Increased Thrombin Activity During Thrombolysis

Dear Sir,

The role of coronary thrombosis in the pathogenesis of acute myocardial infarction (AMI) is well established, and has led to great efforts at early recanalization of the infarct-related vessel. However, in up to 20% of the patients after initially successful thrombolysis, reocclusion by a new thrombus occurs (1). It has not yet been proven in clinical studies that the frequency of reocclusion can be influenced by anticoagulation (2). Therefore it appears to be an important question, whether and at what time during thrombolysis an activation of coagulation is demonstrable.

There are two ways to detect an activation of coagulation by sensitive tests: the measurement of a thrombin-specific split product from fibrinogen, fibrinopeptide A (FPA) and the assessment of the complex thrombin-antithrombin III (TAT) in plasma. For FPA we used an ELISA test kit (Boehringer, Mannheim, F.R.G.). The blood samples were anticoagulated and the in vitro experiments stopped by means of a solution containing 1000 U heparin and 1000 U aprotinin per ml and stored at −70° C. Before assay the fibrinogen was removed by bentonite precipitation. For TAT an enzyme immunoassay recently introduced by Pelzer et al. (3) was used, which has become commercially available (Behringwerke, Marburg, F.R.G.) in the meantime.

The two parameters were determined before (time 0) and 3, 6, 9, 24 h and 1 week after start of treatment in: (i) 12 patients with AMI undergoing thrombolytic therapy (1.5 million units streptokinase [SK] over 30 min i. v., followed 6 h later by a permanent infusion of heparin adjusted according to the thrombin times); (ii) 14 patients with AMI who received no thrombolytic therapy but heparin infusion from the start; (iii) 6 patients with deep vein thrombosis (DVT) who were treated with a permanent infusion of urokinase (UK) over 6 days (100,000 units per h) together with a heparin infusion (500 units per h).

In the AMI and the DVT patients undergoing the thrombolytic therapy the FPA and TAT levels increased to peak values 3 or 6 h after commencing SK therapy, which were significantly higher than the pretreatment values (Table 1). The AMI patients treated with heparin alone exhibited only an insignificant increase in TAT and FPA values. In the further course, both TAT and FPA levels decreased; in the AMI patients earlier than in the DVT patients receiving thrombolytic therapy over several days.

These results suggest that indeed thrombin activity is increased early after the start of thrombolytic therapy. There may be several reasons for this observation. The infarct vessel is likely to expose its thrombogenic surface again after removal of the thrombus (4). Moreover, thromboplastic material may be washed out from the reperfused ischemic myocardium. On the other hand, thrombin absorbed to the fibrin clot may be literated by the fibrinolytic agents and regain its activity (5).

In order to test the latter hypothesis, some in vitro experiments were performed. Clots were prepared by recalcification of 1 ml citrated plasma. They were immersed into heparin (2 units/ml) plasma after rinsing them once in saline, washing them 1 h in 10 times renewed saline, or squeezing them carefully. As shown in Table 2, the TAT levels rose after the addition of the clots (time 0′), with the least increase after addition of the squeezed clots. A further increase occurred after the addition of SK (1000 units/ml). Surprisingly, a small increase also occurred in heparin plasmas without a clot; concomitantly, the FPA levels found in these plasmas increased (Table 3).

The increase of FPA immunoreactivity might have been caused by thrombin action, but also by plasmin digestion of fibrinogen. Though the degradation of the N-terminal A_α-chain is delayed and of only small extent at 60 min (6), some plasmic split products crossreacting with the test kit used may have been present. However, the increase of TAT demonstrates that thrombin is not only released from fibrin thrombi, but may even be generated in vitro in plasma free of a visible clot after activation of fibrinolysis. The latter result was unexpected and is difficult to explain. There are not yet any reports that plasmin or the SK-plasminogen complex might directly active coagulation. Another hypothesis might be that macromolecular derivatives of a threshold plasmic

Table 1 Mean values ± SEM of TAT and FPA levels (both in ng/ml) before treatment (0), peak levels after 3 or 6 h, and levels after 24 h and after 1 week

	0	Peak	24 h	1 week
a) FPA				
AMI, heparin only	6.3 ± 2.4	9.8 ± 3.0	3.4 ± 0.9	4.3 ± 1.0
AMI, SK-treated	7.9 ± 2.0**	16.5 ± 2.3	7.1 ± 2.1**	3.8 ± 1.0***
DVT, UK-treated	8.4 ± 3.6*	23.7 ± 4.9	12.4 ± 4.1	3.3 ± 0.5**
b) TAT				
AMI, heparin only	6.7 ± 2.6	8.6 ± 1.9	4.0 ± 0.7	2.9 ± 0.2**
AMI, SK-treated	8.9 ± 2.1**	16.4 ± 2.0	6.4 ± 1.1***	3.4 ± 0.3***
DVT, UK-treated	10.7 ± 1.3***	32.0 ± 4.1	17.7 ± 3.2*	10.4 ± 2.0**

Differences to the peak values: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 2 TAT levels in heparin plasma before (0) and after (0′) addition of a plasma clot, and 5 and 60 min after addition of SK. Control: heparin plasma without clot and SK

Time	Clot rinsed	Clot washed	Clot squeezed	Control
0	4.2	5.0	4.0	4.0
0′	85.0	70.0	9.0	4.2
5	105.0	80.0	32.0	4.0
60	115.0	90.0	40.0	3.8

Table 3 TAT and FPA levels in heparin plasma without clot before (0) and 5 and 60 minutes after addition of SK or saline

	TAT		FPA	
Time	SK	Saline	SK	Saline
0	2.3	2.3	1.3	1.7
5	2.5	2.2	4.2	1.5
60	4.7	2.4	31.0	1.6

lysis may also be present in normal plasma and may release thrombin upon further plasmin treatment (7).

In conclusion, the data demonstrate an enhancement of thrombin activity during thrombolysis in vivo as assessed by TAT and FPA determination. Since an increase of TAT and FPA was demonstrable both in AMI and DVT patients under thrombolytic treatment, but only an insignificant increase in AMI patients treated with heparin alone, this effect is likely to be related to the treatment itself. The thrombin might stem from several sources; our experiments show that thrombin can be elicited by SK from plasma clots and even from normal plasma. Further studies will be necessary in order to elucidate the significance of the phenomenon for thromboembolic events after thrombolysis, particularly the reocclusion of infarct vessels, and to evaluate the consequences with respect to an appropriate anticoagulation treatment.

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