Editorial

Biochemical basis of novel antiplatelet drugs

Acute myocardial infarction (AMI) and stroke are the leading causes of mortality worldwide.[1] Most therapeutic strategies directed at AMI and stroke target platelets, the circulating cells that play a pivotal role in the initiation, progression and stabilisation of the thrombus. However, currently available antiplatelet agents have been limited by serious bleeding and/or inadequate efficacy[2] necessitating further studies.

There has been significant progress in our understanding of how platelet activation regulates thrombus formation. The platelets in an intact vasculature are relatively inactive and are termed 'resting'. A breach in the endothelium causes platelets to get activated and adhere to the exposed subendothelial matrix proteins through their cell surface receptors. This initiates a signalling cascade causing conversion of platelets from discoid to "spiny-sphere"; platelet-platelet aggregation and degranulation of platelet storage vesicles, the contents of which serve to amplify the response to vessel wall injury by recruiting more platelets. This culminates in plugging of the defect and is termed *haemostasis*. Similar but more exaggerated response occurs in platelets on rupture of an atherosclerotic plaque causing arterial thrombosis.[3] Antiplatelet agents form one of the mainstays of managing arterial thrombosis. While aspirin, an inhibitor of cyclooxygenase continues to be the first line of pharmacological intervention in antiplatelet therapy, the risk of bleeding is significantly exacerbated by its irreversible action. Clopidogrel, the later entrant into this category, acts through the inhibition of the binding of adenosine diphosphate to its platelet receptors. However, it has to undergo cytochrome P450-dependent degradation, leading to genetic variations in its response. Furthermore, platelets can remain activated in some patients through pathways not inhibited by these agents, such as the protease-activated receptor-1 platelet activation pathway stimulated by thrombin. The other recent drugs, prasugrel and ticagrelor, though more effective due to less interindividual variation, are still not without side effects such as bleeding and drug interactions with statins.[2,4]

Currently available antiplatelet drugs interfere with one or more steps in the process of platelet release and aggregation but cannot be dissociated from an increased risk of bleeding.[5] Furthermore, there is a remarkable redundancy in potential triggers/agonists of platelet activation, as well as the signalling cascades that mediate responses to these agonists. Hence, even patients receiving antiplatelet drugs can sometimes continue to experience adverse thrombotic episodes. This diverted the focus of antiplatelet drug research more towards personalised medicine, largely based on genetic tests. However, the progress was hampered by the reports that genetic variants cannot explain the pharmacodynamics completely.[6,7] Hence, it is vital to discover novel antiplatelet strategies that address these challenges. The successful implementation of these strategies aims at reducing the morbidity and mortality due to unwanted platelet activation.

One of the areas being currently explored is the platelet energy metabolism in activated platelets. Regardless of the nature of their stimulus, activated platelets initiate energy-intensive processes such as shape change, integrin activation, aggregation and granule secretion, which sustain the thrombus.[8] At the same time, platelets need to adapt to potential adversities of hypoxia and nutrient deprivation within the densely packed thrombotic milieu. Hence, it is imperative for the activated platelets to alter their energy metabolism to survive and function during arterial thrombosis. The principal metabolic pathways that generate adenosine triphosphate (ATP) in cells are glycolysis that occurs in the cytoplasm and oxidative phosphorylation that occurs in the mitochondria. Although platelets have functional mitochondria, their reserve for oxidative phosphorylation is fairly limited. However, their reserve for ATP generation through glycolysis is enormous. It has been reported that platelet activation is paralleled by an increase in both glycolysis and oxidative phosphorylation.[9] The increase in glycolytic flux is more remarkable[10] and is sustained by an increase in glucose availability, which is facilitated by glucose transporter 3 translocation to the plasma membrane.[11] Thus, stimulated platelets were found to switch their energy metabolism from oxidative phosphorylation to aerobic glycolysis. This switch to aerobic glycolysis is mediated by increased phosphorylation and inhibition of pyruvate dehydrogenase and pyruvate kinase M2 (PKM2) activities by AMP-activated protein kinase and Src family kinase(s), respectively.[10]

The possible reason for the shift towards aerobic glycolysis, particularly the accumulation of glycolytic intermediates, could be to accelerate flux through pentose phosphate pathway (PPP) generating nicotinamide adenine dinucleotide phosphate (NADPH). NADPH, in turn, serves as a substrate for NADPH oxidase generating reactive oxygen species, a known signalling molecule in platelets which is involved in switching the platelet surface integrins $\alpha_{2b}\beta_3$ to the active conformation.[10,12]

Dichloroacetate (DCA), diarylsulphonamide-58 (DASA) and dehydroepiandrosterone (DHEA) interfere with aerobic glycolysis and/or PPP, thus reversing the metabolic alteration that occurs in activated platelets. DCA is an inhibitor of pyruvate dehydrogenase kinase and DASA is an activator of PKM2, both consequently inhibiting aerobic glycolysis. DHEA, an endogenous steroid hormone, is an inhibitor of glucose 6-phosphate dehydrogenase, the rate-limiting enzyme of the PPP. DCA, DHEA and DASA also inhibit agonist-induced platelet responses such as aggregation and granule secretion. The administration of these metabolic modulators significantly delays thrombus formation and prolongs the time needed for complete vascular occlusion in the murine model of ferric chloride-induced thrombosis in mesenteric arterioles. These findings were further validated by the fact that mice pre-treated with small-molecule metabolic modulators were protected from collagen-epinephrine-induced pulmonary thromboembolism.[10]

There is substantial clinical evidence linking higher serum DHEA levels to decreased cardiovascular mortality.[13] DCA is already under clinical trials against various cancers[14,15] and congenital lactic acidosis,[16] and it has been found to be effective in improving cardiac function after ischemia/reperfusion injury in pre-clinical models.[17] DASA, an activator of PKM2, is in pre-clinical stages of development as an antineoplastic drug.[18] Thus, considerable information on the safety and pharmacokinetics of these molecules in humans is already available. This can pave the way for clinical trials of these drugs as antiplatelet/anti-thrombotic agents with a new mechanism of action placing these drugs in the pipeline of improved next-generation antiplatelet agents.

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