Acute thrombotic events, such as myocardial infarction and ischemic stroke, are the leading causes of morbidity and mortality worldwide. Based on the data available in 2010, coronary heart disease alone caused ≈ 1 of every 6 deaths in the United States.¹ Approximately every 34 seconds, an American has a coronary event, and approximately every 1 minute 23 seconds, an American will die from it.¹

Thrombolytic drugs are used to dissolve blood clots to avoid downstream tissue damage after occlusive thrombus formation; however, bleeding complications are a major concern. Therefore, the development of a potential thrombolytic agent that does not lead to bleeding represents a highly desirable strategy. In this issue of Circulation Research, Wang et al² used recombinant technology and successfully generated a novel antibody-targeted fibrinolytic agent that selectively binds to activated platelets and triggers thrombolysis locally without significantly impairing hemostasis. Although the preclinical experiments were performed in murine models, this antibody also cross-reacts with human-activated platelets and both the antibody Fv fragments and urokinase are of human origin, thus this new agent should have great potential in future clinical trials.

Article, see p 1083

Acute thrombosis, such as coronary heart disease, usually occurs after rupture of an atherosclerotic plaque.³ Atherosclerosis is a chronic inflammatory process involving a complex interplay of numerous factors including low-density lipoprotein modification, macrophage recruitment, foam cell formation, and immune responses, interacting with normal cellular elements of the arterial wall.⁴ Recently, studies suggest that platelets are also involved in atherosclerosis. Platelets are now considered immune cells and contribute significantly to inflammation and aspects of the immune response, including leukocyte trafficking, immunoglobulin class-switch, and others,⁵,⁶ which may directly or indirectly affect atherosclerosis. In addition, through their surface scavenger receptors, platelets can internalize lipids. Macrophages phagocytose these platelets and develop into foam cells. Platelets can also interact with circulating progenitor cells to affect their differentiation and foam cell formation.⁷ Most recently, platelet acceleration of atherosclerosis was highlighted in a study that linked platelet production with cholesterol metabolism, providing new insights into the mechanisms of atherosclerosis.¹⁰ Thus, in addition to their central role in thrombosis, platelets are also important players in initiation of atherosclerosis. It is conceivable that antiplatelet drugs may benefit both early and later stages of atherothrombosis.

Platelets are small anucleate cells generated from megakaryocytes in the bone marrow. Platelet response to injury is required to stop bleeding and maintain the integrity of the vessel wall. In the event of vascular injury, endothelial cells are disrupted and expose subendothelial matrix proteins, which initiate platelet adhesion and subsequent platelet activation. Platelet activation may switch glycoprotein (GP) IIb/IIIa from its inactive to active conformation. Adjacent platelets are then cross-linked via fibrinogen and other proteins, resulting in platelet aggregation and formation of a hemostatic plug or thrombus. Although it has been well documented that von Willebrand factor and fibrinogen are required for platelet adhesion and aggregation, thromби still form in mice lacking von Willebrand factor and fibrinogen, even after depletion of plasma fibrinectin.¹¹,¹² Interestingly, no thrombi are found in mice lacking GPIIb/IIIa in the same intravital microscopy thrombosis model, suggesting that GPIIb/IIIa is indispensable for platelet aggregation and thrombus formation. There is no doubt that targeting GPIIb/IIIa, particularly those activated GPIIb/IIIa has been and will likely continue to be an important strategy for control of thrombotic diseases.

After platelet recruitment (the first wave of hemostasis), the second mechanism required to stop bleeding is the coagulation cascade. Through either extrinsic or intrinsic pathways, the coagulation system can be activated and generate thrombin, which converts fibrinogen to fibrin. There are many interactions between these two mechanisms that lead to clotting. For example, platelets (particularly activated platelets) accelerate coagulation by providing a negatively charged phosphatidylserine-rich membrane surface that enhances cell-based thrombin generation,¹⁴ which converts fibrinogen to fibrin. Conversely, thrombin generated via the coagulation cascade is a potent agonist for platelet activation¹⁵–¹⁷ and is important for platelet adhesion and aggregation. Fibrin (especially polymerized fibrin) also stabilizes the platelet plug or thrombi.¹⁸ Although important for hemostasis, inappropriate platelet recruitment and blood coagulation may lead to
thrombosis and vessel occlusion. Unstable angina and myocardial infarction typically result from platelet adhesion/aggregation and fibrin formation at ruptured atherosclerotic lesions in coronary arteries. Therefore, an efficient therapeutic regimen against thrombosis should include the modules that target platelets, inhibit coagulation, and promote fibrinolysis.

Many antithrombotic agents, including antiplatelet and anticoagulant drugs, have been developed and widely used to control thrombosis. Antiplatelet drugs, including ADP receptor inhibitors, thromboxane A2 inhibitors, and GPIIb/IIIa inhibitors, are administered to decrease platelet aggregation and inhibit thrombus formation. Although a GPIb complex inhibitor has not yet successfully entered clinical practice, some progress has been made in developing anti-GPIb humanized monoclonal antibody, 6B4, and snake venom-derived GPIb inhibitor, Anfibanide. Anticoagulant drugs include vitamin K antagonists, heparin, direct factor Xa inhibitors, and direct thrombin inhibitors. However, most antithrombotic agents are used for the prevention of thrombosis or vessel reocclusion and are less effective in fibrinolysis or thrombolysis.

Thrombolysis, a naturally occurring process involving the lysis of blood clots, has been exploited as a means to treat acute thrombosis. Thrombolytic therapies using plasminogen activators (PAs), such as streptokinase, tissue PA (tPA), and urokinase PA (uPA), have been the standard of care for promoting fibrinolysis to restore and maintain perfusion after acute thrombosis. Plasminogen activators convert plasminogen to active plasmin, allowing plasmin to cleave fibrin clots. Although effective, these drugs have well-documented limitations, including a narrow therapeutic window, high neurotoxicity, limited ability to penetrate into thrombi or locally enhance potency, and severe bleeding complications. In addition, the large molecular size of thrombolytics impedes clot penetration, therefore, delaying reperfusion. Furthermore, extensive investigation has revealed that administration of platelet GPIIb/IIIa inhibitors in combination with thrombolytic agents is associated with increased risk of bleeding and only modestly improved mortality. Currently available GPIIb/IIIa inhibitors target the receptor regardless of its activation state. Compared with when initially introduced to the clinic a decade ago, the use of GPIIb/IIIa inhibitors has declined in recent years, mainly because of clinical complications, such as severe bleeding and thrombocytopenia. Consequently, developing a fibrinolysis therapy that specifically targets the site of thrombosis (eg, drug delivery systems that target high shear or activated platelet GPIIb/IIIa) represents a potential breakthrough in thrombolytic and thromboprophylactic treatments for cardiovascular diseases.

In this issue of Circulation Research, Wang et al have introduced a strategy that may address this unfulfilled medical need (Figure). These investigators previously developed conformation-specific small recombinant single-chain antibody fragments (scFvSCES) that only target activated platelet GPIIb/IIIa, avoiding outside-in signaling associated with potential paradoxic platelet activation and allowing effective platelet inhibition without prolonged bleeding time. ScFvSCES likely prevent platelet aggregation through potent blockade of GPIIb/IIIa ligands, such as fibrinogen and other unidentified ligands. Here, to facilitate highly effective and targeted thrombolysis, Wang et al successfully engineered a recombinant fusion protein (scFvSCES-scuPA) that contains single chain uPA (scuPA) fused to scFvSCES.

In vitro studies were performed to confirm that both the scFvSCES and the scuPA retain their individual functions in the fusion protein. First, they demonstrated that scFvSCES-scuPA specifically binds to both platelets and Chinese hamster ovarian cells expressing activated GPIIb/IIIa, and showed that this binding was blocked by abciximab (ReoPro). Competitive flow cytometry assays showed that fibrinogen binding to activated platelets was inhibited by scFvSCES-scuPA. Consistently, scFvSCES-scuPA showed a urokinase-independent platelet inhibition in vitro. Furthermore, the urokinase activity of scFvSCES-scuPA and conversion of plasminogen to plasmin (which directly digests fibrin) by scFvSCES-scuPA were well evaluated. Flow chamber adhesion experiments determined that scFvSCES-scuPA, as expected, targeted activated platelets and fibrin degradation in microthrombi. Overall, the authors provided strong evidence that scFvSCES-scuPA targets activated platelets, therefore facilitating a higher concentration of scFvSCES-scuPA locally, which promotes plasmin generation within thrombi and enhances fibrinolysis.

To further evaluate the prophylactic and therapeutic efficacy of scFvSCES-scuPA in vivo, Wang et al used intravitreal microscopy to study a murine ferric chloride-induced thrombosis model. They demonstrated plasminogen-dependent inhibition of thrombus growth with a nano Doppler flow probe. A significant therapeutic reduction in thrombus size, compared with the same dose of a mutated, nontargeting scFv-scuPA or vehicle, was revealed via real-time molecular ultrasound imaging, an elegant new technique that allows real-time and direct monitoring of thrombolysis. Remarkably, thrombolysis achieved by a low dose (75 U/g) of scFvSCES-scuPA was comparable with the effect of uPA at a significantly higher dose (500 U/g).

To evaluate the safety of scFvSCES-scuPA, the authors performed surgical tail transections and found no significant change in bleeding time at the effective dose of 75 U/g. Thus, scFvSCES-scuPA was an ideal therapeutic agent that allows a low concentration in the systemic circulation (hence eliminating bleeding complications), whereas providing a high concentration localized at the site of the thrombus.

This study represents a landmark advance in the treatment of acute thrombotic events; however, some questions remain that warrant investigation in future studies. First, the scFvSCES-scuPA is a bioengineered fusion protein. Although both scFv and uPA are derived from human genes, which may minimize their antigenicity, the joint regions between scFvSCES heavy and light chains and between the scFvSCES and scuPA may generate linear neoepitopes that may induce an immune response. In addition, the fusion protein may generate some conformational neoepitopes, which may trigger B cell clones for antibody generation. Resulting antibodies may not only decrease the potency and efficacy of the drug but may also trigger side effects, such as the development of allergic reactions and other inflammatory complications. Second, many patients presenting with acute myocardial infarction and ischemic stroke already have vessel occlusion. The current
studies have not performed experiments in a model with pre-existing occlusive thrombi. Third, this study was performed in healthy mice. This may not accurately portray the pathological environments of atherothrombosis, which occurs after rupturing of atherosclerotic lesions. Under hyperlipidemia conditions, circulating platelets may be partially activated or in a hyperactive state. Therefore, scFvSCE5-scuPA may bind to some GPIIb/IIIa of these hyperactive platelets, thus reducing the distribution of the drug to the thrombotic site. Finally, pharmacodynamics and half-life of scFvSCE5-scuPA in humans require further study; however, the recombinant design may allow tailoring the size for specific applications, such as thromboprophylaxis.

In summary, the study by Wang et al represents an important first step in the development of a safe and effective thrombolytic agent devoid of serious bleeding complications. Although some challenges remain ahead, this elegant work successfully generates a novel drug that both targets activated platelets and promotes fibrinolysis. We look forward to the progress as scFvSCE5-scuPA moves along the clinical development pathway.

Acknowledgments
We thank Alexandra H. Marshall for her editing and for valuable comments in preparation of this commentary.

Sources of Funding
We were supported by funding from the Canadian Institutes of Health Research (MOP 119540), Canadian Blood Services, and the Heart and Stroke Foundation of Canada (Ontario).
Disclosures
None.

References

Key Words: Editorials ■ blood platelets ■ fibrinolysis ■ thrombosis ■ thrombolytic therapy

Reheman et al Targeted Thrombolysis 1073
Targeting Activated Platelets and Fibrinolysis: Hitting Two Birds with One Stone
Adili Reheman, Xiaohong Xu, Emily C. Reddy and Heyu Ni

Circ Res. 2014;114:1070-1073
doi: 10.1161/CIRCRESAHA.114.303600
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/114/7/1070

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/