Thrombosis is a dynamic process in which procoagulant activity, which promotes platelet activation and fibrin deposition, is balanced by physiologic anticoagulant and fibrinolytic activity. Historically, the treatment of thrombosis has been based on the use of agents that either inhibit procoagulant activity (i.e., anticoagulants) or induce fibrinolytic activity (i.e., plasminogen activators). However, recent data suggest that the success of antithrombotic interventions requires both inhibition of factors that promote thrombosis and induction of clot lysis. Thus, even when intense fibrinolytic activity is induced in patients with myocardial infarction, increases in procoagulant activity appear to be an important determinant of recurrent thrombosis.\textsuperscript{1-3} Results of recent clinical trials are consistent with the importance of conjunctive therapy with aspirin and heparin in patients given fibrinolytic agents.\textsuperscript{4-9} These data support the hypothesis that the natural history of thrombosis and the response to treatment are mediated by the relative balance of procoagulant and fibrinolytic activity. Recent advances in defining the specific mechanisms that regulate procoagulant and fibrinolytic activity have led to the development of novel agents that offer the potential of even more precise modulation of anticoagulant and fibrinolytic activity as a means of inhibiting thrombosis. One such agent, a recombinant preparation of activated protein C, has previously been shown by Gruber et al\textsuperscript{10} to prevent the formation of platelet-rich arterial thrombi in Dacron grafts in nonhuman primates.

In this issue of \textit{Circulation}, Gruber et al\textsuperscript{11} have reported similar antithrombotic effects when doses of activated protein C lower than those previously required to inhibit thrombosis were combined with urokinase, a plasminogen activator. At two relatively low doses, the antithrombotic effects of inhibition of both procoagulant and fibrinolytic activity with a combination of activated protein C and urokinase was shown to be more effective than the effects of each agent alone at the same doses. By extensively defining the hemostatic effects of the combined regimen, the authors also provided insight into the mechanisms by which interaction of the coagulant and fibrinolytic pathways may determine the success of treatment of arterial thrombosis.

The formation of thrombin is the ultimate consequence of activation of the coagulation system. Thrombin promotes thrombosis by activating platelets, converting fibrinogen to fibrin, and activating factor XIII, which stabilizes the fibrin mesh by inducing cross-linking of fibrin. In addition, thrombin induces anticoagulant activity by activating protein C, a serine protease that inactivates factors Va and VIIIa, two cofactors crucial to activation of the coagulation system.\textsuperscript{12} Activation of protein C requires binding of thrombin to a specific receptor on endothelial cells, thrombomodulin.\textsuperscript{13} Gruber et al\textsuperscript{10} have previously shown that a recombinant preparation of activated protein C inhibits the formation of arterial thrombi in Dacron vascular grafts in nonhuman primates. An important feature of this experimental model is that the platelet-rich thrombi are formed under physiologically relevant shear conditions.\textsuperscript{14} Thrombosis occurs even when animals are treated with aspirin or heparin, a feature similar to that of other models of arterial injury.\textsuperscript{15} In the Dacron-graft model and other models of arterial thrombosis inhibition of thrombin with several recently developed specific inhibitors prevents the accumulation of platelets and the development of thrombosis. Antithrombin agents that appear to be effective include, but are not limited to, hirudin and its derivatives, D-phenylalanyl-prolyl-arginyl chloromethylketone (PPACK) and synthetic inhibitors such as argatroban.\textsuperscript{16-20} These agents share the feature of inhibiting thrombin directly instead of requiring interaction with antithrombin III, as is the case with heparin. The greater efficacy of these inhibitors may be because of the relative inefficiency of heparin-antithrombin III in inhibiting thrombin bound to...
fibrin. Although the specific mechanisms that promote thrombosis on the Dacron grafts have not been defined, the results with activated protein C suggest that inhibition of the formation of thrombin is as effective in preventing thrombosis in this model as inhibiting thrombin activity. In theory, this finding may have important therapeutic consequences because inhibition of the coagulation factors that promote the formation of thrombin would be more efficient than inhibition of thrombin activity. Whether this will result in a lower incidence of bleeding complications or greater therapeutic efficacy remains speculative.

Although thrombosis induced by the Dacron shunt model characterized by Gruber et al has many features that resemble responses to arterial injury, important differences should be appreciated. Arterial injury exposes collagen in the subendothelium, which promotes platelet adhesion and aggregation mediated in part by interaction with von Willebrand factor. Weiss et al have shown that the fibrin deposition decreases whereas the platelet content of thrombi increases at high compared with low shear rates. Therefore, thrombin may play a pivotal role in the procoagulant response to arterial wall injury, but inhibition of platelets may also be necessary to prevent arterial thrombosis. Consistent with this view, increases in thromboxane A2 production consistent with platelet activation have been observed in a canine model of coronary thrombolysis despite potent thrombin inhibition with argatroban. Although argatroban alone was effective in preventing reocclusion, cyclic variation of coronary flow attributable to platelet aggregation continued to be observed and could be abolished by inhibition of thromboxane A2 activity with a specific antagonist. In this preparation, a combination of the thromboxane A2 antagonist and lower doses of the thrombin inhibitor than were necessary when used alone were effective in inhibiting recurrent thrombosis. In addition to platelet interactions with subendothelial components, the mechanisms responsible for procoagulant activity likely differ between Dacron grafts and injured arteries. Activation of coagulation in response to arterial wall injury appears to be mediated by tissue factor, the lipoprotein procoagulant in thromboplastin. Tissue factor is associated with the cell membranes of multiple cell types and forms a complex with factor VIIa that induces activation of factors X and IX. The expression of tissue factor appears to be increased in atherosclerotic plaques, perhaps increasing the procoagulant activity associated with plaque rupture compared with arterial wall injury. The extent to which tissue factor mediates the procoagulant activity associated with the Dacron grafts is unclear and may potentially limit the extent to which results in this preparation can be extrapolated to responses that would occur in patients with thrombotic complications of atherosclerotic vascular disease. Despite these limitations the comparison of antithrombotic strategies in a reproducible, well-characterized primate preparation may yield results that are more clinically relevant than those obtained in nonprimates.

Although the mechanisms responsible for the enhanced antithrombotic efficacy of a combined infusion of activated protein C and urokinase were not clearly defined in the study by Gruber et al, the results of the assays used to monitor the hemostatic effects of this regimen provide some insight into potential interactions between these agents. Urokinase appeared to inhibit platelet activation, judging from the decrease in plasma concentrations of β-thromboglobulin and platelet factor 4, and it prevented platelet deposition in a dose-dependent manner. As noted by the authors, inhibition of platelets by urokinase may reflect elaboration of fibrinogen degradation products that interfere with platelet aggregation. Alternatively, this effect may be specific to urokinase, which has recently been shown to cleave fibrinopeptide B from fibrinogen and induce further proteolysis of fibrinogen, rendering it less clottable. Although not yet characterized, the proteolysis of fibrinogen by urokinase may also have effects on platelet aggregation. However, the finding that concentrations of fibrinopeptide A, a marker of thrombin activity, increase in plasma when urokinase is administered alone suggests that the antithrombotic effects of urokinase may be limited by the induction of procoagulant activity. Similar increases in the levels of fibrinopeptide A occur in patients treated with streptokinase or tissue-type plasminogen activator when heparin is not administered before the fibrinolytic agent. The increases in thrombin activity appear to be due, in part, to activation of factor X by plasmin-mediated increases in the activity of the factor IXa/VIIa complex. Thus, as observed by Gruber et al, the increases in thrombin activity induced by plasminogen activation with urokinase would be expected to be inhibited by concurrent infusion of activated protein C, which inhibits the activity of the factor IXa/VIIa complex and the activation of prothrombin induced by the factor Xa/Va complex. From a clinical perspective, although other mechanisms may account for the increased efficacy of the combination regimen of activated protein C and urokinase, the results of Gruber et al add to the growing body of data that suggest that inhibition of procoagulant activity potentiates the efficacy of fibrinolysis.

The development of activated protein C, as well as other new antithrombotic agents, is a product of the substantial increase in our understanding of the mechanisms that regulate thrombosis. The impact of this knowledge on the treatment of thrombotic vascular diseases has already been considerable. Multiple ongoing trials and the results of studies such as that by Gruber et al offer the promise of even more effective treatment strategies. The challenge will be to determine which of these antithrombotic regimens will increase clinical efficacy while limiting the incidence of bleeding complications.
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Importance of modulating balance of procoagulant and fibrinolytic activity to success of antithrombotic therapy.
P R Eisenberg

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