Dynamic Thrombosis and Thrombolysis
Role of Antithrombins

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In vivo thrombosis after deep arterial injury has been experimentally shown by receptor inhibitors to be mediated predominantly by thrombin activation of platelets and to a lesser extent by thrombin-related fibrin formation. Thrombosis and thrombolysis are dynamic and simultaneous processes that may influence each other, depending on the exposure and concentration of thrombin and administered agents and dosages. The dose-dependent antithrombotic effect of heparin is mediated by its antithrombin action, but is incomplete even at dosages of 500 units/kg administered over 1 hour.\(^1,2\) Because of the critical role of thrombin in the thrombotic and lytic processes and the improved inhibition of thrombin in all locations by small molecular and specific thrombin inhibitors, the articles concerning the specific thrombin inhibitors hirudin and argatroban in the previous\(^3\) and the present\(^4\) issues of *Circulation* are of interest. Haskel et al,\(^3\) in a recent issue of *Circulation*, compared single doses of antithrombin (hirudin and heparin) with antiplatelet (aspirin and antibody to glycoprotein IIb/IIIa platelet membrane receptor) therapy in the acceler-

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ation of thrombosis and prevention of reocclusion. In this issue, Clarke et al\(^4\) have taken an important step in administering another antithrombin agent, argatroban, to nine normal volunteers with and without aspirin.

**Role of Thrombin and Platelets in Arterial Thrombosis**

Specific thrombin inhibition with hirudin or argatroban—which do not directly block other mediators of platelet aggregation such as thromboxane, serotonin, ADP, or collagen—significantly prevent arterial thrombus formation and markedly limit platelet deposition.\(^1,5-7\) However, neither specific thromboxane A\(_2\) inhibitors or serotonin receptor inhibitors, when given alone, prevent arterial thrombosis or reduce platelet deposition in deeply injured arteries as significantly.\(^2,8\) Although thromboxane is a stronger agonist than serotonin or ADP, the three are synthesized and secreted secondarily by the platelet after initial activation by thrombin, collagen, or both.\(^9\) Thus, in the context of acute arterial thrombosis, an evolving priority is the search for agents that block the interaction of thrombin, collagen, or both with platelets.

**Factors That Modulate Arterial Thrombosis and Antithrombotic Efficacy**

The etiology and pathogenesis of arterial thrombosis relate to 1) the vascular substrate for thrombus formation (subendothelium, media or ruptured plaque, prosthetic material, or residual thrombus), 2) rheology of blood flow (high-flow disturbance by stenosis or branching increases platelet deposition, while stasis favors fibrin formation), and 3) the systemic hemostatic-thrombotic state (levels of catecholamines and of various lipoproteins, coagulation, and other factors).\(^10,11\) Thus, these factors relate to the overall thrombogenicity and dictate the types and potency of therapy required for treatment.\(^11\)

Arterial thrombi are by nature predominantly platelet-rich, with a fibrin-rich tail, but even these may differ in etiology, size and type, and potency of therapy required. Thus, experimentally, arterial thrombi after mild endothelial and subendothelial injury (low thrombogenicity by substrate) are not macroscopic or obstructive (unless associated with stenosis), are not well-anchored and are easily washed away, and can be prevented by aspirin.\(^12-14\) Thrombi after deep damage or injury into the media (high thrombogenicity by substrate) are macroscopic and partially (or totally) obstructive, are well-anchored and mimic thrombi associated with plaque rupture (thrombogenic collagen types I and III or exposure of tissue factor), are reduced to 25–30% of control size but not prevented by aspirin, and are totally eliminated by potent specific thrombin inhibition with hirudin or intra-arterial argatroban.\(^1,5,6\) Arterial thrombi associated with prosthetic material such as metal stents are often totally obstructive, have many more associated thrombi if there is predeployment arterial angioplasty and injury, and are not prevented by heparin, aspirin, and dipyridamole or by usual doses of hirudin.\(^15\) These thrombi probably require higher-dose antithrombin therapy be-
cause they have been eliminated from a metal stent deployed within a polytetrafluoroethylene arteriovenous shunt using a thrombin and broad-spectrum serine protease inhibitor, chloromethyl ketone. Residual thrombi are more thrombogenic than is deeply injured artery, especially in the presence of a severe stenosis, and continue to grow during heparin therapy but are inhibited by hirudin and by other specific antithrombins.

In the above-mentioned experimental conditions, the antithrombin action of heparin is limited for three main reasons. First, a residual thrombus contains active thrombin bound to fibrin, which is thus poorly accessible to the large heparin–antithrombin III complex. Second, a platelet-rich arterial thrombus releases large amounts of platelet factor 4, which inhibits heparin. Third, fibrin II monomer, formed by the action of thrombin on fibrinogen, also inhibits heparin. Conversely, molecules of hirudin and other specific antithrombins are at least ten times smaller than the heparin–antithrombin III complex, have no natural inhibitors, and, therefore, have greater accessibility to thrombin bound to fibrin.

Clinically, thrombi in acute coronary syndromes usually anchor in ruptured atherosclerotic plaques that have active matrix-bound thrombin and are platelet-rich, as in experimentally induced deep arterial injury. The clinical response to heparin or aspirin is beneficial but incomplete because 25–40% of these patients have recurrent ischemia; this is consistent with the experimental response to heparin or aspirin. The main substrate for reocclusion after thrombolysis is residual thrombus and the response to heparin or aspirin is also incomplete.

Dynamic Thrombosis and Thrombolysis

The recent report by Haskel et al documents in vivo the simultaneous dynamic processes of thrombosis and thrombolysis; inhibition of platelets but especially of thrombin significantly enhances thrombolysis and prevents rethrombosis. Ongoing thrombosis during thrombolysis has been shown by biochemical markers of thrombosis, including those of thrombin generation (increased fibrinopeptide A and thrombin–antithrombin III complex) and platelet activation (increased plasma and urinary metabolites of thromboxane), and by enhancement of experimental thrombolysis by antithrombotic agents (heparin, thrombin inhibition, and platelet inhibitors), as recently summarized. Platelet-rich thrombi induced by a powerful stimulus, such as those produced in the electrical current model by Haskel et al, the everted artery model by Jang et al, or other deep arterial injury models require a most aggressive antithrombotic and/or platelet inhibitor regimen to enhance thrombolysis and prevent thrombosis. Rethrombosis appears to be mainly related to the substrate of residual thrombus, as previously mentioned, and to the residual stenosis after thrombolysis; in fact, the stenosis relates importantly to the volume and lability of residual thrombus.

The Role of Antithrombins

In the report by Haskel et al, the specific antithrombin hirudin significantly enhanced thrombolysis and totally prevented reocclusion. The response to hirudin was both therapeutic and diagnostic of the thrombin-dependent mechanism of arterial reocclusion. Hirudin is highly specific, potent, and nearly irreversibly (K_D=10^-15 M) binds thrombin in a 1:1 complex. Thus, the blood level of hirudin required for total prevention of thrombosis appears to be an in vivo index of the thrombin content of the thrombotic state or lesion. For example, the blood level of hirudin necessary to inhibit formation of platelet thrombi is fivefold that required to inhibit formation of fibrin thrombi during disseminated intravascular coagulation. Hirudin levels eight to ten times higher are required to prevent thrombus formation on deeply injured compared with mildly injured arteries, and the differential is even greater for venous compared with arterial thrombi. Thus, different doses of specific thrombin inhibitors appear to be necessary for different types of thrombosis. This may have implications for comparison of models of thrombosis and lysis, especially in view of these dynamic processes. Therefore, a description of the stimulus for thrombosis and the depth and extent of arterial injury is important for comparison of results between different laboratories. Moreover, differences between platelet inhibitors and antithrombin therapies may be less apparent in thrombi of lower index of thrombin content and may be greater for the prevention of reocclusion when the index of thrombin content appears to be high.

A synthetic, reversible thrombin inhibitor with lower binding affinity (K_D=10^-9 M), argatroban, reduces but does not eliminate platelet-rich thrombosis in the everted rabbit femoral artery unless administered intra-arterially. It also accelerates thrombolysis, reduces reocclusion after thrombolysis when combined with aspirin, and is experimentally superior to heparin in reducing thrombosis. In this issue of Circulation, Clarke et al report the first phase I study with argatroban in the United States. As with hirudin and argetroban administration in animals, there was dose-dependent prolongation of the activated partial thromboplastin time in humans. The thrombin time will probably be too sensitive for monitoring optimal antithrombin doses for prevention of arterial thrombosis. Future dose–response studies of argatroban alone in arterial thrombosis might show that increased doses may be useful and obviate the need for simultaneous aspirin administration. This possibility is suggested by studies with hirudin and intra-arterial argatroban. However, the lower binding affinity of argatroban compared with that of hirudin may not permit this comparable possibility.

Never before have we had therapy for total prevention of arterial thrombosis. However, we do not know the appropriate dose and duration of therapy needed...
to prevent later thrombosis or, in cases of preexisting thrombus, to complete endogenous lysis (theoretically possible when thrombosis is totally blocked) and prevent thrombosis in animals or humans. To ensure patient safety, to promote intelligent and cost-saving clinical trials, and to minimize future drug cost, dose–response analyses and studies of the duration of antithrombotic efficacy of specific thrombin inhibition alone (initially with aspirin to be safe and practical) need to be completed before combining this potent therapy with a lytic agent in humans.

References
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