Update

Novel therapies in the pipeline: Directions of research into platelet inhibition

Steve M. Cordina, MD

Zeenat Qureshi Stroke Research Center,
University of Minnesota.

Abstract

Background: Ischemic stroke is one of the foremost causes of death and disability in the industrialized world. Apart from both primary and secondary prevention with oral antiplatelet agents, acute treatment is currently limited to recombinant tissue plasminogen activator and interventional therapy. The occurrence of re-thrombosis during and after these interventions clearly indicate the need for further application of novel agents in the treatment of stroke.

Platelet Function: Current antiplatelet agents in use affect platelet aggregation at different steps. The common limiting factor is the observed occurrence of intracerebral hemorrhage in the setting of acute stroke.

Platelet Inhibition: Selective inhibition of glycoproteins has been employed already (GP IIb/IIIa inhibitors) but there are other glycoproteins that can be targeted. This is based on research that shows that monoclonal antibody mediated inhibition decreases the burden of disease in mouse models of stroke. A new drug that targets the A1 domain of activated von Willebrand factor that attaches to GP Ib is potentially another way of solving the thrombosis puzzle with the promise that intracerebral hemorrhage would be limited.

Conclusion: The continuing search for acceptable levels of platelet inhibition during cerebral ischemic events while minimizing the risk of potentially fatal hemorrhagic side effects is leading the way to selective targeting of the platelet signaling cascade. This raises hope that future therapy will be more effective while having a more favorable safety profile.

Key words: platelet aggregation, platelet inhibition, anticoagulation drugs.

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Correspondent: *Steve M. Cordina, MD; Department of Neurology; University of Minnesota; 420 Delaware St. SE, Minneapolis, MN 55455; cord0107@umn.edu*

Abbreviations, in the order used in this report.

GP:	glycoprotein
vWF:	von Willebrand factor
ICH:	intracerebral hemorrhage

Commercial products, in the order referenced in this report.

ARC 1779	Archemix Corp., Cambridge, Massachusetts, USA

Introduction

Ischemic stroke is a one of the foremost causes of death and disability in industrialized countries.[1] Approved treatment of acute stroke is limited to the use of intravenous recombinant tissue plasminogen activator within a narrow three hour period.[2] There is evidence that the use of intra-arterial thrombolytics is beneficial within a 6 hour period.[3] The situation is however still far from ideal. The increased hypercoaguable state in acute strokes can contribute to re-thrombosis despite best medical and interventional therapy. Continuing platelet aggregation in the setting of increased flow, shear stress and also increased thrombogenicity has been implicated in many of these cases.[4-6]

Platelet Function

Platelet antiaggregation agents inhibit the formation of intra-arterial platelet aggregates. The agents in use today include aspirin, clopidogrel, dipyridamole and also GP IIb/IIIa receptor antagonists. These act at various levels of the platelet activation cascade as shown in Figure 1.

The hemostatic cascade is started off by platelet interaction with extracellular matrix that gets exposed at the sites of injury. Collagen is a major player in this in view of its multifactorial involvement, both in supporting platelet adhesion via direct and indirect pathways, and also by being involved in activating the cells that initiate aggregation and coagulation. Platelet adhesion and aggregation is an involved process that includes multiple surface receptors including integrins, G-protein coupled receptors and also immunoglobulin-like receptors. In view of the laminar flow phenomenon seen in small arteries and arterioles, with associated high shear rates,

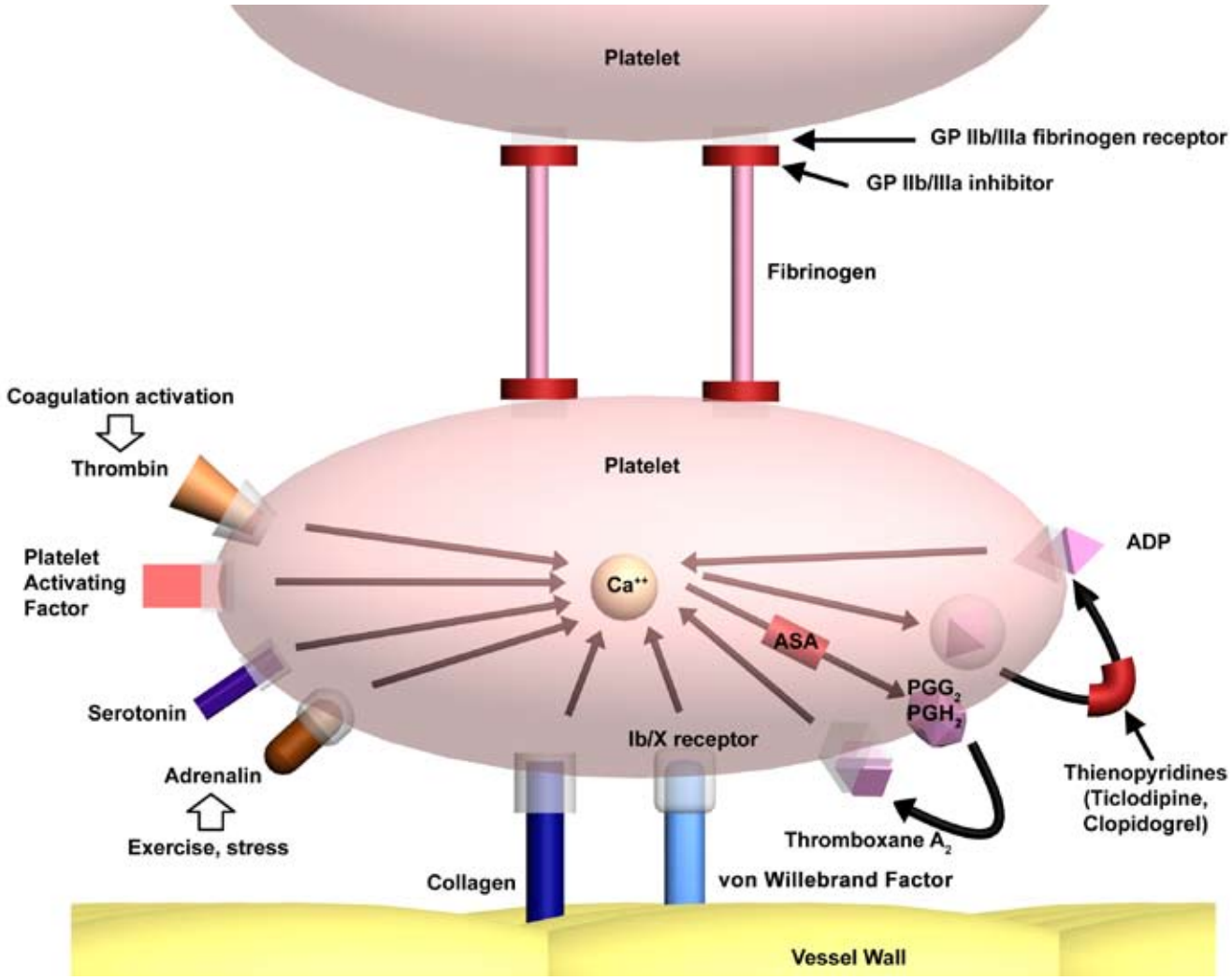

Figure 1. Sites of action of the available antiplatelet agents – aspirin (ASA), thienopyridines, glyocoprotein IIb/IIIa inhibitors,

there is an important initial step that involves the interaction between GP Ib/X and vWF. Their rapid interaction is central to the initial capture of platelets. This is however rapidly reversible, and potentiation of this process involves the recruitment of other molecules between the platelets and the extracellular membrane. This involves primarily integrins. These naturally quiescent surface proteins are activated by GP VI into a high affinity conformation which stabilizes the platelet/vWF/collagen complex.[7]

There already has been substantive research into inhibition of platelet activity in the setting of acute stroke. The benefit of these antiplatelet agents on stroke progression and recurrence can however be outweighed by an increase in ICH and mortality.[8-11] Poor outcome has been one of the drivers into the search for alternative methods of platelet inhibition.

von Willebrand factor

The two mediators of primary hemostasis are vWF and platelets. As mentioned above, platelet activation and aggregation is mediated by vWF when platelets adhere to exposed vascular subendothelial collagen. The simultaneous exposure of tissue factor leads to initiation of the process of secondary hemostasis; i.e., activation of the procoagulant cascade and eventual formation of a hemostatic fibrin clot.[12]

This interaction between vWF and platelets is mediated via the A1 or C3 domain of the vWF. Under the high shear force conditions that are encountered in the arterial circulation, vWF is activated via a physical deformation which exposes its A1 domain and enables its binding to the platelet glycoprotein Ib receptor. It also binds to exposed collagen directly via its A3 domain. During ischemic stress, platelets are exposed to subendothelial matrix proteins. These increase platelet adhesion to vessel walls by stimulating binding of collagens to the GP VI receptor on platelets.[13,14]

Selective GP Ib receptor inhibition

There is experimental evidence that selective inhibition of the GP Ib receptor has a different impact on outcome and risk of ICH as compared with GP IIb/IIIa inhibition.[15] This is on the basis of the molecular properties of vWF. Its A1 domain gets exposed solely under high shear conditions and then binds to the GP Ib platelet receptor, causing platelet aggregation to proceed. There is thus a more precise target of action as compared with irreversible platelet inhibition, as seen under the effect of GP IIb/IIIa inhibitors. GP Ib receptor inhibition leads to an effective vWF-mediated activation pathway inhibition.

Kleinschnitz et al.[15] found that selective GP Ib and VI monoclonal inhibition in a mouse model which then subsequently underwent a temporary total middle cerebral artery occlusion led to decreased infarct volume and absence of any hemor-

rhagic transformation of the stroke. This was in contrast to the hemorrhagic transformations which one can see with final common pathway inhibition. The selective inhibition of both GP Ib and VI was tested both pre- and post-occlusion and benefit was shown in both time frames. This holds promise for possible antiplatelet therapy applications in stroke research and prevention and treatment.

Anti-vWF antagonists

High vWF levels have been also implicated in increased risk of a first ischemic stroke.[16] This further reinforces the need to develop a drug that targets the vWF component of the clotting process. A number of anti-vWF compounds are already available, as monoclonal antibodies or as an aptamers, for example the aptamer *ARC 1779*.

Aptamers are nucleic acid species that have been engineered through repeated rounds of in vitro selection until they bind to pre-determined molecular targets. The usefulness of these compounds lies in their molecular specificity, much like antibodies. The advantage of aptamers over antibodies is that they are easily produced and elicit little to no immunogenic response in live organisms.

ARC 1779 binds competitively to the A1 domain of activated vWF, with a resultant inhibition of interaction with the GP Ib receptor and thus of all vWF-platelet activation pathways. This inhibition is selective in that it is clinically evident only during periods of platelet activation due to pathological thrombosis. A recent in-human evaluation of this compound showed effective vWF and platelet inhibition which was time limited, dose dependent and well tolerated.[17]

Future directions

The selective inhibition of vWF is an avenue of study which could potentially have an important impact in the management of acute stroke. The increased risk of ICH in acute ischemic stroke when GP IIb/IIIa inhibitors are used makes the use of alternative treatments attractive especially if there is potentially a decreased risk of hemorrhage associated with the treatment. More studies of this compound and other vWF-specific antagonists are needed. Only such studies will tell if vWF inhibition is another step towards safer and more effective stroke prevention and reversal.

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