Clinical and basic science articles from Thrombosis and Haemostasis

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In 2009–2010, we have had a great selection of excellent articles published in Thrombosis and Haemostasis, and in this short overview, we present a selection of articles that have caught the Editors’ eye, as well as the scientific community, as reflected by numbers of citations.

Clinical studies

In 2009, the possible interaction of proton pump inhibitors (PPIs) on the antiplatelet effects of clopidogrel caused much debate, and Thrombosis and Haemostasis, added to the discussion. Sibbling et al. (1) reported that the attenuating effects of concomitant PPI treatment on platelet response to clopidogrel were restricted to the use of omeprazole, but no attenuating effects on platelet response to clopidogrel were observed for pantoprazole or esomeprazole. This is perhaps understandable given that PPIs differ in their metabolism properties as well as their potential for drug–drug interactions.

Given the limitations of clopidogrel, attention has been directed towards the new antiplatelet agents. One trial that attracted much interest was the ACAPULCO study, where platelet aggregation with prasugrel (900 mg loading dose, followed by 10 mg od maintenance) was found to be superior to high dose clopidogrel (900 mg loading dose, followed by 150 mg od maintenance) in acute coronary syndromes (2). Nonetheless, platelet response heterogeneity is recognised in thrombus formation, as addressed by Munnin et al. (3). They suggest that at least three subpopulations of platelets are formed in a thrombus: aggregating platelets with (reversible) integrin activation, procoagulant (coated) platelets exposing phosphatidylserine and binding coagulation factors, and contracting platelets with cell-cell contacts. This recognition of such thrombus heterogeneity has implications for the use and development of antiplatelet drugs.

Much interest has also been directed to the new oral anticoagulants (OACs), which would be viable alternatives to the vitamin K antagonists (VKAs, e.g. warfarin) given the significant inter- and intra-patient variability, as well as the significant disadvantage of the VKAs, with the need for monitoring and restrictions on diet, drug interactions and alcohol (4, 5). The new OACs fall into two broad classes, the oral direct thrombin inhibitor (DTI) and the oral factor Xa (FXa) inhibitors.

With the DTIs, Wolowacz et al. (6) published a metaanalysis showing the efficacy and safety of the oral DTI dabigatran for the prevention of venous thromboembolism following total knee atheroplasty. This metaanalysis showed RRs (random effects) for the composite end-point of total VTE and all-cause mortality of 0.95 [95%CI 0.82 – 1.10] and 1.05 [0.87 – 1.26] in analyses of the two (RE-MODEL and RE-NOVATE) and three (RE-MODEL, RE-NOVATE, RE-MOBILIZE) trial analyses, respectively, suggesting that dabigatran extended was non-inferior to enoxaparin 40 mg od, with a similar safety profile. However, heterogeneity between the trials cannot be ruled out.

In the context of stroke prevention in atrial fibrillation (AF), Roskell et al. (7) published a network metaanalysis and indirect comparison of various treatments (placebo, aspirin, aspirin + clopidogrel, VKA) against dabigatran for stroke prevention in AF. Given that a placebo-controlled trial against dabigatran will never be performed, this network meta-analysis provided some insight into efficacy and safety data. Compared with placebo, dabigatran extended 150 mg BID was estimated to significantly reduce the risk of any stroke (ischaemic and haemorrhagic) by 75% (relative risk [RR] 0.25; 95% confidence interval [CI], 0.12–0.51), ischaemic stroke by 77% (RR 0.23; 95% CI, 0.14–0.38), systemic embolism by 83% (RR 0.17; 95% CI, 0.05–0.50) and mortality by 36% (RR 0.64; 95% CI, 0.45–0.91). Dabigatran extended 150 mg BID was estimated to significantly reduce the risk of any stroke compared with aspirin monotherapy by 63% (RR 0.37; 95% CI, 0.20–0.69) and aspirin plus clopidogrel by 61% (RR 0.39; 95% CI, 0.21–0.72). None of this note was an indication of increased intracranial or extracranial haemorrhage with dabigatran extended compared to antiplatelet agents.

Other OACs at earlier stages of development have received some focus. For example, a phase 2 trial of the oral DTI, AZD0837 was reported (8). Also, Weitz et al. (9) published a phase 2 trial with the oral FXa inhibitor, edoxaban, and showed a similar safety profile of edoxaban 30 and 60 mg qd to warfarin in patients with AF. Interestingly, the edoxaban bid regimen in this trial were associated with more bleeding events than warfarin.

With these new OACs, focus has been directed on which coagulation assays can be used to measure an anticoagulation effect (13) and how to manage bleeding and/or overdosage (14). Clearly cost-effectiveness of these new OACs will become an issue, and this journal has contributed to the debate (10).

In the non-cardiovascular arena, the novel paper by Bresin et al. (15) attracted many citations, given that it proposed rituximab as pre-emptive treatment in patients with thrombotic thrombocytopenic purpura (TTP) and evidence of anti-
ADAMTS13 autoantibodies. Whilst TTP is rare, it is still as severe disease characterised by thrombocytopenia, microangiopathic haemolytic anaemia, neurological and renal involvement associated with deficiency of the von Willebrand factor-cleaving protease, ADAMTS13. Their results demonstrated that rituximab used as pre-emptive treatment may be effective in maintaining a sustained remission in patients with anti-ADAMTS13 antibodies in whom other treatments failed to limit the production of inhibitors.

In 2010, we also published a consensus document from the European Society of Cardiology Working Group on Thrombosis, endorsed by the European Heart Rhythm Association (EHRA) and the European Association of Percutaneous Cardiovascular Interventions, on Management of antithrombotic therapy in atrial fibrillation patients presenting with acute coronary syndrome and/or undergoing percutaneous coronary intervention/stenting (16). This provided a systematic review of the evidence base as well as recommendations (see Supplementary Table 1 available online at www.thrombosis-online.com) on how best to manage this complex set of patients, and this document provides the evidence base for the new European guidelines on atrial fibrillation management based on simplifying stroke risk assessment and thromboprophylaxis in real-life clinical practice (17). Other papers in this journal have provided justification for guideline changes, for example, in the value of anticoagulating atrial fibrillation patients at intermediate risk (CHADS2 score = 1) (18).

Basic science

A theme issue devoted to the EMVBM 2009 meeting encompassed a review article by Leroyer et al. (19), who focused on endothelial microparticles (EMP). These complex vesicular structures can be shed by activated or apoptotic endothelial cells and play a key role in the tuning of vascular homeostasis. Multifaceted roles of EMP, notably in coagulation, inflammation and angiogenesis and also the mechanisms that trigger their formation were discussed by the authors, who envision novel therapeutic possibilities based on the inhibition of EMP release.

Several experimental studies in the field of cardiovascular biology also suggest new diagnostic approaches and therapeutic opportunities for patients at high cardiovascular risk. Bai et al. (20) report that low concentrations of immunosuppressant drug FK506 skewed bone marrow-derived macrophage polarisation towards a M2 macrophage phenotype, whereas high concentrations did not. A low-dose FK506 treatment regime protected against atherosclerosis by suppressing T-cell activation and favouring M2 macrophage polarisation. Although a high-dose FK506 afforded a similar T-cell suppressive effect, with a pronounced shift towards Th2-type immune responses, this did not translate in atheroprotection due to hypercholesterolaemia and absent M2 skewing. Hristov et al. (21) evaluated possible associations of circulating monocyte subsets with cardiovascular risk and the extent of coronary artery disease and suggest that the differential profile of monocyte subsets harbours.

In the past decade, the understanding of the role of the Rho GTPases RhoA, Rac1 and Cdc42 has evolved from proteins that regulate specific actin cytoskeletal structures – stress fibers, lamellipodia and filopodia – to complex integrators of cytoskeletal structures that can exert multiple functions depending on the cellular context. Beckers et al. (22) focused on this complexity and consequences of Rho GTPase regulation for endothelial barrier function. The inducers of permeability, thrombin and VEGF, are presented as examples for G-protein coupled and tyrosine kinase receptor-mediated Rho GTPase activation, respectively. These mediators induce complex but distinct networks of Rho GTPase activators, inhibitors and effectors, which alter the endothelial barrier function. An interesting feature herein is that Rho GTPases often have both barrier-protecting and -disturbing functions.

Seijens et al. (23) highlight a role of CD154 in the developing lymphoid system, including the biology of haematopoietic stem cells and lineage-committed T-cell, B-cell, natural killer cell (NK), and dendritic cell (DC) progenitors, as well as clinical and therapeutic implications of CD154 interactions in lymphopoiesis. Aildoudi et al. (24) review platelet factor-4 (PF4), a platelet-derived chemokine combining a pro-atherogenic role with anti-angiogenic effects. High affinity for heparan sulfates is thought to be central to all of PF4’s biological functions. However, other mechanisms include direct growth factor binding, activation of the CXCR3B chemokine receptor isoform present in some vascular cells or binding to lipoprotein-related protein-1. Furthermore, PF4 binds to integrins with affinities similar to matrix molecules. These interactions can explain the effects of PF4 in health and disease.

Compared to the previous two years, Thrombosis and Haemostasis published 25% more articles heralding new animal models to study microvascular dysfunction, atherothrombosis and vascular events in vivo. Mostly interesting results are put forward by studies on the effects of FXa inhibitors on coagulation and bleeding (25) and in a rat model of sepsis induced by colon stent peritonitis (26), on the first murine model of acute thrombus formation after plaque rupture to study atherothrombosis using intravital fluorescence microscopy (27) and studies addressing the role of uPAR in the development of adipose tissue through PI3K/Akt pathway (28).

In July 1982, three cases of acquired immunodeficiency syndrome (AIDS) in men who had haemophilia elicited the immediate notion in Oscar Ratnoff that AIDS was transmissible through blood products. His subsequent work provided important and clear evidence that the AIDS agent was transmissible through pooled plasma products and had rapidly infected many men who had haemophilia. In a Theme Issue related to the Ratnoff Symposium 2009, Dr. Ledermann (29) gives an overview on potential drivers of immune activation in chronic human immunodeficiency virus (HIV) infection such as HIV itself, other co-infecting pathogens, homeostatic responses to cytopenia as well as the translocation of microbial products across a damaged gut mucosal surface. This latter phenomenon is particularly compelling as clinical studies have shown a good relationship between indices of microbial trans-

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location and markers of both immune activation and T-cell homeostasis in chronic HIV infection. Evidence has emerged that these microbial products may also promote and facilitate thrombus formation in HIV infection via induction of monocyte tissue factor expression. Thus, systemic exposure to microbial elements that are translocated through a gut mucosa damaged in the first few weeks of HIV infection may contribute to the pathogenesis of both immune deficiency and the heightened risk for vascular events that have been noted in individuals with HIV infection.

Müthing et al. (30) draw attention to toxin-receptor interactions and the mechanisms of shiga toxin (Stx)-mediated cell injury that induce a prothrombotic response. Binding of Stx to the glycosphingolipid (GSL) globotriaosylceramide (Gb3/Cer/CD77) on endothelial cells followed by receptor-mediated endocytosis is the linchpin in STEC-mediated disease. Only GSLs that associate strongly with lipid rafts appear to carry Stxs retrogradely from the plasma membrane through the Golgi apparatus to the endoplasmic reticulum where they are translocated to the cytosol and exert their toxic function. Thus, the biophysical features of the lipid moiety of GSL receptors may influence its incorporation into certain membrane domains and thereby affect toxin destination. This research will allow us to understand the complex mechanisms of Stx binding and internalisation, and may help to develop new strategies directed at the interruption of the Stx-induced pathological intracellular cascades. DeRoo et al. (31) focused on understanding the basic pathophysiology of post-thrombotic syndrome including clinical characterisation and novel therapeutic options, namely the development of a clinical plasminogen activator inhibitor (PAI)-1. Here, investigators have to deal with a lack of available human specimen, the lack of a gravity animal model of venous hypertension, and limited knowledge of endothelial vascular smooth muscle cell interplay in the venous system.

Granule proteins seeded onto the endothelium by adherent polymorphonuclear neutrophils (PMN) allow direct activation and subsequent adhesion of monocytes (32). In addition, PMN granule components enhance the endothelial expression of cell adhesion molecules, efficiently supporting the arrest of monocytes at inflamed vessels, granule proteins contribute to the fine tuning of the local chemokine network. Proteolytic modification of chemokines as well as enhancement of local chemokine synthesis lead to increased monocyte extravasation. Finally, PMN granules exert direct chemotactic effects, a mechanism which is of special importance in the early recruitment of inflammatory monocytes. Hence, granule proteins modify the monocyte extravasation cascade in a multifaceted manner ensuring the efficiency of these mechanisms.

A steady increase (around 10% per year) in the number of publications submitted under the category New Technologies, Diagnostic Tools and Drugs reflects an important connection between basic and clinical research. Studies by Samama et al. (11, 12) display results of screening existing assays (including commercially available ones) to measure the pharmacodynamics of rivaroxaban, an oral direct factor Xa inhibitor. All clotting and chromogenic assays demonstrated a concentration-dependent effect induced by rivaroxaban. Finally, in the Theme Issue Stem cells, basic principles, labelling techniques, clinical applications, and drawbacks associated with these cells and modalities such as radionucides, magnetic resonance, bioluminescence, and fluorescence imaging were thoroughly discussed (33). Before stem cell therapies can be considered for application in human populations, safety aspects should be meticulously investigated using such innovative technologies.

References