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Review

Atherothrombosis: A widespread disease with unpredictable and life-threatening consequences☆

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Atherothrombosis, characterised by atherosclerotic lesion disruption with superimposed thrombus formation, is the major cause of acute coronary syndromes (ACS) and cardiovascular death. It is the leading cause of mortality in the industrialised world. Atherosclerosis is a diffuse process that starts early in childhood and progresses asymptomatically through adult life. Later in life, it is clinically manifested as coronary artery disease, stroke, transient ischaemic attack, and peripheral arterial disease. From the clinical point of view, we should envision this disease as a single pathologic entity that affects different vascular territories. Available antithrombotic therapy is very safe and efficient but the morbidity and mortality due to atherothrombosis is still unacceptably high. Recent evidence suggests that inhibition of tissue factor or elements in the tissue factor pathway (i.e., factors VIIa and Xa, or thrombin) has the potential to further improve outcomes in atherothrombosis. Here, we will review the most important concepts and advances in the pathogenesis, prevention, and antithrombotic treatment of this widespread disease.

Introduction

Atherothrombosis, defined as atherosclerotic plaque disruption with superimposed thrombosis, is the leading cause of mortality in the Western world. Atherosclerosis is a diffuse process that starts early in childhood and progresses asymptomatically through adult life. Later in life, it is clinically manifested as coronary artery disease (CAD), stroke, transient ischaemic attack (TIA), and peripheral arterial disease (PAD). From the clinical point of view, we should envision this disease as a single pathologic entity that affects different vascular territories. A suggestive analogy is that TIA and intermittent claudication are the unstable angina of the brain and lower limbs, respectively; and stroke and gangrene are the myocardial infarction (Fig. 1).[1]

Endothelial dysfunction is a systemic, reversible disorder considered the earliest pathologic process of atherothrombosis.[2,3] It is involved in the recruitment of inflammatory cells into the vessel wall and in the initiation of atherosclerosis (Fig. 2). Endothelial cells produce cytokines, express adhesion molecules such as ICAM-1, VCAM, and selectins, and assist leukocytes and other blood-derived cells in "homing" and atheroma infiltration. Secondary changes may occur in the underlying media and adventitia, particularly in advanced disease stages. Fatty streaks have been found to be present already in the intima of infants.[4] Lesions progress to fibroatheroma by developing a cap of smooth muscle cells and collagen. Atherosclerotic lesions can progress without compromising the lumen because of compensatory vascular enlargement (positive remodelling).[5] Importantly, lipid-rich lesions leading to acute coronary

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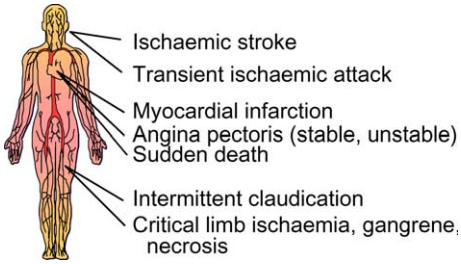

Fig. 1 Major clinical manifestations of atherothrombotic disease.

syndromes (ACS) are often mildly stenotic, due to significant positive remodelling, and therefore are not detectable by angiography. Plaque disruption and subsequent thrombus formation is responsible for the onset of most ACSs and strokes. The magnitude of the thrombotic process triggered upon plaque disruption is modulated by different elements that determine plaque and blood thrombogenicity: local shear rate, tissue factor (TF), apoptotic microparticles, circulating monocytes, and others. The atherosclerotic and thrombotic processes appear to be interdependent and could therefore be integrated under the term "atherothrombosis", a broader term that includes both atherosclerosis and its thrombotic complications (Fig. 1).[1,6]

High-risk atherothrombotic plaque

In spite of a common pathophysiologic pathway, atherosclerotic lesions are very heterogeneous and the "high-risk plaque" of each vascular bed has unique characteristics. Insights into the disease have advanced beyond the notion of progressive occlusion of the coronary artery into the recognition that plaque disruption and superimposed thrombus formation are the leading causes of acute coronary syndromes and cardiovascular death. Consequently, plaque composition (as a determinant of risk of disruption), rather than luminal stenosis, has become the major determinant of this disease.[7]

Histologically, these rupture-prone (also called vulnerable or high-risk) lesions consist of a large core of extracellular lipid, a dense accumulation of macrophages, reduced numbers of vascular smooth muscle cells, and a thin fibrous cap. Hence, is not surprising that these plaques are less stable and have a greater propensity to rupture than the fibrous, collagen-rich plaques. Plaque disruption usually occurs at the weakest point ("shoulder"), where the cap is often thinnest and most heavily infiltrated with inflammatory cells.[8] Once the plaque is disrupted, the highly thrombogenic, lipid-rich core, with abundant tissue factor, is exposed to the bloodstream, triggering the formation of a superimposed thrombus that leads to vessel occlusion and subsequent ischaemic symptoms distal to it.[9]

In contrast with most high-risk coronary plaques, high-risk carotid plaques are considerably more stenotic. They are not lipid-rich but rather heterogeneous and very fibrous. Plaque disruption is often caused by an intramural haematoma or dissection that probably is related to the systolic stroke of blood against the resistance they offer.[10] Although lipid accumulation in the carotid arteries is quite diffuse, a recent study reported the presence of ruptured lipid-rich plaques in patients with TIA and stroke.[11] In addition, the so-called "cryptogenic strokes" also have an atherothrombotic origin. The source of emboli is usually a carotid or aortic thrombus.[12]

Similarly, high-risk plaques of the lower extremities appear to be very stenotic and fibrotic.[13] Available evidence suggests that in PAD, plaque stenosis associated with hyperthrombogenicity of the blood seem to be major contributors to acute ischaemic syndromes (sudden ischaemic pain, gangrene). This is suggested by the

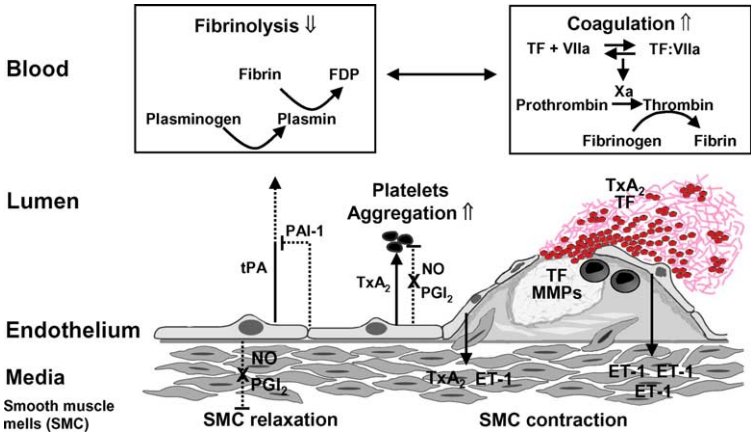

Fig. 2 Normal and abnormal endothelial function. Anti-atherogenic and antithrombotic properties of the endothelium. The endothelium affects vascular homeostasis by regulating vascular tone, thrombogenicity, platelet function, proliferation and migration of smooth muscle cells (SMC), and vasomotion. Normally functioning endothelium (left) produces several substances that maintain normal shear conditions by balancing the production of vasodilators (nitric oxide [NO]) and vasoconstrictors (endothelin 1 [ET-1]), impeding excessive platelet aggregation (NO and prostacyclin [PGI2]), and balancing the coagulation system by controlling fibrin production (TF pathway inhibitor/TF) and fibrinolysis (tissue plasminogen activator [tPA]/plasminogen activator inhibitor-1 [PAI-1]). Dysfunctional endothelium (right) favours macrophage adhesion and migration (monocyte chemotactic protein-1 (MCP-1)), and plaque growth, and induces vasoconstriction → = induces; - - - - - = inhibits; FDP = fibrin-degrading product; MMP = matrix metalloproteinase; TXA_2 = thromboxane A_2; VEGF = vascular endothelial growth factor.

high prevalence of known causes of a hyperthrombotic state of the blood, such as diabetes, cigarette smoking, and dyslipidaemia,[14] in PAD patients.[15]

Conversely, high-risk plaques in the thoracic aorta frequently contain a high proportion of extracellular lipids and are characterised by a shift toward greater macrophage content relative to smooth muscle cells in the cap. At autopsy, aortic plaques from persons who died of ischaemic heart disease often have ulceration and mural thrombosis.[16] Recent aortic plaque characterisation by magnetic resonance imaging (MRI) has confirmed their lipid-rich composition.[17]

Over the last decades, pathologic studies have shown an increased number of vasa vasorum in advanced atherosclerotic lesions. A correlation between the extent of vasa vasorum neovascularisation and severity of atherosclerotic disease has been demonstrated in human coronary arteries. Therefore, this observation indicates that the vasa vasorum might play a role in atherogenesis as a regulator of plaque progression and instability. Nevertheless, whether neovascularisation precedes or follows plaque development is still not known. Interestingly, a recent experimental study demonstrated that the angiogenesis inhibitor angiostatin reduces macrophage accumulation and progression of advanced atherosclerosis.

High-risk blood

Two thirds of ACSs are caused by the disruption of a high-risk atherothrombotic plaque with superimposed thrombus formation. In one third of ACSs, particularly in sudden coronary death, there is no rupture of a high-risk atherothrombotic plaque but only a superficial erosion of a markedly stenotic and fibrotic lesion.[18]

Thrombus formation in such cases may depend on a hyperthrombogenic state triggered by systemic factors. Indeed, several cardiovascular risk factors, including elevated LDL cholesterol, cigarette smoking, and hyperglycaemia, have been associated with increased blood thrombogenicity.[19]

Circulating tissue factor has been associated with increased blood thrombogenicity in patients with unstable angina[20] and chronic coronary artery disease. Blood levels of tissue factor have also been shown to predict outcome in patients with unstable angina.[21]

Several lines of evidence support the hypothesis that circulating apoptotic cells and cellular microparticles may play a significant role in blood thrombogenicity. Patients with ACS have elevated levels of circulating tissue factor,[21] and there is evidence that acute thrombosis may be initiated by membrane-bound circulating tissue factor originating from activated or injured cells.[22] It is believed that a major source of blood-borne tissue factor could be the circulating microparticles, which are endowed with potent procoagulant potential, attributable to the presence of phosphatidylserine on their surface.[23] A significant increase in the number of circulating endothelial cells, some of them apoptotic, has also been reported in patients with ACS.[24] The circulating procoagulant microparticles may also contribute to the blood thrombogenicity of patients with hyperlipidaemia or high blood glucose concentrations; these vascular risk factors are known to be responsible for increased apoptotic activity in vitro.[25]

Elevated LDL cholesterol levels have been found to increase blood thrombogenicity and the growth of thrombi under defined rheology conditions.[26] Reducing LDL cholesterol levels with statins has been shown to decrease thrombus growth by approximately 20%.[27] The extent to which this antithrombotic effect contributes to the reduction of total vascular events, including death, coronary events, and stroke, is a matter of debate.[28]

Diabetic patients, especially those with poorly controlled diabetes, have increased blood thrombogenicity.[29] Platelets from patients with diabetes have been shown to have increased reactivity and hyperaggregability and expose a variety of activation-dependent adhesion proteins.[30] Abnormal platelet function is reflected by increased platelet consumption and increased accumulation of platelets on the altered vessel wall. The increased procoagulant activity in diabetes is also attributed to leukocytes, which may, in part, activate the tissue factor pathway[31] and contribute to the high blood thrombogenicity.[30]

Fibrinogen concentration was found to be associated with increased blood thrombogenicity.[32] Several of the classic risk factors have been shown to modulate fibrinogen levels. Fibrinogen levels tend to be higher in patients with diabetes, hypertension, obesity, smoking habit, and sedentary lifestyles.[33,34] However, further clinical trials are needed before it can be determined whether fibrinogen is directly involved in the pathogenesis of atherothrombosis or is merely a marker of the degree of vascular damage.

As previously described, lipid-rich atherosclerotic plaques contain tissue factor associated with macrophages within the lesion,[35] which may account, in large part, for the high thrombogenicity of these lesions. In addition, specific inhibition of the tissue factor pathway by its physiologic inhibitor, tissue factor pathway inhibitor (TFPI), significantly reduces plaque thrombogenicity.[36]

Early detection with noninvasive imaging technology

As discussed above, culprit lesions are often mildly stenotic owing to significant positive remodelling and, therefore, not detectable by angiography. Given the importance of plaque composition rather than degree of stenosis, over the last decade there has been a substantial improvement in different noninvasive imaging modalities that allow full characterisation of atherothrombotic plaques.[6] Use of these imaging techniques to detect subclinical pathology and as a surrogate marker may supplement or improve cardiovascular risk assessment, especially in patients with intermediate cardiovascular risk.

Ultrasound

Measurements of carotid and aortic wall thickness, as well as qualitative and quantitative analysis of atherothrombotic plaques, can be made by ultrasound. Hypoechoic heterogeneous plaque is associated with both intraplaque haemorrhage and lipids, whereas hyperechoic homogeneous plaque is mostly fibrous.[37]

The North American Symptomatic Carotid Endarterectomy Trial and the Asymptomatic Carotid Artery Stenosis Study have shown that the degree of stenosis plays a significant role in producing stroke.[38] Real-time B-mode ultrasound with Doppler flow imaging has emerged as the modality of choice for examining the carotid arteries. Real-time B-mode ultrasound can be used to measure the intima-media thickness of large- and medium-size arteries, such as the carotid, femoral, or radial. Several studies have found that carotid and aortic atherosclerosis are markers for coronary atherosclerosis.[39,40] Patients with symptomatic CAD have increased intima-media thickness compared with asymptomatic controls.[41] Carotid wall thickening was also found in patients with silent ischaemia.[42] The relationship between intima-media thickness and the severity of CAD is rather constant. In addition, large prospective studies have demonstrated that intima-media thickness is a useful marker of CAD progression. For example, the Cardiovascular Health Study[43] found associations between carotid intima-media thickness and the incidence of new myocardial infarction or stroke in patients 65 years of age. Prevention trials of lipid-lowering treatments using intima-media thickness as a surrogate endpoint have shown that retardation in the progress of intima-media thickness correlates with a reduction of clinical endpoints.[44] Recently intima-media thickness was compared with C-reactive protein, a well known inflammation marker, and was found to be an independent and accurate predictor of ischaemic stroke.[45]

Magnetic resonance imaging

High-resolution MRI has emerged as the potential leading noninvasive in vivo imaging modality for atherosclerotic plaque characterisation.

Recently, Cai et al.[46] published a classification of carotid atherothrombotic lesion with in vivo, multicontrast MRI. The authors found a strong correlation between the classification of the American Heart Association and the one obtained with MRI. More recently, Yuan et al.[11] reported on the presence of a ruptured fibrous cap (identified with MRI) in patients who had experienced a stroke or TIA within 90 days. In addition, the use of gadolinium provides additional information, since it allows the identification of neovascularisation in atherothrombotic plaques and may distinguish a fibrous cap from a necrotic core.[47]

Experimental studies in a pig model showed that the difficulties of coronary wall imaging are due to a combination of cardiac and respiratory motion artefacts, nonlinear course, small size, and location of the coronary arteries.[48] Our group extended the black-blood MRI methods used in the human carotid artery and aorta to imaging the coronary arterial lumen and wall.[49] High-resolution black-blood MRI of both normal and atherosclerotic human coronary arteries was performed. The difference in maximum wall thickness between the normal subjects and patients ($\geqslant$40% stenosis) was statistically significant.[49] This coronary plaque MRI study[49] was performed during breath-holding to minimise respiratory motion. We have shown recently that MRI can be used to measure the effect of lipid-lowering therapy (statins) in asymptomatic, untreated, hypercholesterolaemic patients with carotid and aortic atherosclerosis.[50] (see Fig. 3) Atherosclerotic plaques were assessed with MRI at different time points after initiation of lipid-lowering therapy. Significant regression of atherosclerotic lesions was observed. Despite the early and expected hypolipi-

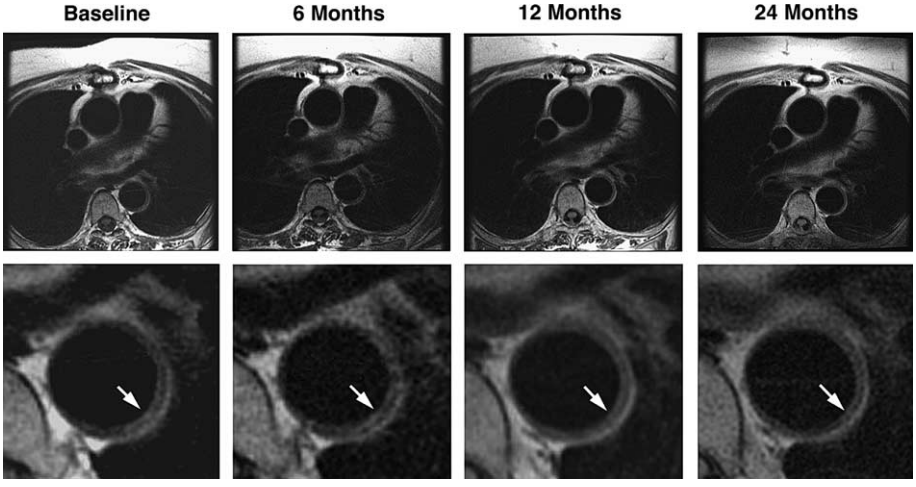

Fig. 3 Effect of lipid-lowering by simvastatin on human atherosclerotic aortic lesions. T2-weighted magnetic resonance images of the same patient scanned at different time points (baseline and 6, 12, and 24 months after simvastatin) and detail of the descending aorta (bottom). A significant reduction in vessel wall area and plaque thickness without changes in the lumen was demonstrated after 12 months of treatment. The arrow indicates the maximal plaque thickness.

daemic effect of the statins, a minimum of 12 months was needed to observe changes in the vessel wall. No changes were detected at 6 months. In agreement with previous experimental studies, there was a decrease in the vessel wall area and no change in the lumen area at 12 months.[50]

Ankle-brachial index

The ankle-brachial index is a very simple noninvasive method for assessing the patency of the lower-limb arterial system and screening for the presence of PAD. Measurement of the ankle-brachial index is a simple procedure and requires only an ordinary blood pressure cuff and a Doppler ultrasound sensor. The ankle-brachial index is calculated from blood pressure measurements in the brachial artery in both arms and the left and right posterior tibial arteries and dorsalis pedis arteries. Low ankle-brachial index values (<0.90) are considered evidence of PAD, and progressively lower ankle-brachial index values indicate more severe obstruction. Low ankle-brachial index values are also considered to be indicative of generalised atherosclerosis.[51]

Biomarkers of atherothrombosis

In recent years, a number of biomarkers have been proposed as significant predictors of atherosclerosis and its thrombotic complications (Table 1). Among them, C-reactive protein is one of the most studied. C-reactive protein is an acute-phase reactant that increases in inflammatory states. A growing body of evidence suggests that even small increases in C-reactive protein are predictive of future vascular events in apparently healthy, asymptomatic individuals.[52] Recently, Danesh et al.[53] reported a meta-analysis of 14 prospective studies on C-reactive protein and the risk of nonfatal myocardial infarction or death from coronary heart disease. The analysis comprised 2557 cases and a mean follow-up of 8 years. The adjusted risk ratio was 1.9 (95% confidence interval, 1.5—2.3) for the development of CAD amid the patients in the top tertile of baseline C-reactive protein concentrations compared with those in the bottom tertile. In addition, several studies demonstrated that C-reactive protein predicts recurrent events or increased mortality in patients with stroke, ACS, stable angina, and PAD.[52,54–56]

Among the prothrombotic markers, fibrinogen is one of the most studied.[57] It is a circulating glycoprotein that acts at the final step of the coagulation cascade. Aside from its role in thrombosis, a number of other functions have been postulated, including regulation of cell adhesion and migration, vasoconstriction, stimulation of platelet aggregation, and determination of blood viscosity.[58,59] Epidemiologic evidence supports an independent association between elevated fibrinogen and cardiovascular morbidity and mortality. Two recent meta-analyses involving 18 and 22 prospective studies demonstrated strong, statistically significant, risk ratios for the individuals in the upper tertile of baseline fibrinogen levels compared with those in the bottom tertile (risk ratio, 1.8, 95% CI 1.6—2.0, and odds ratio, 1.99, 95% CI 1.85—2.13, respectively).[33,53] Additionally, other studies demonstrated an independent association between fibrinogen and stroke and PAD.[34,60,61]

Table 1 Biomarkers in atherothrombosis

Inflammation markers	Thrombosis markers
C-reactive protein	Fibrinogen
Interleukins	von Willebrand factor
CD40 ligand	Plasminogen activator inhibitor 1
Serum amyloid A	Fibrinopeptide A
Vascular and cellular adhesion molecules	Prothrombin fragment 1 + 2

Antithrombotic approaches

Treatment of atherothrombotic patients must include the management of cardiovascular risk factors and antiplatelet treatment for the prevention of thrombotic complications. Secondary prevention of an ischaemic event in the index territory will provide primary prevention for other arterial beds that are still clinically silent. The aims of antiplatelet therapy are, firstly, to prevent the occurrence of acute ischaemic events through inhibition of platelet thrombus formation and, secondly, to protect distal tissues by inhibiting microembolisation. Due to the systemic nature of the disease, antiplatelet therapy (which has shown consistent benefit across all arterial beds) is essential for optimal prevention of ischaemic events in atherothrombotic patients.[62]

The significance of thrombosis in atherothrombotic disease is evidenced by the fact that antithrombotic therapy has reduced the relative risk of cardiovascular events by up to 25%.[63] Several landmark trials have established the efficacy of aspirin in atherothrombosis (Table 2). Remarkably, the ISIS-2 study found that the effect of aspirin in acute myocardial infarction (MI) was comparable to the effect of a fibrinolytic agent (streptokinase).[64] A recent meta-analysis by the Antithrombotic Trialists Collaboration suggests that the use of aspirin should be expanded to populations such as those with diabetes, peripheral arterial disease, carotid disease, and end-stage renal disease. They also concluded that there is no additional benefit by using chronic aspirin doses higher than 75 mg.[62] The Thrombosis Prevention Trial (TPT)[65] demonstrated a 20% relative reduction in the combined endpoint of coronary death and nonfatal MI with a dose of aspirin of 75 mg.

In the antiplatelet armamentarium, clopidogrel represents a critical advance and several clinical trials have been carried out with this drug (Table 3). A daily 75 mg dose of clopidogrel was compared with a daily 325 mg dose of aspirin in patients with cardiovascular disease in the CAPRIE[66] (Clopidogrel versus Aspirin in Patients at Risk of Ischaemic Events) trial. After an average of 1.9 years follow-up, the data demonstrated a statistically significant 8.7% relative risk reduction in the composite

Table 2 Landmark acetylsalicylic acid trials

Trial	Patients	Treatment
ISIS-2	17,187 men with suspected MI	ASA 160 mg daily vs. placebo
US Physicians Health Study	22,701 healthy physicians	ASA 325 mg every other day
Thrombosis Prevention Trial (TPT)	5085 high-risk men from UK	ASA 75 mg daily vs. placebo
Hypertension Optimal Treatment (HOT)	18,790 hypertensive men and women	ASA 75 mg daily vs. placebo

Table 3 Landmark clopidogrel trials

Trial	Patients (*n*)	Treatment
CURE	17,187	ASA 160 mg daily vs. placebo
CAPRIE	22,701	ASA 325 mg every other day vs. placebo
CREDO	5085	ASA 75 mg daily vs. placebo
PCI-CURE	18,790	ASA 75 mg daily vs. placebo

endpoint of MI, ischaemic stroke, and vascular death. This is noteworthy when one takes into account that aspirin, which itself has a marked effect compared with placebo, was used as an active control.

Clopidogrel for the Reduction of Events During Observation (CREDO) was a multicenter, double-blind study of patients with stable and unstable angina who were undergoing percutaneous coronary intervention. The trial demonstrated the safety and efficacy of clopidogrel treatment before the procedure, and the beneficial effect of prolonged (1 year) versus short-term (1 month) antiplatelet therapy.[67]

The combination of aspirin and clopidogrel has a synergistic effect in preventing thrombus formation. The CURE[68] (Clopidogrel in Unstable angina to prevent Recurrent Events) trial tested the efficacy of this combination compared with aspirin alone. The results showed a 20% relative risk reduction of the composite endpoint of nonfatal MI, stroke, and cardiovascular death in the combination group. Patients assigned to the dual antiplatelet treatment had higher rates of major bleeding, but no increase in life-threatening bleeding. A subgroup analysis of patients who underwent percutaneous coronary intervention (PCI) during the CURE trial, PCI-CURE,[69] demonstrated that pretreatment (mean = 10 days) with clopidogrel and aspirin before percutaneous coronary intervention, as well as long-term treatment (mean = 8 months), was useful in reducing ischaemic events. Many studies with clopidogrel are still ongoing (Table 4).

There have been 5 large randomised trials of oral glycoprotein (GP) IIb/IIIa antagonists in patients with CAD: OPUS-TIMI 16,[70] EXCITE,[71] SYMPHONY,[72] SYMPHONY II,[73] and BRAVO.[74] Although these agents once held great potential, all the clinical trials consistently demonstrated an increased mortality with GP IIb/IIIa agent compared to placebo. A recent meta-analysis of 4 trials showed a significant 37% increase in mortality with the use of a GP IIb/IIIa antagonist.[75]

Future pharmacologic targets

Despite the important beneficial effect of antiplatelet therapy in atherothrombotic disease, the mortality of this pathology is still unacceptably high. In order to address this problem, novel antithrombotic agents have been developed for inhibiting the tissue factor metabolic pathway. Specifically, interest is focused on thrombin inhibitors[76] and factor Xa inhibitors.[77] A summary of the antithrombotic drugs under development is presented in Table 5.

Thrombin inhibitors not only prevent thrombin prohaemostatic reactions, such as thrombin-induced platelet aggregation and secretion, but also the synthesis of some mediators (tissue factor, thrombomodulin, endothelin) from the vascular endothelium. Moreover, they interfere with some of the "pleiotropic" effects of thrombin, including fibroblast proliferation and mitogenesis of smooth muscle cells. A recent experimental study assessed the antithrombotic effect of ximelagatran in comparison with hirudin and enoxaparin in healthy male volunteers. The effect of ximelagatran on thrombin generation and platelet activation was comparable with the one observed in the active controls.[78]

In the SPORTIF II[79] trial, a dose-guiding study, ximelagatran at 20, 40, and 60 mg twice daily was compared with warfarin in patients with nonvalvular atrial fibrillation. One ischaemic stroke and one TIA occurred in the ximelagatran arm versus two TIAs in the warfarin arm. No

Table 4 Ongoing clopidogrel trials

Study	Patients	Maximum follow-up	Patients (*n*)	Results expected
ACTIVE	Atrial fibrillation	48 months	~14,000	2007
CAMPER	PAD (post-angioplasty)	30 months	~2000	2006
CHARISMA	Cardiovascular or cerebrovascular disease, PAD, or major risk factors	42 months	~15,200	2005
COMMIT (CCS-2)	Acute MI	4 weeks	~45,000	2005
CLARITY	Acute MI	4 weeks	3000	2004
MATCH	TIA or ischaemic stroke	18 months	7601	2004

Table 5 Antithrombotic drugs under development or recently approved for clinical use

Therapeutic agent	Mechanism of action	Model	References
Tissue-factor inhibitors			
Tissue-factor-pathway inhibitor**	Direct binding to factor Xa	Animal	Oltrona et al.[86]
	Inhibition of TF:VIIa complex	In vitro	Badimon et al.[36]
Gene transfer of TFPI	Local expression of TFPI	Animal	Atsuchi et al.[87]
			Golino et al.[88]
Monoclonal antibodies	Inhibition of TF activity	Animal	Ragni et al.[89]
Active site-inactivated factor VIIa	Competitive inhibition of TF-dependent	Animal	Banner et al.[90]
	activation of factor Xa	In vitro	Jang et al.[91]
Nematode anticoagulant protein c2	Inhibition of TF:VIIa complex	Human	Lee et al.[92]
		Human	Moons et al.[93]
Factor Xa inhibitors			
DX9065	Direct inhibition of factor Xa	Human	Shimbo et al.[83]
		Human	Dyke et al.[77]
Fondaparinux	Indirect inhibition of factor Xa	Human	Turpie et al.[94]
		Human	Buller et al.[95]
		Human	Bauer et al.[96]
		Human	PENTUA[84]
		Human	PENTALYSE[85]
Idraparinux (SanOrg34006)	Indirect inhibition of factor Xa	Human	PERSIST investigators[97]
		Human	Reiter et al.[98]
Thrombin inhibitors			
Hirudin	Direct thrombin inhibitor	Human	OASIS 1 & 2[99,100]
Bivalvidurin	Direct thrombin inhibitor	Human	CACHET[101]
Argatroban	Active-site thrombin inhibitor	Human	ARGAMI[102]
Melagatran	Active-site thrombin inhibitor	Human	METHRO I, II, III[103,104]
Ximelagatran (oral)	Active-site thrombin inhibitor	Human	METHRO I, II, III[103,104]
		Human	SPORTIF I, II, III, V[79–81]
Low-molecular-weight heparins			
Enoxaparin		Human	ESSENCE[105]
Dalteparin		Human	FRISC I, II[106]
Nadroparin		Human	FRAXIS[107]
Bemiparin		Human	Navarro-Quilis et al.[108]
Antiplatelets			
AR-C69931MX	P2Y12 antagonist	Human	Storey et al.[109]
YD-3	Protease-activated receptor 4 antagonist	In vitro	Wu et al.[110]
AJvW-2	Anti-vW factor monoclonal antibody	Animal	Kageyama et al.[111]

major bleeding occurred in the ximelagatran group compared to one case in the warfarin group. The authors concluded that ximelagatran up to 60 mg twice daily was well tolerated without need for coagulation monitoring. SPORTIF III and V trials are designed to compare ximelagatran versus warfarin in high-risk atrial fibrillation patients (similar to SPORTIF II) with nonvalvular atrial fibrillation. In the SPORTIF III trial, patients with nonvalvular atrial fibrillation were randomised (open-label) to ximelagatran 36 mg twice daily or warfarin (INR 2—3). In the ximelagatran group there was 41% relative risk reduction ($p < 0.018$) in stroke or systemic embolism. In summary, ximelagatran was as effective as warfarin and caused less bleeding.[80] The major disadvantage is the elevation of liver enzymes in a small proportion of patients. The ongoing SPORTIF V (double blind) trial will provide additional information on the efficacy and safety of ximelagatran in the same patient population.[81]

Recently, the ESTEEM randomised, controlled trial assessed the efficacy of oral ximelagatran for secondary prophylaxis after myocardial infarction. Oral ximelagatran significantly reduced the risk for the primary endpoint compared with placebo from 16.3% (102 of 638) to 12.7% (154 of 1245) (hazard ratio. 76, 95% CI 59—0.98, $p = 0.036$) for the combined ximelagatran groups versus placebo. There was no indication of a dose response in the ximelagatran groups. Major bleeding events were rare, 1.8% (23 of 1245) and 0.9% (6 of 638) (hazard ratio 1.97, 95% CI 80—4.84) in the combined ximelagatran and placebo groups, respectively. The authors concluded that oral direct thrombin inhibition with ximelagatran and aspirin is more effective than aspirin alone in preventing major cardiovascular events during 6 months of treatment in patients who have had a recent myocardial infarction.[82]

Several factor Xa inhibitors have been made available (Table 5). Fondaparinux and DX-9065a are the two most studied. Shimbo et al.[83] investigated the antithrombotic effect of DX-9065a in an *ex vivo* model of arterial thrombosis and compared its effect with enoxaparin. The

authors concluded that the antithrombotic effect was better than enoxaparin and without the significant prolongation of the standard coagulation parameters observed with enoxaparin. The recently published XANADU[77] trial describes the first human study on the safety of DX-9065a in patients undergoing percutaneous coronary intervention. In both studies the drug was infused parenterally. We strongly believe that future research should be focused on developing an orally active factor Xa inhibitor.

The Double-Blind Dose-Ranging Study of Fondaparinux (Pentasaccharide) in Unstable Angina (PENTUA) trial examined different doses of Fondinaparux versus enoxaparin in patients with acute coronary syndromes. A total of 1147 patients were enrolled and 1134 of them were randomised to either enoxaparin or one of four different doses of fondaparinux. Along with the treatment drug, all patients were also on acetylsalicylic acid (ASA) and 95% on beta-blockers. Patients were treated for 3–8 days, or until coronary revascularisation. The primary endpoints were death, myocardial infarction, or recurrent ischaemia at 9 and at 30 days. At the end, there was no significant difference between the enoxaparin and fondinaparux groups. The primary endpoint was reached in 40.2% of the enoxaparin group versus 37% of the combined fondaparinux groups. It was interesting that the low dose (2.5 mg) of fondaparinux showed the best results, with only a 30% endpoint outcome at 9 days and 33.8% at 30 days.[84]

In the PENTALYSE trial, fondaparinux was studied as an adjunct to thrombolysis in patients with acute myocardial infarction. Three hundred thirty-three patients with acute ST-elevation MI were enrolled. Patients were all treated with ASA and alteplase and they were randomised to either continuous unfractionated heparin for 48–72 h or to low, medium, or high doses of fondaparinux daily for 5–7 days. Both heparin and fondaparinux were infused before alteplase. Coronary angiography was done at 90 min and repeated 5–7 days after alteplase infusion. Patients who needed intervention at 90 min were excluded $(n = 115)$ from the study. At 90 min there was no significant difference of TIMI (Thrombolysis in Myocardial Infarction) grade 2 or 3 flow in any group. At the second angiogram on day 5–7, the fondinaparux groups had a reocclusion rate of 0.9% versus 7.0% for unfractionated heparin. At the end of the 30-day follow-up, revascularisation was done in 51% of the heparin group while it was only needed in 39% of the patients in the fondinaparux groups. A higher number of revascularisations were carried out in the heparin group on days 2 to 5, when fondinaparux was still being administered. At 30 days there was no difference in mortality in any of the groups. There was a lower incidence of transfusion in the fondaparinux groups (3.3% vs. 7.1%).[85]

Conclusions

The major conclusions of this paper are:

1. Atherothrombosis is a diffuse disease affecting different arterial beds but with a similar aetiopathogenesis. Regardless of the vascular territory affected, the therapeutic goals must be the same: (A) to aggressively treat cardiovascular risk factors and (B) to reduce thrombogenic potential.
2. High-risk or vulnerable blood is as important as the vulnerable plaque in determining the offset of at least one third of acute coronary syndromes.
3. The use of noninvasive imaging technology makes it possible to detect atherothrombotic disease in a preclinical stage and, therefore, to implement adequate preventive interventions.
4. Available antithrombotic therapy is very safe and efficient but the morbidity and mortality due to atherothrombosis is still unacceptably high.
5. Recent evidence suggests that inhibition of tissue factor or of elements in the tissue factor pathway (i.e., factors VIIa and Xa, or thrombin) has the potential to further improve outcomes in atherothrombosis.

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