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From Evolution of Platelets to Their Role in Heart Attacks

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Recent years have seen renewed interest in the possible role of infection and inflammation in the pathogenesis of atheromatous disease in man and there are likely to be shared messengers involved in regulating platelets and the inflammatory process. A retrospective study of evolutionary biology can serve to remind us of potentially important systems and cellular interactions within the regulatory and pathological processes in our current state of evolution and suggest novel therapeutic approaches. This is particularly true of the cardiovascular system. The platelet has accumulated a variety of functions and regulatory mechanisms during its remarkable evolution in man and mammals. Understanding these will remain the goal of much future research.

Key words: Evolution, platelets, heart attacks

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INTRODUCTION

Heart attacks are common in the industrialized world and a growing problem in the developing countries. Considerable effort has been made to discover the pathological processes behind it. Earlier studies showed that thrombosis is always present in patients dying after heart attacks[1]. The role of platelets in such events is now well documented and the antiplatelet therapy forms a central component in the management of thromboembolic disorders.

The platelets are abundant in circulation; there are between 200-400×10^9 L^{-1} of blood and they have a life span of about 10 days in humans. Their main function is to control bleeding (haemostasis). They evolved from primitive cells called haemocytes, which mainly had a protective role, preventing infection as well as playing a part in haemostasis. In healthy individuals, a fine balance is maintained in regulating platelet function, preventing excessive blood clotting (thrombosis) on the one hand and excessive bleeding (haemorrhage) on the other. This process is altered in people suffering from heart attacks. We take a step to look at evolutionary changes resulting in human platelets leading to heart attacks with available antiplatelet therapy.

Evolution of platelets: Blood developed from interstitial fluid and started to circulate as 'haemolymph' in the open circulatory systems of primitive organisms. Invertebrates use three main mechanisms to control the loss of heaemolymph: conversion of the plasma to a more gelatinous fluid (gelation), cellular aggregation, or a combination of both[2].

Haemostasis in fish is achieved by means of circulating thrombocytes, which are the equivalent of mammalian platelets. When stimulated, they undergo morphological changes analogous to those seen in their mammalian counterparts. Substances which stimulate mammalian platelets to aggregate tend to have a similar effect on thrombocytes. As in mammals, the plasma protein fibrinogen is required for aggregation and mammalian fibrinogen can substitute for the same molecule from fish, suggesting sequence homology across classes[3]. Despite these similarities there are also some important differences. In fish, amphibians, reptiles and birds, thrombocytes, which are nucleated and diploid, differentiate directly from progenitor cells in the bone marrow. This is not true of mammals. In fact, fish do not have bone marrow *per se*. The thrombocytes also differ from mammalian platelets in their intracellular composition and their responses to some stimuli[4]. Despite this, recent work on haemostasis in fish has reveraled similarities between fish and mammals, including a possible equivalent of human thrombotic disease (furunculosis).

Structure and function in mammals: Mammalian platelets are about 5 μm in diameter, 1 μm in thickness and with a volume of 5-10 fl. they are disc-shaped and contain a single peripheral microtubule coil, along with an action on membrane skeleton, which serves to maintain their shape. The plasma membrane contains numerous surface invaginations (the open canalicular system) which act as pathways for the transport of substrances into the platelets and as conduits for the discharge of their contents. Platelets are characterized by intracellular granules (called α-and dense granules); these contain substances such as platelet factor 4, β-thromboglobulin and platelet derived growth factor in α-dense granules and ADP, ATP and 5-hydroxytryptamine in dense granules. When they are secreted in response to platelet activation, α-granule contents promote vascular smooth muscle cell growth and proliferation, while dense granule contents promote further platelet activation.

Because it is relatively easy to obtain human blood, rather more is known about human platelet function than about platelet function in other mammals. However, some species differences in responsiveness have been identified; for instance unlike human platelets, bovine platelets[5] and those from African elephants lack a well-developed open canalicular system and do not produce pseudopodia when activated[6]. Their aggregatory process is also more readily reversible than that of human platelets and relies on the involvement of other chemical mediators. It has therefore been postulated that they represent a different type of mammalian platelet. Platelet responsiveness is also different in aquatic mammals such as the killer whale and dolphin[7] as compared to man. Drastic pressure changes activate human platelets, but aggregatory responses in dolphin and killer whale platelets to standard agonists are attenuated compared to humans; this may have a protective purpose, preventing thrombosis during diving and resurfacing.

Human platelet biology: Under normal conditions platelets circulate through the human body as quiescent discs, but when the endothelium of a blood vessel is damaged and the underlying structures are exposed, platelets adhere to the extracellular matrix components. One of these, collagen, is a strong stimulus to platelet activation and aggregation.

The vascular endothelium lines every blood vessel from the aorta (circa 2.5 cm in diameter in man) down to the smallest capillary (circa 10 μm in diameter) and as such

is a large, continuous structure. Its main functions are to maintain vascular integrity, prevent trombosis and ensure the fluidity of the circulating blood. This is achieved by the secretion of substances such as nitric oxide and prostacyclin, both of which have very short half-lives *in vivo,* or via enzymes (which convert pro-aggregatory substances like ADP to adenosine, which is inhibitory). The endothelium is held together by specialized tight junctions, sealing the vessel and preventing the blood and its components from coming into contact with surrounding tissues[8].

The endothelium may be damaged as a result of mechanical injury, chemical damage (eg by lipids) and changes in shear stress as a result of partial blocking of the blood vessels[8].

During haemostasis/development of thrombosis, there are several distinct phases in the platelet response: (1) adhesion to the damaged endothelium as a result of exposure of underlying collagen; (2) shape change, from disc to spiny sphere, caused by the eversion of membrane invaginations and projection of pseudopodia, resulting in exposure of membrane surface receptors; (3) aggregation of platelets, forming a thrombus and resulting in recruitment of other platelets to the site and (4) degranulation or release of intracellular granule contents (termed 'platelet secretion' or 'the platelet release reaction'), which serves to augment the thrombotic process[8,9]. As more and more platelets are recruited, these changes have the potential to impair or even arrest the flow of blood in the vessel.

Platelets do not have a nucleus and are therefore more correctly described as 'formed elements' rather than 'cells'. Although mRNA is present, their ability to synthesise proteins is limited and since they have few mitochondria, there is little intracellular oxidative metabolism. Platelets derived from the cytoplasm of megakaryocytes, cells located in the bone marrow, which supply them with their proteins, ATP and glycogen[10]. Megakaryocytes can undergo endomitosis, doubling their DNA content without dividing. As a result, they are polyploidy, having a DNA content between 2N and 64N[11] this means that platelets with different DNA contents and different functional capabilities, can be released from mature megakaryocytes.

Platelets derived from more polyploid megakaryocytes are thought to have increased haemostatic activity and so mammals can respond to changing haemostatic demands by producing platelets with enhanced aggregatory potential[4]. This works well in healthy individuals, but can be a problem in disease. The advantage of having anucleated platelets is that more efficient use is made of the same genetic material and that a larger number of haemostatically active platelets can be produced, as compared to what would be possible for nucleated thrombocytes. Four consecutive endomitoses can create a 32 N megakaryocyte from a diploid megakaryoblast; such a megakaryocyte can liberate 3000 platelets, whereas four mitotic cell divisions from a diploid precursor would produce only 16 nucleated thrombocytes[4]. Quite why megakaryocytes became polyploid is not clear and is the subject of much speculation, but the advantages to mammals in terms of haemostasis are important.

Therapy directed at megakaryocytes may be possible in the future. Among the antiplatelet drugs currently in use, much less is known of their possible effects on megakaryocytes. Aspirin has the same effects on megakaryocytes as it does on platelets; since megakaryocytes, like platelets, possess membrane receptors for different agonists, it is probable that these platelet precursors may be accessible to pharmacological manipulation. Drugs may either inhibit or stimulate the production of megakaryocytes, resulting in fewer or more platelets, or may inhibit the reactivity of megakaryocytes and that of their progeny, the platelets.

Heart disease: Several studies suggest that platelets play a role in atherosclerosis and the development of heart disease[11,12]. The ability of heart as an efficient pump is dependent on an adequate blood supply to its muscular walls (the myocardium). When myocardial blood supply is reduced (ischeamia), the contractile function of the heart is impaired. Prolonged ischeamia may result in cellular damage which, if prolonged, leads to cell death (infarction) and contractile failure predisposing the heart to potentially fatal changes in heartbeat rhythm (ventricular arrhythmias).

Atherosclerosis (Fig. 1) leading to myocardial damage is a gradual and slow process. It starts in childhood from an initial low grade inflammation, as a result of endothelial damage (a vascular fatty streak). The initial platelet aggregatory response is seen as an attempt to repair the damage and prevent the inflammation from leading to more platelet. If the damage is severe, or occurs over a larger area, a fibrous plaque may develop. It would then involve more inflammatory mediators like macrophages and deposition of oxidized low density lipoproteins forming foam cells along with connective tissue and smooth muscle cell as well. This atherosclerotic plaque adheres to the coronary vessel wall. Plaque rupture exposes the highly thrombogenic core, which triggers platelet activation and the development of the atherosclerotic plug. This, in turn, may further restrict or prevent coronary perfusion causing ischeamia. The

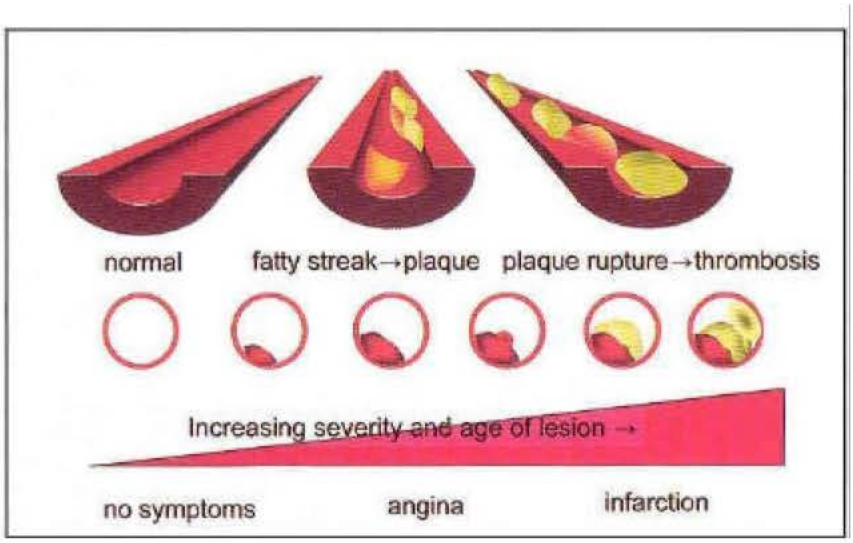

Fig. 1: The atherosclerotic process in the coronary circulation. Vascular fatty streaks can develop within the first 20 years of life and may progress to fibrous plaques and possibly to atherosclerotic plaques over the next 20 to 40 years. Although many people may be symptom-free at this stage, others may experience chest pain (angina) associated with the impaired blood flow to the muscular walls of the heart

formation of underlying plaques may have taken years to develop, but it is the sudden superposition platelet aggregatates which is now thought to be central to coronary vessel occlusion and ischeamic injuries to heart. In support of this, the frequency of myocardial infarction and sudden death in man is known to increase during the early hours of the morning and this correlates with increase ability of platelets to aggregate. Also, the incidence of atherosclerosis is known to increase with age and this is associated with observations that platelets from elderly people are more sensitive to aggregatory stimuli[11]. Activated platelets can further complicate myocardial ischeamia by releasing substances which may alter the electrophysiological properties of the heart, promoting changes in the heartbeat i.e. arrhythmias[13].

Antiplatelet therapy: Platelets play a central role in both the short and long term manifestations of atherothrombosis. In acute coronary syndrome, there is steep rise in cardiovascular events followed by an incremental rise over the long term. This long term event rate is also related to persistent platelets activation and thrombin generation. There is therefore the need to optimize both short and long term antiplatelet and antithrombotic strategies. Several drugs have been developed and are actively used to inhibit platelet function in heart disease. Conventional drugs like aspirin have platelet inhibiting properties via platelets Cyclooxygenase-1 isoenzyme inhibition i.e. responsible for the production of proaggregatory compounds like thromboxane-A2. Aspirin also affects the ability of fibrinogen to interact with platelets and form clots and may alter red blood cell platelet interaction which is all involved in thrombus formation[12]. The ADP receptor antagonists, ticlopidine and clopidogrel, inhibit the early steps of platelet activation, degranulation and release of prothrombotic and inflammatory mediators. Another class of drugs platelet glycoprotein IIb/IIIa inhibitors like abciximab and epitifibatide are derived from monoclonal antibodies; these inhibit the binding of fibrinogen to platelet membrane receptors-the 'final common pathway' of platelet aggregation. But these drugs are very expensive.

Nowadays, a new strategy of combined and sequential administration of different antithrombotic agents is receiving growing attention and seems also to be promising. In the CURE trial, the initial treatment with clopidogrel in addition to aspirin and anticoagulation led to a 20% relative risk reduction for an endpoint of death, MI and stroke[14]. Further studies and clinical trials need to be done to explore the pros and cons of such combination therapies.

The reocclusion and restenosis of coronary arteries means the most difficult issue and task for anti thrombotic treatment modalities. These requirements accelerated the development of novel anti thrombotic agent, the disintegrin family, the monoclonal directed against platelet adhesion and aggregation, the application of hirudin and its analoges and new generation of thrombolotic agents[15]. But these are very expensive

drugs as well. The search continues for new oral antiplatelet agents; these will need to be more potent, free from side-effects and suitable for use by ambulatory patients in the chronic phase of ishceamic heart disease. They would also have to be relative inexpensive.

Heart disease usually occurs in older individuals and is widely thought to be the cumulative result of modern living (a diet high in saturated fats and a sedentary, stressful lifestyle). It is also likely that haemostatic mechanisms evolved before man became susceptible to heart disease and that if these lifestyle factors are addressed, the problem would recede naturally. A lot of effort has been directed towards this end, but recent studies suggest that the situation may not be that simple. A link between genetic variation in platelets and heart disease has been reported, suggesting that this may be a new genetic risk factor for heart disease, although further confimatory work is required[16]. This involves identification of polymorphisms in the genes encoding fibrinogen and platelet membrane receptors. The polymorphism is associated with myocardial infarction and coronary artery disease and augmented expression of platelet membrane receptors following stimulation, suggesting greater platelet reactivity in these patients.

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