Cardiovascular controversies

Thrombin plays a pivotal role in vascular re-occlusion after PTCA and coronary thrombolysis

Giancarlo Agnelli *

Istituto di Medicina Interna e Medicina Vascolare, Università di Perugia, Via Enrico dal Pozzo, 06126 Perugia, Italy

Keywords: Thrombin; Acute coronary syndromes; Thrombosis; Thrombolysis; Coronary angioplasty; Restenosis

Acute coronary syndromes are the clinical presentations of complex biochemical events which in most cases culminate in intracoronary thrombosis. A central role in the process of intravascular thrombosis is played by thrombin, a glycosylated trypsin-like serine protease with multiple action in vascular biology [1]. In patients with acute coronary syndromes thrombin is generated by the exposure of tissue factor after fissuring or rupture of a coronary plaque. Tissue factor leads to thrombin generation through the activation of the coagulation factors VII and IX and the prothrombinase complex. Once generated, thrombin initiates a positive feed-back autocatalytic loop by directly activating factors V and VIII, thus amplifying its generation by about 1000-fold. At substrate level, other than activating factors V and VIII, thrombin cleaves fibrinogen to fibrin and activates factor XIII which results in formation of stable, cross-linked fibrin clot. Thrombin binds to a specific receptor on the platelet surface initiating a number of intracellular processes, including ADP and thromboxane A2, release and expression of GP IIb/IIIa receptors, which result in platelet aggregation. Thrombin receptors have been cloned from human platelets and vascular smooth muscle cells [2,3]. The activation of thrombin receptor on vascular smooth muscle cells is followed by most of the multiple cellular effects of thrombin, mainly by smooth muscle cell mitogenesis and migration. High concentrations of thrombin are present at the time and the site of mechanical injury or PTCA and persist throughout the period of vascular formation. The restenosis process appears to be triggered by the formation of a "neointima" composed mainly by migrating and proliferating smooth muscle cells. This response to injury appears to be initiated by thrombin [4] which seems to be an important mediator of vascular lesion formation or restenosis after PTCA. Actually, the expression of the thrombin receptor in injured arteries is strikingly increased over the level of expression observed in normal arteries [5]. The up-regulation of the expression of smooth muscle cell thrombin receptor is a very early event in the formation of vascular lesion and continues during neointimal development. In normal baboon carotid arteries, thrombin receptor mRNA is undetectable, while 30 days after surgical endarterectomy it is markedly increased compared to normal control vessels [6]. The expression of thrombin receptor mRNA and the resulting mitogenic effect mediated by thrombin is inhibited by TR-R9, a polyclonal anti-thrombin receptor antibody [6]. Further experimental studies are necessary to estimate the clinical potential of these observations. The question of whether thrombin inhibition will prevent neointimal proliferation in humans is currently being tested in clinical trials.

Thrombin activity is controlled by three major regulatory mechanisms. Thrombin is inhibited by antithrombin III and heparin cofactor II, two naturally occurring inhibitors. Thrombomodulin binds thrombin and changes its conformational structure so that it becomes unable to cleave fibrinogen and activate factors V, VIII and platelets but able to activate protein C. Activated protein C joins with protein S to inactivate factor Va and factor VIIIa. Finally, thrombin induces the release of plasminogen activator. Thus, thrombin generation is under tight control and local vascular injury might result in widespread thrombosis only if the inhibitory mechanisms fail.

Exogenous thrombin inhibitors inhibit prothrombin activation in plasma by delaying the onset of prothrombinase

* Tel. (+39-75)5783395 or 5722905; Fax (+39-75)5722011.
generation. The principal mechanism by which direct and indirect thrombin inhibitors delay the onset of prothrombinase generation is the reduction of endogenously generated thrombin. By the inhibition of endogenous thrombin, thrombin inhibitors delay the onset of the activation of factor VIII and factor V and thereby inhibit the generation of prothrombinase. The importance of inhibiting thrombin-mediated amplification reactions for effective anticoagulation becomes readily evident when the activity of pure endogenous anti-Xa inhibitors and pure thrombin inhibitors are compared. Selective thrombin inhibitors, such as dermatan sulphate and hirudin, are approximately ten times more effective than pentasaccharide, a selective factor Xa inhibitor. Even when direct inhibitors of factor Xa and thrombin inactivate the respective enzymes with the same Ki, the direct thrombin inhibitors inhibit intrinsic and extrinsic prothrombin activation more effectively than the direct factor Xa inhibitors. Whatever the mechanism, current information demonstrates that α-thrombin inhibition is more important for preventing prothrombin activation than increased factor Xa inhibition.

Various pieces of experimental evidence support the role of thrombin generated or exposed during thrombolytic treatment for myocardial infarction in inducing immediate or delayed re-thrombosis. At least two mechanisms responsible for increased thrombin activity during thrombolytic treatment have been elucidated. Thrombin is endowed in the residual wall standing thrombus and a "paradoxical" thrombin-stimulating effect of thrombolytic agents has been observed. Concerning the first mechanism, it is now clear that thrombin is incorporated in active form into the forming thrombi. Plasma contains sufficient prothrombin to generate 150,000 units of α-thrombin per liter, corresponding to 1.37 µM. During the activation in whole blood or recalcified plasma only approximately 10% of the potential α-thrombin is achieved, despite more than 80% consumption of the proenzyme. This apparent discrepancy is believed to be largely due to the incorporation and sequestration of active α-thrombin into fibrin clots. In clots undergoing lysis, fibrin-bound active thrombin is exposed and potentially released, promoting continuing fibrin formation and activation of platelets as well as of the coagulation system. This effect of thrombin has been confirmed in experimental animals where the selective thrombin inhibitor hirudin is able to "passivate" thrombus-bound thrombin, so achieving a persistent antithrombotic activity [7]. Concerning the second of the two mechanisms, in vitro thrombin activity increases in response to activation of plasminogen with streptokinase or rt-PA as a consequence of plasmin-mediated activation of the coagulation system [8-12]. Whatever its mechanism, thrombin generation can be easily estimated through the measurement of plasma concentration of specific markers of thrombin activity. Plasma levels of fibrinopeptide A (FPA), thrombin-antithrombin III complexes (TAT) and prothrombin fragment 1+2 measured during and after thrombolytic therapy have been found to be predictive for clinical outcome after thrombolytic therapy [10]. Plasma concentrations of FPA rapidly increase in patients receiving thrombolytic treatment. This increase in FPA is markedly reduced by the administration of intravenous heparin. An increase in TAT plasma levels during thrombolytic therapy has been observed in patients with nonsuccessful thrombolysis and early re-thrombosis, while patients with persistent patency exhibited a decrease in TAT plasma level [12]. Similar results have been obtained measuring plasma prothrombin fragment 1+2 before and after thrombolytic therapy [11].

Further evidence for the role of thrombin in vascular re-occlusion derives from the antithrombotic activity of hirudin and other thrombin inhibitors in experimental models of arterial thrombosis. Hirudin is effective in preventing arterial thrombosis produced by electrical, chemical injury and, probably of greater clinical relevance, by balloon angioplasty. In this last model, an intravenous infusion of hirudin completely inhibited thrombus formation. This result has been confirmed in rabbits. The role of thrombin inhibitors in potentiating thrombolytic agents has been clearly established [13]. Hirudin has been shown to accelerate thrombolysis following rt-PA and to prevent reocclusion in a canine model [14] whereas hirulog accelerated thrombolysis and prevented reocclusion in a rat model [15]. There is further experimental evidence supporting the therapeutic role of selective thrombin inhibitors in coronary intervention. Hirudin prevents platelet aggregation and fibrin deposition in a pig model of carotid angioplasty [16]. The same thrombin inhibitor prevents restenosis at 28 days in a rabbit angioplasty model, suggesting a potential role of thrombin in the proliferation of smooth muscle cells [17] as suggested by the finding of increased thrombin receptor expression in atherosclerotic human arteries [5]. Furthermore, hirudin is more effective than heparin in reducing both platelet and fibrin deposition on coronary stents [18].

Data from heparin-comparative clinical trials assessing the value of selective thrombin inhibitors, although absolutely preliminary, are quite controversial. After an initial report suggesting a high efficacy of these compounds [19], the more recently reported TIMI 9B has shown negative results. However, the results of these studies still probably reflect the need for the proper identification of an effective and safe dose.

References


