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Dynamic and Structural Formation of a Thrombus: The Inciting Event of Arterial Ischemic Stroke

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Abstract

Arterial ischemic stroke occurs as a result of abnormal clinical circumstances that alter hemostasis and cause thrombosis, either within a vessel or as an embolic event. Understanding normal hemostasis, including differences between children (developmental hemostasis) and adults, will provide background for determining the pathophysiology of stroke and potential treatments.

Keywords

thrombus; arterial ischemic stroke

Introduction

Hemostasis can be described as the reparative process for damaged vasculature in the closed high pressure circulatory system.¹ Regulatory mechanisms exist to contain the reparative process and restore vascular integrity. However, if these mechanisms are overwhelmed, excessive amounts of thrombin are produced, resulting in thrombosis.¹ To provide a starting point for understanding thrombus formation, our discussion is limited to the most salient participating hemostatic components known to date.

Normal Hemostasis

Hemostasis is maintained through vessel wall blood composition, and flow. Advances in our understanding of the physiology of hemostasis have often resulted from the study of mouse models.¹

The endothelium of the vessel wall plays a major role in ensuring patency of vasculature by secreting 3 thrombo-regulating substances. Nitrous oxide^{2, 3} and prostacyclin⁴ inhibit platelet adherence, and ectonucleotidase CD39 degrades adenosine triphosphate directly into adenosine monophosphate, bypassing adenosine diphosphate production; thus, adenosine diphosphate-induced aggregation via P2Y1 receptor is inhibited.⁵

Vessel wall damage may occur as a result of disruption or more subtle endothelial cell alterations as hypothesized by Furie and colleagues,¹ resulting in activation of hemostasis. When vessel wall damage occurs, platelet activation occurs via 2 separate pathways. One pathway is invoked by collagen while the other is invoked by tissue factor. Either or both pathways may predominate depending on the type of vasculature damage or disease

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entity.^{6,7} Exposed collagen in the subendothelial matrix interacts with platelets, resulting in adhesion by 2 mechanisms: binding to von Willebrand factor followed by platelet glycoprotein 1b-IX-V and through direct interaction with platelet glycoprotein VI.⁸ Shear force influences which receptor plays the foremost role in platelet tethering to the vessel wall, but glycoprotein VI and glycoprotein 1b are essential for platelet adhesion.^{6,9,10} The integrin $\alpha_2\beta_1$ also plays a lesser role in platelet adhesion.^{11,12}

Tissue factor in microparticles^{13, 14} circulates in its inactive form^{15, 16} and requires activation, which has been hypothesized to occur through protein disulfide isomerase released by activated endothelial cells and platelets.^{1,14} Transformed tissue factor causes activation of factor VII and factor IX. The combination of activated factor X and factor VIII, known as the tenase complex, causes activation of factor X. Activated factor X then combines with factor V to form the prothrombinase complex, which in turn activates a small amount of prothrombin to thrombin. The minute amount of thrombin generated is a catalyst that activates factors V and VIII to their most active cofactor forms, factor VIIIa and factor Va. Large amounts of thrombin are then generated as a result of the continued action of the tenase and prothrombinase complexes (Figure 1¹⁷). Thrombin activates fibrinogen to form non-polymerized thrombus, but also activates platelets (Figure 2¹⁸) through cleaving protease activating receptor 1, causing platelet granule release (adenosine diphosphate stored in the dense granules, and serotonin and thromboxane A₂ stored in the α granules) and further platelet activation. Adenosine diphosphate activates platelets through receptors P2Y₁ and P2Y₁₂ (Figure 2). Serotonin activates platelets through 5 hydroxytryptamine 2A receptors (Figure 2). Thromboxane A₂ activates platelets through the thromboxane receptor (Figure 2).

In addition to activating tissue factor, protein disulfide isomerase activates glycoprotein IIb/IIIa ($\alpha_{IIb}\beta_3$) on platelets which binds to the ligands fibrinogen and von Willebrand factor; thus promoting platelet recruitment to the thrombus and platelet-platelet interactions.^{19–21} Increased affinity for the ligands occurs when platelets bound to the damaged vessel wall are activated and glycoprotein IIb/IIIa undergoes a transformational change.²² Blood flow shear rates determine the binding ligand, with low and high shear rates binding with fibrinogen and von Willebrand factor, respectively.⁸

Microparticles circulate in the blood and carry proteins associated with the cell type they were derived from (eg, leucocytes, platelets, endothelial cells, smooth muscle cells, and monocytes). Activated platelets expose the protein P selectin²³ on their surface that binds to circulating microparticles derived from monocytes that have the counter receptor displayed, P selectin glycoprotein ligand 1 (PSGL-1).¹³ Through this mechanism, tissue factor exposed on these microparticles^{13, 14} is responsible for further thrombin generation and thrombus extension.

Developmental Hemostasis

Compared with adults, children have decreased physiologic levels of proteins involved in hemostasis (factors XII, XI, X, IX, VII, II), fibrinolysis (plasminogen and tissue plasminogen activator), and inhibition of hemostasis (antithrombin, proteins C and S) and fibrinolysis (plasminogen activator inhibitor-1). As a result of these differences, known as developmental hemostasis,^{24,25} children generate less thrombin than adults when hemostasis is activated.²⁶ As a result, idiopathic thrombosis is rare in children; however, thrombosis does occur and may even be life-threatening in a number of high-risk pediatric cohorts.

Challenges with Pediatric Arterial Ischemic Stroke

The etiology of childhood arterial ischemic stroke not completely understood and appears to be multifactorial. Consequently, studies evaluating specific therapeutic agents become challenging. The ideal therapeutic agent should inhibit thrombus formation/extension without causing hemorrhage while maintaining hemostasis. Current agents have a single mode of action, either inhibiting platelet activity or hemostasis, or actively promoting clot degradation (fibrinolysis). These therapeutic approaches are too primitive for the complexity of modulating hemostatic balance.

Multiple receptors participate in platelet activation; thus, single-target therapeutic agents may not protect against all mechanisms of platelet activation. Furthermore, anticoagulation prevents thrombus formation or extension, which is associated with increased risk for hemorrhage and which may have devastating consequences in children with arterial ischemic stroke. In addition, few pharmacokinetic and pharmacodynamic studies using current therapeutic agents have been performed in children to determine and guide dosing and duration of therapy.

Conclusions

Further elucidation of the mechanism(s) of pediatric arterial ischemic stroke are necessary to provide guidance to determine the most effective therapeutic agent(s), and dosing regimens, followed by the establishment of safety and efficacy profiles.

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Figure 1.

Coagulation phase of hemostasis.

The coagulation phase of hemostasis occurs in three phases: initiation, amplification and propagation.

Abbreviations: TF, tissue factor; vWF, von Willebrand factor. Adapted from Reference 17.

Figure 2.

Platelet receptors and important signaling molecules leading to platelet activation. Soluble agonists stimulate G protein-coupled receptors, triggering pathways involving the corresponding G proteins. Cross-linking of GPIIb or CLEC-2 results in activation of phospholipase (PL) Cγ2. Abbreviations: TF, tissue factor; TxA₂, thromboxane A₂; TP, TxA₂ receptor; PAR, protease-activated receptor; RhoGEF, Rho-specific guanine nucleotide exchange factor; PI3K, phosphoinositide-3-kinase; AC, adenylyl cyclase; PIP₂, phosphatidylinositol-4,5-bisphosphate; PIP₃, phosphatidylinositol-3,4,5-trisphosphate; IP₃, inositol-1,4,5-trisphosphate; DAG, diacylglycerol. With permission from Reference 18.