The overall adverse event profile in the apixaban arms, including reported serious and non-serious adverse events, was comparable to conventional therapy. Major bleeding occurred in 1.3-3% of patients treated with apixaban Twice-daily dosing of apixaban is anticipated to provide greater clinical benefit than once-daily dosing based on the phase II study results. In the phase II study, among the range of once and twice daily doses tested, the regimen of 2.5 mg administered twice daily (5 mg total daily dose) provided a better benefit/risk profile than other apixaban dosing regimens in the study when both VTE/death events and bleeding were considered. An ambitious Phase III apixaban development program, ADVANCE, has been initiated for the prevention and treatment of venous and aterial thromboembolic disease, which will initially involve more than 30,000 patients.

MECHANISMS IN HEMOSTASIS AND THROMBOSIS II

BLOOD CLOTTING ON MEMBRANE NANODOMAINS

J.H. Morrissey, V. Pureza, R.L. Davis-Harrison, S.G. Sligar, Y.Z. Ohkubo, E. Tajkhorshid
Departments of Biochemistry, Chemistry and Pharmacology, College of Medicine, University of Illinois at Urbana-Champaign, Urbana, USA

Blood clotting involves a series of enzyme activation reactions, each of which requires the coordinated assembly of a serine protease, its cognate regulatory protein (protein cofactor), and protein substrates on a membrane surface. In particular, the outer leaflet of the membrane must contain exposed anionic phospholipids such as phosphatidylserine (PS) in order to support the assembly and function of blood clotting reactions. In spite of the critical nature of membrane binding in blood clotting, our understanding of how the membrane contributes to catalysis, or even how blood clotting proteins interact with phospholipids, is incomplete. In addition, membranes containing mixtures of PS and neutral phospholipids have been shown to spontaneously coalesce into PS-rich membrane microdomains in the presence of plasma concentrations of calcium ions. We therefore hypothesize that blood clotting proteases partition into these PS-rich microdomains. Unfortunately, however, little is known about how membrane microdomain composition influences the activity of blood clotting proteases. Our laboratories are developing and applying new technologies for studying membrane proteins, in order to gain insights into how blood clotting protease-cofactor pairs assemble and function on membrane surfaces. Our studies include the use of a novel, nanometer-scale lipid bilayer system (Nanodiscs), which permits us to assemble blood clotting protease-cofactor pairs on stable bilayers containing from 65 to 250 phospholipid molecules per leaflet (in which the phospholipid composition is under strict experimental control). This system allows insights into how local (nanometer-scale) changes in phospholipid bilayer composition modulate TF:VIIa activity, and how different phospholipid types can synergize to support blood clotting reactions. We have also applied detailed molecular dynamics simulations of nanoscale bilayers to provide atomic-scale models of how membrane-binding domains in blood clotting interact with PS in bilayers. This work was supported by grants from the National Institutes of Health and the Roy J. Carver Charitable Trust.

GAS6 AND PROTEIN S, VITAMIN K-DEPENDENT LIGANDS FOR THE AXL-FAMILY OF RECEPTOR TYROSINE KINASES

B. Dahlbäck

Department of Laboratory Medicine, Lund University, University Hospital, Malmö, Sweden

Vitamin K-dependent protein S has multiple functions. It serves as a cofactor to activated protein C in the degradation of coagulation cofactors FVa and FVIIIa. In addition, in human plasma it forms a high affinity complex with the complement regulator C4b-binding protein (C4BP) giving the complex the ability to bind to negatively charged phospholipid membranes, e.g. exposed on the surface of apoptotic cells. Binding of free protein S and protein S-C4BP complexes to apoptotic cells provides local regulation of both coagulation and complement systems on the apoptotic cell surface. Protein S is homologous to Gas6 (growth arrest specific protein 6), which is a ligand for a family of tyrosine kinase receptors that includes Tyro3 (Sky), Axl and Mer (TAM family). Numerous studies have shown the Gas6/TAM system to regulate cell survival, proliferation, migration, adhesion and phagocytosis. Consequently, altered activity/expression of its components has been detected in a variety of pathologies such as cancer and vascular, autoimmune and kidney disorders. Each member of the TAM family possesses distinct expression profiles as well as discrete functions. A hallmark of the Gas6/TAM system is the unique ability of Gas6 to tether its Gla domain to the negatively charged membranes of apoptotic cells, which results in the stimulation of phagocytosis of the apoptotic cells. Removal of apoptotic cells is an essential process for normal development and tissue maintenance. Protein S bound to apoptotic cells can also stimulate the phagocytosis, but the receptors involved in this process are not yet elucidated. Preliminary results suggest that protein S can bind to Tyro3 and Mer but not to Axl. Importantly, Gas6 bound to the apoptotic cells stimulate the phagocytosis by macrophages while actively suppressing inflammatory responses. The clearance of apoptotic cells is important for regulating tissue homeostasis, inflammation, and autoimmune responses. Animals with mutations or loss of the TAM receptors exhibit phenotypes reflective of impaired phagocytosis and a hyperactive immune response with widespread accumulation of apoptotic cells and autoantibody production. This presentation will provide on overview of the TAM family of tyrosine kinase receptors and the emerging role of Gas6 and protein S as ligands and regulators of this receptor family.

POLYPHOSPHATE MODULATES CLOT STRUCTURE

S.A. Smith and J.H. Morrissey

Departments. of Biochemistry & Internal Medicine, College of Medicine, University of Illinois at Urbana-Champaign, Urbana, USA

Polyphosphate is a linear polymer of inorganic phosphate that is present in platelet dense granules and is secreted by platelets upon activation. We recently reported that polyphosphate is a potent hemostatic regulator, serving to activate the contact pathway of blood clotting and accelerate factor V activation.[1] In our previous studies, we found that polyphosphate did not alter clotting times initiated by thrombin, so it appeared that polyphosphate exerted all its procoagulant effects upstream of thrombin. We now report that polyphosphate enhances fibrin clot structure in the presence of physiologic concentrations of calcium ions. We found that fibrin clots formed in the presence of polyphosphate had up to threefold higher turbidity, exhibited higher mass-length ratios, and had substantially thicker fibers in scanning electron micrographs. Furthermore, the ability of polyphosphate to enhance fibrin clot turbidity was independent of factor XIIIa activity. When plasmin or plasminogen and tissue plasminogen activator was included in clotting reactions, fibrin clots formed in the presence of polyphosphate exhibited prolonged clot lysis times. And finally, specific staining techniques demonstrated that polyphosphate is physically incorporated into fibrin clots. *Conclusions*. Polyphosphate alters polymerization of fibrin, resulting in fibers of higher mass-length ratio that are lysed more slowly. This effect is calcium-dependent and is enhanced by preincubation of fibrinogen with calcium and polyphosphate. Release of polyphosphate from activated platelets or infectious microorganisms may therefore enhance fibrin clot structure. Polyphosphate may also be a useful additive to surgical fibrin sealants. This work was supported by grants from the National Institutes of Health and the Roy J. Carver Charitable Trust.

Reference

1. Smith et al. Proc Natl Acad Sci USA 2006;103:903-8.

NEW ANTICOAGULANT DRUGS

A.G.G. Turpie

Department of Medicine, HHS-General Hospital, Hamilton, Canada

New anticoagulants that do not require coagulation monitoring, preferably oral, are required as alternatives to heparin, low molecular weight heparins and the vitamin K antagonists. A large number of targets involved in clotting, including regulatory enzymes, and serine protease mediators are under investigation. Treatment of various conditions with recombinant forms of naturally occurring proteins, such as tissue factor pathway inhibitor (tifacogin), activated protein C (drotrecogin alpha) and soluble thrombomodulin (ART-123), has been investigated, with varying degrees of success. Fondaparinux, a subcutaneous, indirect Factor Xa inhibitor, is approved for the pre-

vention and treatment of venous thromboembolism (VTE) and in some countries for the treatment of acute coronary syndromes. Idraparinux, a subcutaneous, long-acting, indirect Factor Xa inhibitor, is in development. Direct inhibition of thrombin or Factor Xa with synthetic, small molecules is an attractive strategy for the development of novel anticoagulants. After the withdrawal of ximelagatran, dabigatran is now the furthest advanced oral, direct thrombin inhibitor, and an extensive phase III clinical trial programme (the RE VOLUTION trials) has recently determined its efficacy and safety in the prevention of VTE in major orthopaedic surgery. Direct Factor Xa inhibitors in development that show clinical promise in various indications include rivaroxaban, apixaban, betrixaban, LY-517717, YM150, and DX-9065a and its derivative Du-176b. Of these rivaroxaban and apixaban are the furthest advanced. Rivaroxaban has a favourable efficacy and safety profile, relative to enoxaparin, for the prevention of VTE after major orthopaedic surgery as shown in the RECORD trials. The results of trials of rivaroxaban for the treatment of proximal deep vein thrombosis are expected soon. It is likely that these new anticoagulants will revolutionize oral anticoagulant therapy.

BLEEDING DISORDERS

SURGERY AT HIGH RISK OF BLEEDING

A. De Gasperi

2° Servizio Anestesia Rianimazione e Trapianti Addominali, Ospedale Niguarda Cà Granda, Milano, Italy

"Some loss of blood is always inevitable".

In spite of sharp and innovative advances in surgical techniques, massive blood loss is still one of the major perioperative complications, able to impact on both morbidity and mortality.[1] Major thoraco-abdominal vascular surgery, major liver surgery including transplantation, neurosurgery, spine surgery, obstetrics complicated by postpartum hemorrhage are among the surgical procedures at risk of critical bleeding (defined as severe, unexpected, and uncontrollable bleeding), a condition able to raise mortality rate from <1% to above 20%.[2,3] Management of critical perioperative bleeding includes prompt evaluation, early and (hopefully) correct diagnosis, timely, appropriate and, not infrequently, multimodal approach for the treatment.[1,4,5] Complex haemostatic derangements and the related bleeding complications have to be managed understanding the specific physiological change(s), thus giving a solid rationale to the treatment, which could not be "simply" or only symptomatic (*"give blood and FFP"*) but causative (*"find the defect and treat it"*).[6] Generally speaking, perioperative bleeding could be subdivided in "surgical" and "non -surgical or haemostatic bleeding".[1,4,5] The so called "surgical" blood loss, responsible for up to 70% of the cases is mainly attributable to surgical technical problems and is characterized by uncontrolled bleeding at the operative site, where the problem is usually confined.[1,7] Proper patient selection and anticipation of technical problems may substantially contribute in reducing surgical bleeding in high-risk patients (eg. patients with portal hypertension or frequent bacterial peritonitis in major liver surgery; reoperations in case of previous extensive cardiac, thoracic, or abdominal surgery). The ''*non-surgical or haemostatic bleeding*'', on the contrary, is usually due to a dysfunction/failure in one (or more) of the phases of the haemostatic system: it appears as a generalised oozing with spontaneous, multiple sites bleeding (traumatized tissues, puncture sites, surgical wounds etc) without any apparent single bleeding point.[1] Main causes of the "non surgical bleeding" are:[1,4,5,6]

a) pre-existing, previously undetected bleeding disorder;
b) changes induced by drugs (e.g.: aspirin, clopidogrel, NSAIDs, warfarin, LMWH, UFH);
c) coexisting pathologies (eg: liver failure, chronic renal failure);
d) haemostatic derangements induced by:
- the surgical procedure itself (cardiopulmonary bypass, orthotopic liver transplantation, prostate surgery, fusion spinal surgery, neurosurgery, complicated obstetrics)
- massive blood loss and massive transfusion in complicated surgery.

Since mechanical treatment of this kind of hemorrhage by clipping or vessel ligation is frustrating and useless at