1. ABSTRACT

Worldwide, cardiovascular disease (CVD) is a leading cause of death. Endothelial dysfunction is now recognized to be a key platform for the pathophysiological effects of atherosclerosis. It is now well accepted that atherosclerosis is not merely a benign and passive process, but is in fact a dynamic and progressive disease arising from a combination of endothelial damage/dysfunction, inflammation, thrombosis and coagulation leading to potential clot-related vessel occlusion. The between inflammation, thrombosis and coagulation in the pathogenesis of CVD is more than simply association, as it clear that these processes are critically influenced by one another. In this preface we present a basic overview of the evidence in support of this relationship, which will be expanded upon in sequential chapters. In addition we briefly discuss a number of novel anticoagulants which not only reduce coagulation, but have ancillary antiinflammatory properties, thus further supporting the triad of inflammation, thrombosis and coagulation in the development of CVD.

2. INTRODUCTION

Cardiovascular disease (CVD) is currently the leading cause of death and disability in the developed world, and is predicted to soon overtake infectious disease as the pre-eminent cause of death worldwide (1). In particular, myocardial infarction (MI) related to coronary artery disease (CAD) represents the single greatest contributor to this enormous health burden (2).

However, arterial thrombosis on disrupted atherosclerotic lesions can manifest as other well recognised cardiovascular events, such as stroke and acute limb ischaemia. Thrombin, fibrin and platelets are the prominent components of the thrombi that occlude arteries and may also participate in the initiation and progression of the atherosclerotic plaque (3,4).

Whereas previously considered a relatively bland process, our current understanding of atherosclerosis suggests that the latter is a dynamic and progressive disease arising from a combination of endothelial damage/dysfunction, inflammation and coagulation which can ultimately lead to clot-related vessel occlusion (5,6). Indeed, the inflammatory response involves not only the arterial smooth muscle and endothelial cells, but also leukocytes and platelets derived from the blood. Also, endothelial damage/dysfunction is considered the earliest pathological signal of atherosclerosis (7). Many cardiovascular risk factors, such as hypertension, smoking, diabetes and elevated cholesterol impair endothelial integrity and may trigger atherosclerosis (‘atherogenesis’) without the need for physical endothelial injury per se (8-11).
Figure 1. Simplified illustration showing the interaction between inflammation, coagulation and thrombosis.

We are honoured to introduce this comprehensive review issue of ‘Frontiers in Bioscience’, outlining the unique relationship between blood coagulation, inflammation, and thrombosis, and its implications in cardiovascular disease. This is even more pertinent in recent years as our understanding of the complex pathophysiology of these processes has dramatically increased. This has allowed for the introduction of several novel anticoagulants that - in addition to reducing thrombosis - also appear to have a dual anti-inflammatory role, providing further evidence to support the inflammation/coagulation/thrombosis concept.

The process of clot formation (thrombogenesis) is a complex process involving the initiation and propagation of coagulation with simultaneous platelet activation and thrombosis (12). In the original ‘cascade’ system, the process of coagulation was thought to involve two parallel but separate pathways, known as the intrinsic and extrinsic system (13). It is now known that this two-pathway model is somewhat simplistic and outdated, and does not accurately reflect the events of in vivo haemostasis; consequently, the original, simple two-pathway model to clot formation has been replaced by the tissue factor (TF) pathway (formerly known as the extrinsic pathway) - where TF/factor (F)VII is the key protagonist – and the intrinsic system (activated when FX (Hageman Factor) comes into contact with negative charges underlying the endothelium) providing an ancillary propagation and amplification role (14).

The initiation of coagulation is triggered when TF becomes exposed on the plasma membrane leading to its interaction with FVII, or its active form, FVIIa, to form the enzymatically reactive TF-FVIIa complex (Figure 1) (14,15). TF is itself a lipid-dependent transmembrane glycoprotein that is sequestered in the circulation in quiescent endothelial cells and monocytes (14,16). In addition to its initiation by trauma, there is also increasing evidence to support a pivotal role of inflammation as a key trigger for the TF pathway (17). Also, the TF/ FVIIa complex activates a series of clotting factors (FVIIIa and FIXa) leading to the activation of activated X (Xa). TF/FVIIa with FV as a cofactor and calcium then form the prothrombinase complex, leading to the conversion of prothrombin (FII) to thrombin (FIIa), platelet activation and the subsequent conversion of fibrinogen to fibrin (18).
which will be addressed by Dr McVey in article 2 (15). This system is simultaneously counterbalanced by a series of activated coagulation inhibitors (tissue factor pathway inhibitor (TFPI), antithrombin (AT) and the Protein C pathway) working in tandem with the competing fibrinolytic pathway, acting to control unrestrained coagulation (figure 1) (19).

Not only does inflammation lead to activation of coagulation, but the converse is equally true, whereby coagulation itself leads to activation of a number of parallel processes culminating in vascular inflammation (20). The vulnerable plaque model of coronary artery disease provides an excellent example of the crosstalk that potentially occurs between inflammation and coagulation. Expression of coagulant material, notably TF, by inflammatory cells in the unstable plaque can also initiate coagulation and thrombin generation, resulting in the formation of a platelet-fibrin thrombus, and further stimulation of inflammatory pathways.

Coagulation activation induces a series of signalling pathways that mediate both thrombosis and a variety of inflammatory responses. The issue of coagulation-dependent thrombosis and coagulation dependent inflammation will be discussed in detail in sequential articles. The key mechanism for this process is via activation of protease activated receptors (PARs), located on endothelial cells, mononuclear cells, platelets, fibroblasts and smooth muscle cells (21,22). All PARs belong to a family of seven-transmembrane G-protein-coupled receptors, which have the pecuniary feature of serving as their own ligand. Consequently proteolytic cleavage of the PAR receptor leads to autoactivation of the same receptor by its own N-terminus, which acts as a ‘tethered ligand’ (as the ligand is attached to the receptor itself) leading to transmembrane signaling (23).

PARs appear to link tissue injury to appropriate cellular responses. Also, PAR activation can induce cellular production of several inflammatory cytokines - such as tumour necrosis factor (TNF-α), IL(interlukin)-6, IL-8 IL-1β, etc – and cause the upregulation of inflammatory responses in macrophages and the production of adhesions molecules, such as ICAM (intercellular adhesion molecule)-1 and VCAM (vascular adhesion molecule)-1 (24,25). In addition, stimulation of PARs on endothelial cells leads to mobilization of von Willebrand factor (vWF) and P-selectin from Weibel-Palade bodies, as well as the production of platelet activating factor and subsequent platelet activation (26,27).

Thus, coagulation can stimulate various inflammatory pathways, but inflammation per se can stimulate coagulation and thrombosis. Some of the key concepts of this process will be reviewed in this series. In cell cultures, both endothelial cells and monocytes can be induced by inflammatory markers – such as C-reactive protein, MCP-1, IL-1, IL-6 and TNFα - to produce TF, shifting the vascular environment to a more prothrombotic state (28,29). These cells can also release blood borne TF in microparticles that further contribute to haemostasis (30,31).

Perhaps the best in vivo evidence supporting the important role of cytokines in mediating thrombosis is the association of coagulopathy with sepsis, which has been shown to be largely driven by bacterial endotoxin stimulation of the de novo expression of TF on circulating monocytes (32). Administration of pro-inflammatory cytokine inhibitors (eg. anti-IL-6 or anti-TNFα antibodies) can even prevent abnormal coagulation during systemic infection (33,34) Inflammation can also lead to reduced activation of natural anticoagulant pathways, further enhancing thrombosis (20).

The role of platelet aggregation and adhesion involves a number of important interactions between platelets, fibrin and leukocytes. Furthermore, we now know that platelets provide a fundamental role in atherothrombosis and the targetting of platelet interactions has represented a fundamental advance in the treatment and prevention of coronary artery disease, which will be discussed in detail in a sequential article. In patients with CVD, vascular intimal injury associated with endothelial denudation and plaque rupture exposes subendothelial collagen and vWF, which support prompt platelet adhesion and activation (35). Circulating platelets can adhere either directly to collagen or indirectly via the binding of vWF to the glycoprotein (GP)Ib/FIX complex on the platelet surface (36,37).

P-selectin is a membrane adhesive glycoprotein contained within platelet alpha granules and Weibel-Palade bodies of endothelial cells (38). Following cellular activation, P-selectin is rapidly mobilised to the plasma membrane and facilitates interactions between platelets and leukocytes and between endothelial cells and leukocytes, the latter leading to leukocyte rolling (39). This interaction between platelets and leukocytes also links haemostatic/thrombotic and inflammatory responses. Indeed, following platelet activation, P-selectin can directly increase TF expression on monocytes (40). This process is a key component in atherosclerotic plaque development and subsequent thrombotic events (41).

Glycoprotein IIb/IIIa (GPIIb/IIIa) plays a major role in the regulation of platelet adhesion and aggregation during haemostasis. Platelet activation leads to conformational changes within GPIIb/IIIa (“inside-out” signalling), which increases the affinity of the receptor for its primary ligand, fibrinogen (42). Bound fibrinogen then acts as a bridging molecule facilitating the interaction of adjacent platelets. Upon fibrinogen binding, GPIIb/IIIa on the platelet undergoes further conformational changes and through a process termed "outside-in" signalling the receptor signals in to the platelet ultimately resulting in acceleration of the aggregation process (42).

TF is the key protagonist of the coagulation pathway and we have previously demonstrated its importance in inflammation and hemostasis (14,15). It is also increasingly appreciated that TF has a fundamental role in intracellular signalling and cell proliferation (43). The TF content of atherosclerotic plaques has been shown to be a major determinant of its thrombogenicity and TF
levels are increased in patients with acute coronary syndromes (44,45). In fact, mononuclear cells on atherosclerotic plaques appear to be primed to express more TF than native circulating mononuclear cells, which probably relates to sustained exposure to proinflammatory cytokines in the plaque, such as IL-6, and monocyte chemoattractant protein and platelet-derived growth factor (46).

Given, the multiple complex roles of TF, and its critical role in the initiation of coagulation and contribution to inflammation, there has been a huge amount of research interest in the development of specific TF/FVIIa inhibitors. A wide array of strategic approaches to inhibiting TF/FVIIa complex has been tried and developed, with some in their very early infancy. Antagonists include active site inhibited FVIIa, TF mutants, anti-TF antibodies, anti-FVII/FVIIa antibodies, naturally-occurring protein inhibitors, peptide exosite inhibitors, and protein and small molecule active site inhibitors (47). The implications of these potential therapies in anti-inflammation and antithrombotic therapy will no doubt be a topic of future discussion. We expect that there will be a rapid future expansion in research into the targeted inhibition of TF/FVIIa in the setting of CVD, (in particularly coronary artery disease) and in the modulation of sepsis.

We have so far demonstrated the importance of inflammation in the development of atherosclerosis, thrombosis and coagulation. This process is known to be orchestrated by the interactions between inflammatory cells (such as platelets and T and B lymphocytes) and vascular cells (such as endothelial cells and smooth muscle cells). Following cellular activation or apoptosis these cells release vesicles shed from the blebbing plasma membrane called microparticles (MP) (48). Whilst once considered merely inert debris reflecting cellular activation or damage, MP are now acknowledged as important cellular effectors involved in cell-cell crosstalk (49).

These MP have both cell surface proteins (with negatively charged phospholipids on their surface) and cytoplasmic components from the original cell. MPs differ in their size, shape, cellular protein content (including tissue factor) as well as in their procoagulant and proinflammatory properties (48). They have potent proinflammatory effects, promote coagulation and affect vascular function (50). For example, both platelets and platelet-derived microparticles can lead to activation of endothelial nuclear factor-xB and nuclear factor- xB-regulated genes that play important roles in chemotaxis and transmigration of monocytes (51,52). Since these processes are critically involved in the development of CVD, coupled with the fact that increased numbers of circulating MPs have been identified in a variety of CVD states strongly supports a role for MPs in the pathogenesis of CVD (53-57).

MPs are known to be are present in low numbers in health with dramatically increased numbers being found in the presence of a number of CVD states. Flow cytometry has evolved as the gold standard technique for their quantification and identification (58). Recent findings have demonstrated that leukocyte-derived microparticles, bearing both tissue factor and the platelet adhesion marker P-selectin glycoprotein ligand 1 circulate in the blood and accumulate in the developing platelet-rich thrombus following vessel wall injury (31, 59). Once activated, platelets themselves can release various pro-inflammatory cytokines (such as CD40 ligand and IL-1β) and chemokines (such as RANTES and platelet factor-4), which further amplifies the inflammatory process (60-62). The importance of MP in inflammation, vascular remodelling and thrombosis will be further discussed in this special issue.

Current anticoagulation practice involves the use of drugs that inhibit thrombin directly or indirectly by interacting with other clotting factors. Anticoagulants in current use can broadly be divided into the heparins (unfractionated and low molecular weight), vitamin K antagonists (VKAs) and new agents, such as the factor Xa inhibitors and thrombin inhibitors. VKAs antagonists, most notably warfarin have represented the mainstay of anticoagulation for the last 50 years. Unfortunately, whilst highly effective anticoagulants, VKAs are beset by a multitude of problems that have limited their use (63).

Given its unique position at the convergence point of the original intrinsic and extrinsic (TF/FVIIa) pathways in the coagulation system, FXa represents an interesting therapeutic target for both antithrombotic and anti-inflammatory drug development (64-66). The physiological role of FXa is in the cleavage of prothrombin to thrombin. The FXa inhibitors are a novel class of anticoagulants, which are synthetic versions of the five sugar sequence of heparin and are thus known as pentasaccharides. They have specific inhibitory activity for FXa, which is largely a reflection of their very small molecular size and unlike heparins, they have no affect on IIa (67). The pentasaccharides can inhibit FXa either directly (for example tick anticoagulant peptide, ZK-807834 and DX-9065a) and indirectly by forming a tight bond with antithrombin (for example, the subcutaneous agents fondaparinux, idraparinux and the oral FXa inhibitor razaxaban) (68-70). Both low molecular weight and unfractionated heparin can to some extent be considered indirect Xa inhibitors, as they inhibit both Xa and thrombin by indirectly by increasing the antithrombin III activity.

Of note, FXa inhibitors have several advantages over traditional VKAs and heparins, such as a predictable anticoagulant profile without the need for routine anticoagulant monitoring, few drug interactions and a very low incidence of heparin induced thrombocytopenia (71).

With respect to cardiovascular disease, there have been a number of promising data. For example, the XaNADU-1B Investigators demonstrated the efficacy of the synthetic reversible FXa inhibitor DX-9065a in reducing both thrombin generation and fibrin formation among patients with stable coronary artery disease (72). Furthermore, in a phase II trial of 402 patients, there was a non-significant tendency toward a reduction in ischemic
Blood clotting, inflammation, and thrombosis in cardiovascular events

events and bleeding with DX-9065a compared with heparin in patients with acute coronary syndromes (73). In addition, the PENTUA (Pentasaccharide in Unstable Angina) and PENTALYSE (Pentasaccharide as an Adjunct to Fibrinolyis in ST-Elevation Acute Myocardial Infarction) trials provide data to support the safety and efficacy of the FXa inhibitor fondaparinux in the setting of unstable angina and ST-elevation myocardial infarction respectively (PENTUA Study (74-77).

It would seem plausible, given the demonstrated interrelationship between inflammation and coagulation and the composite role of FXa in this process, that inhibition of FXa may also have the capacity to attenuate inflammation (66). In rat models, FXa can induce TF expression in human peripheral monocytes and inhibition of FXa by DX-9065a reduces TF expression in the liver of rat endotoxemia (78). Furthermore, in another rat model, the FXa inhibitor DX-9065a showed a protective effect on the microcirculation of endotoxemic rats by attenuating leukocyte-endothelial interaction, with suppression of both excessive coagulation and cytokine production appearing to play an important role (79).

In contrast to all heparin products which act indirectly via antithrombin to inhibit both thrombin and FXa, the direct thrombin inhibitors (DTI) bind to thrombin specifically and inhibit its catalytic activity without involvement of AT. Drugs in this class (all administered parenterally) include lepirudin (a recombinant hirudin), desirudin (a recombinant desulfato hirudin), ximelagatran (AstrZeneca®) specifically and inhibit its catalytic activity without FXa, the direct thrombin inhibitors (DTI) bind to thrombin indirectly via antithrombin to inhibit both thrombin and inhibition of FXa by DX-9065a reduces TF expression in the liver of rat endotoxemia (78). Furthermore, in another rat model, the FXa inhibitor DX-9065a showed a protective effect on the microcirculation of endotoxemic rats by attenuating leukocyte-endothelial interaction, with suppression of both excessive coagulation and cytokine production appearing to play an important role (79).

Smaller DTIs, such as melagatran, offer the advantage of inhibition of both free circulating and clot bound thrombin (84,85). DTIs probably provide more effective inhibition of thrombus progression than unfractionated and low molecular weight heparins that inhibit free thrombin only (86,87). In addition, DTIs have few plasma protein and platelet interactions, do not bind to PF4 on platelets, so their activity is preserved in the vicinity of platelet-rich thrombi and they consequently do not cause heparin-induced thrombocytopenia (88).

With respect to CVD, ximelagatran has proven efficacy in the prevention of stroke among patients with non valvular atrial fibrillation, however concerns over its hepatic side effects, have curtailed its widespread approval (89). Other DTIs such as Bivalirudin have proved promising as an adjunct in percutaneous coronary intervention (90,91).

DTIs are able to neutralize thrombin, by occupying its catalytic binding sites, its fibrinogen binding sites or both (92). However, whilst the anticoagulation properties of DTI have been thoroughly investigated, the anti-inflammatory potential of these drugs has not yet been explored. Whilst fibrinogen is an acute phase protein it can itself act as an inflammatory trigger by stimulating the expression of proinflammatory cytokines (such as TNF-α and IL-1β) on mononuclear cells and induce the production of chemokines (IL-8 and MCP-1) by endothelial cells (93,94). Increased blood levels of circulating fibrinogen are also associated with long-term risk of thrombosis, as well as an increased risk of cardiovascular disease (95). Thus, as DTIs inhibit the conversion of fibrinogen into fibrin, they have the capacity to influence inflammation, by augmenting neutrophil activity (96). Furthermore, by inhibiting the availability of thrombin, DTI have the capacity to interrupt its well known proinflammatory actions (97).

The protein C anticoagulant pathway serves as a major system for controlling thrombosis, limiting inflammatory responses, and potentially decreasing endothelial cell apoptosis in response to inflammatory cytokines and ischemia (98).

Indeed, several components of the protein C anticoagulant pathway can reduce the inflammatory response. Under physiological conditions, for example, protein C is activated by thrombin bound to endothelial cell-membrane thrombomodulin (99). Thrombomodulin is itself a membrane protein with several domains, and the binding of thrombin to thrombomodulin results in a significant increase in the activation of protein C and prevents the binding of thrombin to thrombomodulin in results in a significant increase in the activation of protein C and prevents the binding of thrombin to thrombomodulin in other cellular PARs on platelets and inflammatory cells (100,101). Binding of protein C to the endothelial protein C receptor also results in a further 5-fold augmentation of the activation of protein C by the thrombomodulin-thrombin complex (102).

Activated protein C (APC) regulates coagulation activation by proteolytic cleavage of the essential cofactors FVIIa and FVa. Furthermore, APC has a number of anti-inflammatory effects and has been shown to inhibit endotoxin-produced production of TNF-α, IL-1β, IL-6 and IL-8 by cultured monocytes and macrophages (103). Given the important dual anticoagulant and anti-inflammatory role of APC, it would seem highly plausible that it could have potential therapeutic benefits, particularly in disease states that involve the activation of both systems. Current research in this field with respect to cardiovascular disease is still in its infancy and is a highly evolving area. Current research utilizing APC, in the setting of cardiovascular disease has mainly centred on ischemia-reperfusion injury and ischaemic stroke; however, we expect there to be a significant expansion in therapeutic options of APC in the setting of CVD in the future (104,105).

There are four identified PARs - PARs1, 3 and 4 bind thrombin whilst PAR-2 binds to several trypsin-like serine proteases, including the TF-FVIIa complex, and are thus important mediators of both inflammation and thrombosis (106,107) PAR1 is the primary thrombin receptor in human and animal cells, and whilst there has been a great deal of interest in the development of specific antagonists of the protease-activated receptors, achieving such goals remains extremely challenging (108).
Blood clotting, inflammation, and thrombosis in cardiovascular events

Considerable efforts have been directed at developing specific antagonists of the first elucidated member of this receptor family, namely the thrombin receptor, PAR-1 109).

In rat models, RWJ-58259 is a potent and selective inhibitor of PAR-1, and has been shown to inhibit thrombin-induced intracellular calcium signalling, vascular smooth muscle cell proliferation and arterial injury-induced stenosis post balloon angioplasty (110). However, significantly less effort has been directed at the second member of the family, PAR-2, due in part to lack of clarity concerning its activating protease(s), and uncertainty concerning its physiological and pathophysiological roles in disease pathways (111).

PAR1 inhibitors are able to selectively inhibit most of the cellular effects of thrombin. PAR1 antagonists have the relative advantage over DTIs of not inhibiting the enzymatic action of thrombin in the coagulation cascade, and hence they have minimal bleeding side-effects (112,113).

In conclusion, there have been extraordinary advances in our understanding of the complex pathophysiology of coagulation, thrombosis and inflammation. These processes do not happen in isolation, and there is a clear interaction between all 3 processes, in which thrombosis and coagulation can act as triggers for inflammation and vice versa. Atherosclerosis is a prime example of these systems working in tandem, and thus represents a unique opportunity for further research into the concepts of blood coagulation, thrombosis and inflammation. Furthermore, this increased understanding has created exciting opportunities for the development of novel drugs that can interrupt these synergistic pathways with consequent genuine therapeutic potential.

3. REFERENCES

Blood clotting, inflammation, and thrombosis in cardiovascular events


Blood clotting, inflammation, and thrombosis in cardiovascular events


Blood clotting, inflammation, and thrombosis in cardiovascular events


100. Ye J, N.L. Esmon, C.T. Esmon and A.E. Johnson: The active site of thrombin is altered upon binding to thrombomodulin. Two distinct structural changes are detected by fluorescence, but only one correlates with protein C activation. *J Biol Chem* 266, 23016-21 (1991)


**Key Words:** Blood clotting, inflammation, thrombosis, coagulation, Review

**Send correspondence to:** Professor G.Y.H. Lip, Haemostasis, Thrombosis and Vascular Biology Unit, University Department of Medicine, City Hospital, Birmingham B18 7QH, UK, Tel: 0121 507 5080, Fax: 0121 554 4083, E-mail: g.y.h.lip@bham.ac.uk

http://www.bioscience.org/current/vol11.htm