UNIT NO. 1

ATHEROTHROMBOSIS – KEY CONCEPTS AND PATHOGENESIS

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ABSTRACT

Over the past decade, there has been considerable improvement in the understanding of processes leading to atherothrombosis. The concepts of plaque stability, recurrent plaque rupture and intraplaque haemorrhage have been better elucidated. Molecular techniques have identified several novel mediators and recognised the contributions of inflammation and immunomodulation. The atherosclerotic plaque is no longer thought to be a passive clogging of the vessel as the wall thickens but an active biological entity

INTRODUCTION

While the age-standardised incidence of cardiovascular diseases may be falling for both men and women in several developed and some developing countries, the health burden is expected to increase. This somewhat contradiction may be explained in part by an aging population and also, in part due to a rising prevalence of obesity, diabetes and other lifestyle-related diseases. By 2020, cardiovascular diseases will be the commonest cause of death worldwide. A similar trend is observed in Singapore. Based on our Ministry of Health Report, in the year 2002, heart diseases are the second commonest cause of death (24.2%), next to cancer (28.0%). When taken together with stroke (8.8%), which is cerebrovascular disease, cardiovascular diseases become the principal cause of death in Singapore (33.0%).

For a number of years, the evolution of atherosclerosis was believed to be attributed to a gradual narrowing of the vessel lumen from deposition of fatty substances. The occurrence of acute coronary events, including myocardial infarction and sudden cardiac death, have been largely attributed to an abrupt occlusion of a critical coronary vessel with a pre-existing high grade narrowing. Conversely, subsequent other investigators found that a substantial number of patients with coronary occlusion had previous narrowing of <50%. Indeed, recent experimental, clinical and pathological findings have further enhanced the understanding of the underlying processes, in particular, the role of thrombus in accelerating the progression of atherosclerosis. In fact, the pre-eminence of thrombus formation has transformed to the current concept of atherothrombosis. This modification in the understanding of disease processes has led to advances in the modalities in prevention and treatment.

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CURRENT CONCEPT OF ATHEROTHROMBOSIS

Virchow's Triad Revisited

In 1845, Rudolf Ludwig Karl Virchow, at the age of about 25 years, published his treatise on haemostasis and thrombosis. The German physician stated that the nature of the vessel wall, local rheologic characteristics of flow, and systemic coagulation factors in the circulating blood are the key determinants for clot formation. What is known as Virchow's Triad still remains as the fundamental considerations for thrombosis today. Although the work focussed on thrombus formation, the complex interactions between thrombosis and atherosclerosis have been increasingly recognised.

Vessel Wall

Although most physicians are aware that intimal alterations have been observed among teenagers who died from non-vascular causes, focal intimal lesions have been found in human foetus as well. To streamline the nomenclature of disease progression, the American Heart Association established specific plaque types based on histologic characteristics (Fig. 1). Under these phases, the risk for thrombosis is heightened in advanced lesions, particularly during plaque disruption, and is partly due to the higher composition of prothrombotic substances such as tissue factor and fatty gruel. Indeed, certain coronary risk factors such as smoking elevates the content of tissue factor in the vessel wall, and exposure of the fatty gruel to circulating blood induces clots 6 times larger than other components of the vessel wall.

Blood Flow

Disruption to blood flow can occur gradually when the atheromatous plaque builds up in the vessel wall. Acute flow disturbances may occur as a result of vasoconstriction, plaque rupture or clot formation. As these changes reduce the size and alter the geometry of the lumen and increase flow velocity, shear forces on the vessel wall increase correspondingly. At the site of peak shear stress, usually at the apex of the narrowing, platelets are deposited, and may subsequently initiate the coagulation process. Simultaneously, substances released by platelets enhance vasomotor tone and further reduce flow. Several coronary risk factors such as smoking, hyperlipidaemia and diabetes mellitus have been shown to be associated with endothelial dysfunction which may promote vasoconstriction and platelet deposition.

Systemic Factors

Several systemic factors, including oxygenation, changes in hormonal and glycaemic status, are known to induce a hypercoaguable state. Previously, the occurrence of acute coronary syndromes was believed to be the result of plaque

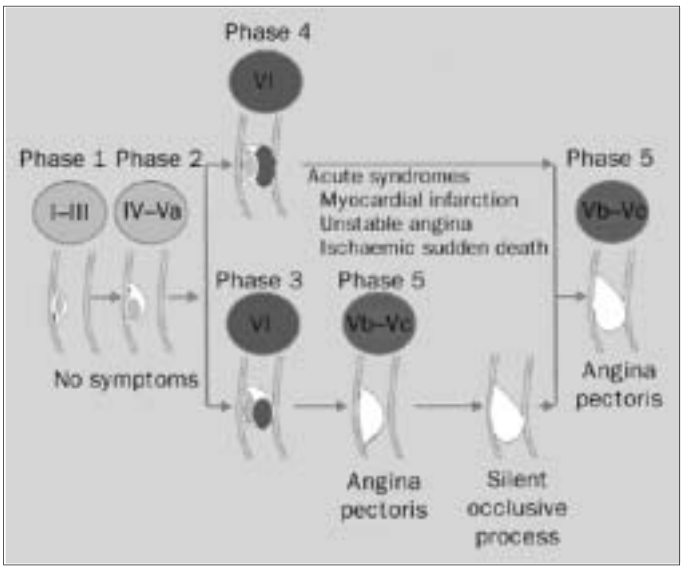

Fig 1: Phases and lesion morphologic characteristics of progression of coronary atherosclerotic according to gross pathological and clinical findings. Early lesions are fibrolipid plaques (phase 1) which may gradually increase in thickness (phase 2). At the same time, there may be vascular remodeling, leading to enlargement of the vessel lumen. Hence, the initial thickening of the vessel wall may not compromise on the luminal area. Formation of thrombus or haematoma may result in angina pectoris (phase 3) or an acute coronary event (phase 4) depending if the lumen is occluded and the presence of collateral circulation. The formation of thrombus may be a dynamic process, in which intrinsic fibrinolysis may dissolve the clot. Furthermore, the patient may be asymptomatic, even if the occlusion was complete. Subsequently, phase 3 and 4 lesions may progress to phase 5, which are fibrotic narrowings and progress gradually to occlusive lesions.

Roman numerals indicate lesion type (I to III = early lesions, IV to V = advanced lesions):

I = isolated macrophage-foam cells
II = multiple foam-cell layers
III = isolated extracellular lipids
IV = atheromatous or fibrolipid plaques with confluent lipid pools (atheroma)
Va = atheromatous or fibrolipid plaques with fibromuscular tissue layers and atheroma
Vb = advanced lesions with calcifications
Vc = advanced lesions with fibrous tissue
VI = complicated advanced lesions with surface defects, haemorrhage, or thrombi deposition

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rupture. However, approximately 20% to 30% of these incidents have been attributed to plaque erosion or fissure, especially among women and smokers, in a recent study. For these events to occur, increased systemic thrombotic tendency is believed to be a major contributor.

The understanding of mechanisitc pathways for certain coronary risk factors has improved substantially in the recent years. Lipoprotein (a), which is structurally similar to plasminogen, reduces formation of plasmin and impairs fibrinolysis. Increased low-density lipoprotein levels promote thrombus formation and propagation. Smoking induces the release of catecholamines, and heightened catecholamine levels increase platelet and vasomotor reactivity. Elevation of several coagulation factors such as fibrinogen has also been associated with various manifestations of atherosclerotic disease.

Plaque Rupture and Fissure

A mechanism responsible for the sudden onset of coronary heart disease is disruption of the atherosclerotic plaque. Several pathohistological features have been characterised in a plaque which is likely to rupture (Fig. 2). Generally, there is a thin fibrous cap with a large (>40% of the volume) lipid core and abundance of macrophages and T lymphocytes. Other investigators suggested that mast cells may also play a role in plaque instability. These cells are found near sites of rupture or erosion and express tumour necrosis factor-a (TNF-a), aid in the formation of foam cells and activate matrix metalloproteinases. These enzymes weaken the structural integrity, especially at the "shoulder" of the plaque, and increase the propensity of the fibrous cap to break or rupture. Once the cap is breeched, substances contained in the lipid core, including cellular and extracellular tissue factor, fibrous accumulation of lipids, dead foam cells and erythrocytes, which are highly thrombogenic, are exposed to the blood. Naturally, a clot is rapidly formed.

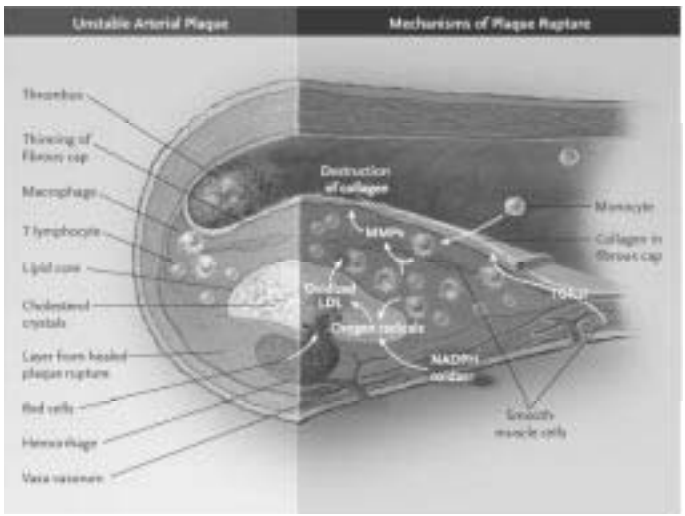

Fig 2: An Unstable Arterial Plaque and the Mechanism of Plaque Rupture.

The stability of atherosclerotic plaques is provided by extracellular matrix and a thick fibrous cap. This unstable plaque has a thin fibrous cap and thrombus at the shoulder, many inflammatory cells, and a large lipid core. The synthesis of collagen by smooth-muscle cells is stimulated by growth factors, such as transforming growth factor b (TGF-b). Inflammation in plaques, with the accumulation of macrophages and T lymphocytes, leads to the release of matrix metalloproteinases (MMPs), which digest collagen and cause thinning of the fibrous cap. The necrotic lipid core grows as a result of the accumulation of lipids in extracellular matrix, the death of lipid-laden macrophages, and perhaps the accumulation of erythrocyte membranes after intraplaque haemorrhage from the vasa vasorum. Oxygen radicals, generated from many sources, including NADPH (reduced nicotinamide adenine dinucleotide phosphate) oxidase and inflammatory cells, oxidise low-density lipoproteins (LDL) and cause necrosis of cells. Repetitive cycles of plaque rupture and healing, which may be clinically silent, produce layers in the advanced plaque (Heistad DD. Unstable coronary-artery plaques. N Engl J Med 2003; 349:2285-2287).

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The clinical manifestations of plaque rupture and fissure were previously thought to be either acute coronary syndromes or sudden cardiac death. Recently, clinically *silent* plaque rupture has been described in as many as 10% of persons who died from other non-cardiac causes. Occlusive and non-occlusive plaque ruptures have been increasingly recognised. Furthermore, the occurrence of an acute coronary event is also dependent on presence of collateral circulation and possibly ischaemic preconditioning. Conversely, fatal myocardial infarction may not always arise from thrombotic occlusive plaque rupture or fissure. There may be particulate distal embolisation or vasospasm resulting in myocardial necrosis. Nonetheless, plaque rupture and fissure remain the principal mechanistic pathway for thrombotic vascular occlusion. Subclinical events may lead to plaque expansion and subsequent fibrosis and narrow the lumen. Consequently, these patients may present as angina pectoris. Therefore, instead of gradual reduction in lumen by enlargement of the atherosclerotic lesion, plaque rupture may accelerate the progression of the disease.

Role of Intraplaque Haemorrhage

Another important pathway for plaque progression has been recently described among patients with sudden cardiac death. Vasa vasorum are tiny vessels which are found on the adventita of large vessels. They provide the blood supply to the vessel wall. However, in advanced lesions and increased thickness, new vessels are readily formed from the adventitia and lumen. Unfortunately, these tiny vasculatures have weak walls and break easily. Sometimes, intraplaque haemorrhage may occur leading to a sudden expansion of volume. These events may occur in cycles, layering down fibrous tissue sequentially which subsequently encroach the lumen of the vessel. The erythrocytes released into the vessel wall are believed to play a critical role in providing the lipid composition, attracting macrophages and possibly activating an immune response. Subsequently, the lesion enlarges and progression of the disease is accelerated.

INFLAMMATION AND APOPTOSIS

Inflammatory reaction is part of our body's response to an adverse stimulus. The widespread application of molecular techniques in cell and vascular biology has improved our understanding of the role of inflammation in atherothrombosis. To build up the lesion, migration, localisation and function of several cell types are required. Intercellular communication is provided by a variety of cell adhesion molecules (CAMs) and cytokines to co-ordinate these complex processes. A significant proportion of these signals come from the inflammatory reaction. The various CAMs recruit specific cell types, such as macrophages and lymphocytes, and attach them to the target sites before facilitating their entry into the vascular wall. Correspondingly, cytokines such as TNF-a, C-reactive protein (CRP) and the interleukins, enhance the inflammatory processes and further develop the atheromatous plaque through its various intercellular interactions. In addition, these inflammatory mediators have been shown to be associated with endothelial dysfunction which promotes the process of atherosclerosis.

Furthermore, coagulation factors are recognised to exert non-thrombotic actions directly on vascular cells. Mediated through receptors on smooth muscle and endothelial cells, thrombin is a potent mitogenic and chemotactic pro-inflammatory stimulus. Likely, processes of atherosclerosis are the result of an intricate interplay of coagulation and inflammation.

Commonly seen in the atheromatous plaque are several cell types, particularly macrophages, which are undergoing apoptosis, also described as "*clean*" death. Unlike what was expected, they are located frequently in areas rich in inflammatory cells and pro-inflammatory cytokines, likely attributed to an inefficient and overloaded *usual* clearance system. Conversely, there are less apoptotic cells in areas with large amounts of anti-inflammatory cytokines. This interesting relationship suggests that apoptosis and inflammation may play a critical role in plaque instability, rupture, and subsequent thrombosis. Indeed, the phospholipid, phosphotidylserine, which is part of the cell membrane, externalises following apoptosis for phagocytosis. Notably, the same substance is found on the surface of stimulated platelets and is used as a platform for the coagulation cascades. Furthermore, the exteriorised phosphotidylserine may activate tissue factor and initiate the extrinsic clotting pathway. Currently, there is some evidence suggesting that intravascular apoptoptic cellular elements may be promoting thrombosis as well.

IMMUNE SIGNALS

Given the current understanding of thrombosis, inflammation and accumulation of lipids, cells and extracellular elements in atherothrombosis, the role of immune signals have been recently elucidated. Majority of this work has focussed on the receptors CD40 and CD154 (previously known as CD40 ligand), which are traditionally thought be expressed on B and $CD4^+$ lymphocytes, respectively. Currently, CD40 and CD154 have been found on several other different cell types, including macrophages, smooth muscle and endothelial cells. Indeed, the ligation of CD40 between CD154 is involved in numerous biological functions are critical in atherogenesis. CD40 signaling has been shown to crucial in the initiation, progression and late acute thrombotic complications of the atherosclerotic plaque in several animal models. Recently, patients with acute coronary syndromes and high serum levels of soluble CD40 ligand were associated a greater occurrence of death or myocardial infarction at 6 months. Further insights into the interactions

between CD40 and CD154 may unravel potential targets for prevention, identification and treatment of individuals with atherothrombosis.

CLINICAL PREDICTION AND INFLAMMATION MARKERS

Of the several inflammatory markers studied, including soluble P-selectin, soluble ICAM-1, interleukin-6 and interleukin-1 receptor antagonist, TNF-a, CRP has been most extensively evaluated. Patients admitted to hospital for acute coronary syndromes with elevated levels of CRP (>3 mg/dL) were more likely to suffer from death, myocardial infarction or undergo coronary revascularisation procedures than those with lower levels (<3 mg/dL). The temporal relationship in the trend of CRP suggested the rise was related to an underlying inflammatory reaction rather than myocardial necrosis. Indeed, the value of CRP in predicting adverse outcomes has been demonstrated in several other clinical scenarios, including healthy individuals (Fig. 3), stable angina, myocardial infarction and patients receiving revascularisation procedures. Importantly, the level of CRP has been shown to be stable over a period of 5 years.

Notably, treatment with statins, which lowers serum cholesterol, also reduces CRP levels independently, suggesting an anti-inflammatory action. Indeed, the protection afforded by statins was greater among those with heightened levels of CRP. Similarly, aspirin prevented more myocardial infarction events among apparently healthy men with CRP levels in the highest quartile (relative risk reduction, 56%; p=0.02) than in the lowest quartile (relative risk reduction, 18%; p=0.8). Unlike statins, it remains uncertain if aspirin lowers CRP levels.

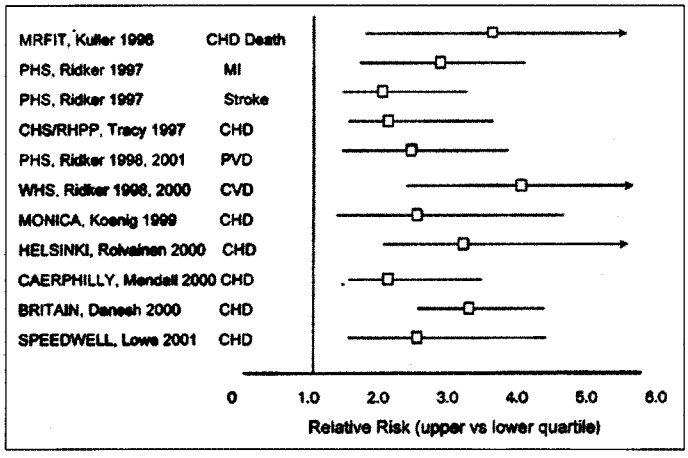

Fig 3: Prospective studies of C-reactive protein (CRP) as a marker of future cardiovascular risk among individuals without known coronary disease.

Risk estimates and 95% confidence intervals are calculated as comparison of top versus bottom quartile within each study group (Blake GJ, Ridker PM. Novel clinical markers of vascular wall inflammation. Circ Res 2001; 89:763-771).

CRP = C-reactive protein; CHD = coronary heart disease; MI = myocardial infarction; PVD = peripheral vascular disease; CVD = cardiovascular disease.

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Although patients with various traditional coronary risk factors, such as diabetes, obesity and cigarette smoking, were associated with increased levels of CRP, the level of CRP provided additional prognostic information on risk prediction. Moreover, the combination of lipid profile and CRP assay improves the ability to predict future adverse cardiovascular events. For example, even among women with low-density lipoprotein cholesterol level of <3.33 mmol/L (130 mg/dL), the relative risk was 4.1 (p=0.0002) comparing those in the highest to the lowest quartile.

Unfortunately, there is considerable overlap in the level of CRP between "normal" and "disease" limiting its discriminatory power. Furthermore, several other non-related conditions such as infections or major trauma can raise CRP levels. Assays in these patients should defer 2 to 3 weeks after the event. The value of CRP level is also limited in patients with chronic inflammatory disease such as rheumatoid arthritis or systemic lupus erythromatosus. It appears that individuals with low CRP levels have a low likelihood for subsequent cardiovascular events. However, caution has to be exercised when interpreting those with high levels, and the assay may need to be repeated. Nonetheless, high-risk individuals who do not meet the current treatment guidelines for lipids in particular, CRP levels may provide additional information on risk assessment.

CONCLUSION

Throughout the past decade, our understanding of atherothrombosis has increased considerably. In particular, the role of inflammation is better elucidated. Atherosclerosis, once thought to be a gradual accumulation of lipid plaque, is currently recognised as a complex interplay among a variety of interacting processes and pathways. Nonetheless, in current clinical practice, traditional coronary risk factors account for a significant proportion of the likelihood of an individual to develop cardiovascular disease. Therefore, individualised aggressive management of these conditions remains the cornerstone for the reduction of cardiovascular burden in our society. Advancement in our knowledge of atherothrombosis and the availability of such information to patient and doctor alike will provide new targets of diagnostic, prognostic and therapeutic potential.

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ATHEROTHROMBOSIS ALL CLOGGED UP, MAKING SENSE OF IT

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To understand atherothrombosis, we need to revisit its definition. Atherothrombosis is the process of thrombus formation on an atherosclerotic plaque. The clinical expression of atherothrombosis in vascular disease can be acute coronary syndromes (myocardial infarction and unstable angina), transient ischaemic attack and stroke or peripheral artery disease. Thrombosis in vessels is also a common problem in various cancers and after major surgery where there is prolonged immobilization after the procedure.

Pathogenesis

Atherothrombosis is a progressive disease characterized by the accumulation of lipids, fibrous materials and minerals in to the arterial wall leading to narrowing of the arterial lumen. 150 years ago in 1845, Virchow described the process of atherogenesis based on three prerequisites: (1) abnormal blood flow, (2) vessel wall abnormalities, and (3) blood constituent abnormalities. Know as the Virchow triad, this concept has evolved today with modern technology and knowledge of endothelial function, flow characteristics, blood constituents, haematological factors, clotting factors, and platelet physiology.

Atherosclerosis plaque formation is no longer thought of as a passive clogging of the vessels as the vessel walls thickened. It is in fact an active biological entity brought on by inflammatory process that occurs in the wall of the vessels filled with lipids. The plaque contains intrinsic vascular wall cells (endothelial and smooth muscle wall cells), and inflammatory leucocytes (monocytes/macrophages and T-lymphocytes). The interactions among these cells are critical in atherogenesis. This process of atherogenesis develops over many years even decades. Early lesion formation may even occur in adolescence.

The process of inflammation participates in all phases of atherothrombotic disease of lesion initiation, lesion progression, and thrombotic complication. The process of atherogenesis starts with leucocytes migration into the intrinsic layer of the vessel. The accumulation of leucocytes and subsequent death lead to a lipid core covered by a fibrous cap (atheroma). Once the atheroma is well established, it crosses the threshold to clinical manifestations such as unstable angina and acute myocardial infarction. Thrombotic complication of the atheroma in the cerebrovascular or peripheral arteries results in transient ischaemic attack or stroke and critical limb ischaemia respectively.

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Thrombosis of the atheroma results from weakening of the fibrous caps and the enhanced thrombogenecity of the lipid core. The weakening of the fibrous caps and the subsequent plaque disruption is thought to be caused by the ability of the inflammatory cells in the intima in inhibiting the production of collagen by the smooth muscle cells and the release of proteolytic enzymes capable of degrading collagen and other structurally important constituents of the fibrous cap[1,2].

Diagnostic tools

Identification of vulnerable atherosclerotic plaque and measurement of the extent of atherosclerosis are subjects of ongoing studies and experimentation. The tools available range from invasive angiographic techniques to non-invasive techniques such as ultrasonography, magnetic imaging techniques and analysis of coronary calcium.

Atherosclerosis is a systemic disease affecting not only the heart but also brain and peripheral vessels. Epidemiological studies show that peripheral arterial disease (PAD) is a marker for systemic vascular disease. PAD can be diagnosed accurately, quickly, and non-invasively in most patients in the office setting through Ankle – Brachial Index (ABI). The ABI has emerged as one of the most useful early warning markers of diffuse atherosclerosis. This is a simple technique that is easy to perform using a handheld Doppler and Sphynomonometer.

As we understand more about the biology of atherothrombosis, we need to move beyond standard cholesterol screening if we are to appreciate the promise of preventive early intervention therapies. While hyperlipidemia, hypertension, and diabetes, as well as the behavioral risk factors of smoking and diet, remain major critical modifiable risk factors for vascular disease, we have learnt over the years that many haemostatic and thrombotic markers such as lipoprotein (a), D-dimer, and homocysteine, inflammatory markers such as C-reactive protein (CRP), fibrinogen, and interleukin-6, and genetic markers are all part of the evolving understanding of cardiovascular risk.

Antithrombotic therapy

At the present moment the therapeutic approaches available are often unable to prevent short and longer term progression of disease and ischaemia. This is because the agents available are not potent enough to block thrombus formation.

The agents presently available[3] are:

1. Antiplatelet agents; (a) aspirin, (b) dipyridamole, (c) ticopidine and clopidogrel, and (d) glycoprotein IIb/IIIa

receptor inhibitors e.g., abciximab (intravenous)
2. Anticoagulant drugs: (a) warfarin, and (b) heparin
3. Thrombolytic agents: (a) Streptokinase, and (b) Tissue plasminogen activator.

Unfortunately, the agents are not potent enough and are unable to block all the factors contributing to the process of atherothrombosis. Some cannot be administered for extended period of time without risk of causing bleeding.

Other strategies

Behavioral modification and therapeutic lifestyle changes still remain important actions to take to prevent atherosclerosis (diet, exercise, weight control, stop smoking and control of hypertension and diabetes). For those individuals who are symptomatic of heart, brain and peripheral artery atherothrombosis, specific aggressive intervention will be needed.

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