Editorial Comment

Thrombin Inhibitors in Unstable Angina: Rebound or Continuation of Angina After Argatroban Withdrawal?*

JAMES T. WILLERSON, MD, FACC, WARD CASSCELLS, MD
Houston, Texas

In this issue of the Journal, Gold and colleagues (1) describe important observations concerning the use of the thrombin inhibitor argatroban in patients with unstable angina. Essentially, they noted that angina recurred in 21% of the patients, generally between 4 and 8 h after cessation of argatroban therapy. This editorial discusses the clinical implications and potential mechanisms of this finding.

Current Focus on Thrombin Inhibitors

Role of thrombin in cardiac events. Argatroban, a synthetic derivative of arginine, is one of several inhibitors of thrombin at various stages of preclinical development and clinical testing. These drugs are attracting interest for several reasons. First, it is now well established that coronary thrombus develops in the majority of acute myocardial infarctions and in many, perhaps most, cases of unstable angina (for review, see Ref. 2). Moreover, patients with unstable angina have elevated coronary sinus levels of the platelet products thromboxane A\(_2\) and serotonin (2), as well as elevated blood levels of fibrinopeptide A (3), which is cleaved from fibrinogen when the latter is proteolytically converted to fibrin by thrombin. Heparin and aspirin have each reduced the mortality rate of myocardial infarction and of unstable angina (4-7), further confirming the role of thrombin and of platelets in the pathogenesis of these syndromes. In addition, thrombotic reocclusion has proved to be a limiting factor in approximately 5% of angioplasty procedures and 10% of thrombolytic interventions in myocardial infarction (8).

Limitations of current antithrombotic agents. Thus, a second reason for the current interest in thrombin is the recognition of the need for better antithrombotic regimens. For example, warfarin is slow to act, has little effect on the extrinsic pathway, must be taken orally and interacts with many other drugs. Recombinant tissue plasminogen activator, by activating plasmin, activates the coagulation system by cleaving prothrombin and Factors V and X (9,10). Plasmin also activates platelets (see later) (11). Aspirin inhibits prostaglandin endoperoxide synthesis (cyclooxygenase), thereby inhibiting the formation of thromboxane A\(_2\), itself a platelet agonist and vasoconstrictor (12-14). However, other agonists such as thrombin, adenosine diphosphate, serotonin, platelet activating factor, epinephrine and collagen are not inhibited by aspirin; these act not only through the phospholipase A pathway, but also through phospholipase C and perhaps other pathways, thereby partially circumventing the aspirin effects (2,10,15). Moreover, even relatively low doses of aspirin can inhibit endothelial synthesis of prostacyclin (16). Ticlopidine has been approved by the Food and Drug Administration for transient ischemic attacks in aspirin-sensitive patients but is burdened with a slow onset of action and a small risk of neutropenia (12). Heparin, which largely acts by accelerating the proteolytic inactivation of thrombin and activated Factor X by antithrombin III (17,18), is at a disadvantage in established thrombosis because it is neutralized by platelet factor 4 and thrombospordin, and it has reduced efficacy against thrombin bound to fibrin or to platelets. In addition, heparin is a heterogeneous group of molecules extracted by a variety of procedures and subject to lot to lot variability. Finally, the requirement for antithrombin III limits its utility in the occasional patient with reduced levels of antithrombin III (9,19).

Role of thrombin in coagulation and platelet systems. A third reason for the recent focus on thrombin inhibitors is that it is now understood to be not only the first step in the conversion of fibrinogen to fibrin, but also a key regulatory step (together with activation of Factor VII and tissue factor, or of Factor X) in the coagulation cascade. Moreover, thrombin plays a critical role in the amplification of the cascade, activating Factors V and XIII, which eventually leads to the generation of more thrombin. In addition, thrombin activates Factor XIII to promote clot retraction (20,21). These effects can all be demonstrated in vitro and, although the in vivo regulation of this system is not yet completely understood, it is noteworthy that the high risk period after myocardial infarction is characterized by ongoing, low grade activation of thrombin (22).

Finally, thrombin is one of the most potent known agonists for platelet release and aggregation (20). Thus, whereas any platelet inhibitor is an indirect inhibitor of coagulation (because platelet surfaces accelerate the coag-
lation enzymes and platelets promote stasis), thrombin is directly involved in both the platelet and coagulation systems.

The recent cloning of the platelet thrombin receptor has indicated a unique mechanism of action (23). Thrombin cleaves a specific sequence in the extracellular domain of the receptor, releasing a small peptide that serves as the ultimate ligand for the receptor. (An interesting inference is that, because trypsin activates the thrombin receptor and plasmin has a similarly broad spectrum of action, it is likely that plasminogen activators activate platelets by cleaving the thrombin receptor in addition to generating thrombin. Thus, it may prove to be important to begin administration of antithrombin agents such as argatroban before starting thrombolytic therapy.

A receptor of similar sequence has been cloned from rat smooth muscle cells, and it remains to be shown whether these differences are a function of the species or whether there are cell-specific thrombin receptor isoforms (24). Such a possibility is suggested physiologically by the difference in responsiveness of different types of endothelial cells to thrombin (25). There is another thrombin-binding molecule on platelet surface—GP Ib, which activates protein kinase C in response to von Willebrand factor (26).

It is possible that these two receptors on platelets and cells, and this one catalytic site on thrombin, mediate all of thrombin's actions: proteolytic activation Factors II, V, VIII and XIII and protein C, platelet aggregation, vasoconstriction, neutrophil adherence, monocyte chemotaxis, mitogenesis for endothelial and smooth muscle cells and lymphocytes, and stimulation of production of prostanoids via platelet-activating factor, platelet-derived growth factor and nitric oxide (23). Thus, the effects of a thrombin antagonist like argatroban could be diverse.

**Actions of current thrombin inhibitors.** The thrombin inhibitors that have received the most attention are hirudin (a leech peptide whose sequence is similar to one near the catalytic site of the thrombin receptor) and the related hirulog and hirugen, as well as the synthetic inhibitors PPACK, DuP714 and argatroban. Experiments to date indicate that these inhibitors block most if not all of thrombin's actions. This observation is noteworthy because it was not obvious that the catalytic site and receptor site would be physically or functionally linked: Fibrin binds thrombin without inhibiting thrombin's catalytic action and several reports have indicated that thrombin can serve as a mitogen for smooth muscle cells, despite inactivation of the catalytic domain (27). Argatroban binds to a hydrophobic pocket near the catalytic site of thrombin with moderately high affinity (28). Its $K_i$ for inhibition of catalytic activation of fibrinogen is 19 nM, and it is almost as potent in inhibition of platelet aggregation ($K_i$, 40 nM) (29). Because argatroban is small and binds to a different binding site from that of fibrin, it inhibits clot-bound thrombin just as hirudin does (30). In experimental models of thrombolysis, argatroban prevents thrombosis and accelerates lysis as well as or better than heparin (31).

The Current Study

Mechanisms of renewed angina after argatroban administration. The report by Gold and colleagues (1) in this issue of the Journal represents an important step in characterizing the clinical effects of argatroban. After a 4-h intravenous infusion of 0.5 to 5 g/kg (with or without heparin), 9 of 43 patients experienced an episode of angina, in most cases to 8 h after cessation of the infusion. These episodes were responsive to nitroglycerin and generally uncomplicated. Although some readers might be struck by the fact that all 43 patients were free of angina during the infusion, the investigators have taken a cautious approach and have emphasized the "rebound." This term is used because of a fourfold increase in concentration of thrombin-antithrombin III complex at 2 h after infusion compared with the pretreatment baseline values. However, there was no rebound in levels of fibrinopeptide A. Whether the renewed angina after argatroban infusion represents a true rebound or simply represents its recurrence after the withdrawal of thrombin inhibition in some particularly refractory patients is not clear. However, the results do clearly demonstrate an important role for thrombin in the pathophysiology of unstable angina in humans.

**Role of continued generation of thrombin and increased thrombin-antithrombin III complex.** The data of Gold et al. (1) indicate that one factor contributing to renewed angina after argatroban infusion is the continued generation of thrombin during the infusion. This observation suggests that the positive feedback loop (wherein thrombin activates Factors V and VIII, eventually causing activation of prothrombin to thrombin) is not critical for the sustained generation of thrombin in patients with unstable angina. Alternatively, thrombin's activation of fibrinogen may be more completely inhibited than that of Factors V and VIII by a given dose of argatroban. In addition to continued generation of thrombin, a second factor in the thrombin-antithrombin III rebound can be inferred from the data of Eidt and colleagues (32), who reported that argatroban displaces thrombin from antithrombin III. Thus, as argatroban is a competitive (reversible) inhibitor and is largely cleared by 1 h, an increase in thrombin-antithrombin III complex at the end of the infusion is to be expected, assuming that there are no dramatic changes in the binding or degradation of the thrombin-argatroban complex.

**Other thrombin-related mechanisms.** Other mechanisms that may well contribute to the reactivation of angina after cessation of argatroban infusion include a possible up-regulation of thrombin receptors on the platelets, as platelet receptors are subjected to posttranslational regulation by cyclic nucleotides. Inhibition of thrombin may also prevent thrombin's antithrombotic actions, which are mediated by activation of protein C and stimulation of production of nitric oxide and prostacyclin (19).

**Role of cessation of heparin therapy.** Another mechanism
that should be considered relates to the recent report by Theroux et al. (33) of a reactivation of angina 5 to 15 h after the cessation of heparin administration in patients with unstable angina. In the present study by Gold and colleagues (1), six of the nine patients developing recurrent angina had been receiving heparin until approximately 4 h before argatroban infusion. In contrast, of the 34 patients who had no recurrent symptoms, only 12 had previously been taking heparin. Thus, some of the reactivation of angina in the present study might reflect discontinuation of the heparin as well. Moreover, increased clearance of antithrombin III during heparin infusion would tend to lower the baseline measurements of thrombin-antithrombin complex before argatroban infusion. By the time the complex was measured again (2 h after cessation of the infusion and 10 h after discontinuation of heparin), antithrombin III levels would be increasing. Thus, the calculated fourfold increase in thrombin-antithrombin III complex after argatroban may be in part artifactual.

Clinical Implications of the current findings. The clinical implications of these data are that infusions of argatroban should not be discontinued abruptly but should be tapered after beginning treatment with aspirin or another effective platelet inhibitor. Jang et al. (34) found that the addition of aspirin to argatroban therapy acted to maintain arterial patency after thrombolytic therapy in a canine model. Fitzgerald and Fitzgerald (31) found that addition of a thromboxane A2 receptor antagonist to argatroban further reduced cyclic flow variations (attributed to transient platelet aggregations and vasoconstrictor responses) in a canine model.

However, concomitant aspirin therapy will not prevent the increase in thrombin levels after discontinuation of argatroban. The increased thrombin could activate platelets (through pathways not blocked by aspirin) and generate fibrin. Thus, patients must be closely monitored for recurrent ischemia during and after the administration of a thrombin inhibitor, such as argatroban. In the canine model of cyclic flow variations, Eist et al. (32) found that argatroban blocked cyclic flows only if given in the 1st 30 min to 2 h. When treatment was begun after 3 h of cyclic flows, the argatroban (and heparin) were largely ineffective. However, cyclic flows were subsequently blocked by the addition of antagonists of the serotonin and thromboxane A2 receptors. Thus, clinical studies need to be performed that evaluate the benefit afforded by the more direct thrombin antagonists when they are given relatively late after the onset of unstable angina.

Inhibition of the final steps (platelet aggregation, formation of fibrin) is naturally attractive in a system with multiple and partially redundant pathways. However, as the molecular defects in thrombosis are characterized, it may be possible to select specific therapies based on specific mechanisms in individual patients. For example, it is clear that at least some patients with unstable angina have increased generation of serotonin and thromboxane A2 (2). Some patients with recurrent venous thrombosis have deficiencies in antithrombin III, elevated plasminogen activator inhibitor I or deficient protein C or protein S (22). Some patients after myocardial infarction continue to generate thrombin and fibrin. High levels of fibrinogen, hematocrit, platelet count, low density lipoprotein and lipoprotein(a) appear to be risk factors for thrombosis (22). Thrombosis itself probably creates a local "heparin deficiency" due to binding of cell surface heparan sulfate by thrombin and degradation of heparan by platelet-derived endoglycosidases (35). However, at the present time, individualized therapy is impractical. Moreover, the likelihood is that multiple mechanisms are operating in many patients. These factors act to limit the efficacy of monotherapies as exemplified in this report by Gold et al. (1). Thus, in the foreseeable future, we are likely to be using double and triple therapy, with increasingly specific agents, to treat patients with unstable angina and to prevent its development in patients at risk.

References


