrombosis than hemostasis.
ment of the hemostasis while it exerts its effect at the site of generation. Any APC that diffuses into the systemic circulation does not cause systemic hemostasis impairment because it is progressively inhibited by plasma protease inhibitors similar to the inhibition of free thrombin.

The challenge for the endogenous protein C system similar to the way we routinely utilize the endogenous plasminogen system for antithrombotic therapy is that the natural protein C activator (PCA) enzyme, thrombin, cannot be safely infused as a systemic agent. We therefore engineered a new PCA enzyme by introducing two mutations, W215A and E217A into human thrombin. The ability of this neoenzyme, termed WE, to recognize and cleave fibrinogen and platelet PAR is severely restricted while it still can activate protein C reasonably well after binding to thrombomodulin (Figure 4).

Unlike other anticoagulants, circulating thrombomodulin-dependent PCAs have no direct anticoagulant activity and do not directly impair hemostasis. The danger of excessive protein C activation and significant systemic anticoagulation upon overdose is inherently limited by finite protein C and TM supply (Gruber et al. 2002). Our studies in primates clearly demonstrated the antithrombotic efficacy and hemostatic safety of WE treatment in the vascular graft thrombosis model (Gruber et al. 2002). Since a natural enzymatic system is used, the efficacious weight-adjusted doses of WE is very low (0.06 to 0.23 nmoles/kg/hour) compared to equally effective but antihemostatic doses of intravenous low molecular weight heparin (70 to 290 nmoles/kg/hour).

Potential indications for injectable recombinant PCAs include acute ischemic stroke, myocardial infarction, interventional cardiology (e.g., stenting), acute thrombo-embolism, major surgeries and trauma.

Summary and Future Directions

Research must continue to refine our understanding the differences in thrombosis and hemostasis (Colman. 2006). These efforts will help us identify the optimal molecular targets for thrombosis-specific intraluminal antithrombotic therapy. At least two areas of research in this direction has now shown promise. Both topical anticoagulation by thrombomodulin-dependent endogenous protein C activators and systemic anticoagulation by antibodies or other inhibitors of the contact phase (FXI, FXII) activity have shown safety and efficacy. The validity of these approaches need further verification in additional animal models. Characterizing the differences between platelet activation by collagen, other proteins of the extracellular matrix (ECM), and thrombin in wounds but essentially thrombin only on thrombi that protrude into the vessel lumen might lead us to the discovery of ECM-resistant, thus safer platelet inhibitors. Fibrinolytic agents that dissolve blood clots but are inactive in the environment of hemostatic plugs would prove useful as safe treatments for existing thrombi.

References