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R. KORBUT, R. J. GRYGLEWSKI

PLATELETS IN FIBRINOLYTIC SYSTEM

Department of Pharmacology,
Medical College of Jagiellonian University, Cracow, Poland

Platelets play a vital role in mediating the activity of plasma fibrinolytic system. They have both the potential to inhibit as well as to activate fibrinolysis. Just as platelet can affect thrombolysis, thrombolytic agents can have reciprocal effects on platelet function. Accumulating evidence indicates that thrombolysis induced both by streptokinase and t-PA results in rapid activation of platelets, the phenomenon being possibly responsible for reocclusion of arteries after successful thrombolysis. However, caution is required in comparing the results of the various studies because of differences in the thrombotic models employed, with the major variables being the mechanism of thrombus formation *(in vivo* or *in vitro)*, the platelet concentrations and the doses of investigated agent. Various studies indicate that adjunctive therapy with anti-platelet agents, such as inhibitors of cyclooxygenase, inhibitors of thromboxane A_2-synthase and activators of platelet cyclic-AMP or -GMP may lower the dose of the thrombolytic agent required to attain reperfusion.

Key words: *Platelets, fibrinolysis, PAI-1, streptokinase, t-PA, plasmin, prostacyclin, aspirin, thromboxane A_2-inhibitors, c-AMP, c-GMP*

INTRODUCTION

Conflicting *in vitro* data have been obtained on the interactions between platelets and the fibrinolytic system, making it difficult to predict whether platelets facilitate or inhibit thrombolysis (1). Fibrinolytic effects include the presence of tissue plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) associated with platelets, the ability of platelets to bind plasminogen even under basal conditions, but with significant enhancement upon thrombin stimulation, and release of thrombospondin, a plasminogen-binding protein, from α-granules. It is not surprising therefore that under certain experimental conditions fibrin thrombi formed in the presence of platelets are more readily lysed than thrombi formed in their absence.

Platelets also have the potential to inhibit fibrinolysis by a variety of mechanisms, including releasing plasminogen activator 1 (PAI-1) and

α_2-antiplasmin from α granules and supplying factor XIII, which can both cross-link fibrin itself into a form that resists thrombolysis and cross-link α_2-antiplasmin to fibrin, resulting in localization of this inhibitor of thrombolysis in the thrombus. It has been suggested that platelets inhibit the binding of t-PA to fibrin through a mechanism related to clot retraction (2). On a more long-term basis, platelets may modify the fibrinolytic components in endothelial cells by release of growth factors such as TGF β from their granules (3).

It is difficult to extrapolate *in vitro* observations to predicting whether platelets augment or oppose *in vivo* thrombolysis, and in fact even a single model of fibrinolysis *in vitro* can demonstrate that low concentrations of platelets augment thrombolysis, whereas high concentrations of platelets inhibit (4). However, static *in vitro* models are unlikely to simulate the *in vivo* situation, wherein an ongoing supply of new platelets can contribute their α-granule contents to an evolving thrombus as they undergo activation. In the light of the above we have developed *in vivo* method for simultaneous monitoring of thrombolysis or thrombogenesis in anaesthetized cats under flow conditions (5). Using an extracorporal circulation in which the stream of arterial blood from the cat is continuously superfusing a collagen strip, we have demonstrated that endogenous prostacyclin is responsible for maintaining of thromboresistance while nitric oxide (NO) for controlling vascular tone, and these endogenous mediators do not interact with each other in any respect (6). Intravenous injections or infusions of t-PA or streptokinase (SK) produced an ε-aminocapronate-inhibitable pathway a biphasic effect on thromboresistance. The early distinct thrombogenic phase was followed by thrombolysis. Endogenous or exogenous prostacyclin were capable to abolish thrombogenesis and to enhance thrombolysis by t-PA and streptokinase (7). One newer *in vivo* model of platelet-rich thrombi using everted blood vessel segments demonstrated resistance to thrombolysis with t-PA (8). Adding a monoclonal antibody directed at GPIIb/IIIa to the t-PA dramatically augmented thrombolysis and prevented rapid reocclusion. This raises the possibility that the 10% to 25% of blood vessel that fail to reperfuse with thrombolytic therapy are the ones that are particularly rich in platelets. However, direct evidence for this hypothesis is not yet available.

EFFECTS OF THROMBOLYSIS ON PLATELET FUNCTION

Just as platelets can affect thrombolysis, thrombolytic agents can have reciprocal effects on platelet function, through either direct or indirect mechanisms (9). Accumulating evidence indicates that thrombolysis induced by both SK and t-PA results in rapid activation of platelets, as judged by nearly

immediate increases in thromboxane A_2 production and platelet aggregability. *In vitro* plasmin at high doses can cause platelet aggregation, perhaps by means of direct proteolysis of membrane receptors or proteolysis of an important protein regulator of platelet aggregation (10), but since plasmin-induced aggregation does not cause significant increases in thromboxane A_2, it is uncertain whether this is the mechanism that operates *in vivo*. In fact the mechanism by which SK and t-PA show pro-aggregatory and pro-thrombotic action has been a matter of considerable debate. Various actions of SK might be implicated as *primum movens* in the formation of thrombi, however, we have considered that the solitary explosive generation of plasmin is the most feasible explanation (7, 11). In our hands streptokinase and t-PA produced prothrombotic and thrombolytic phases both of which were abolished by ε-aminocaproic acid and aprotynin and therefore we believe in the "plasmin link" in SK-induced thrombogenesis. This acute thrombosis can be associated with activation of platelets by a sudden rise of plasmin in plasma. Perhaps this phenomenon constitutes one of mechanisms of "early hazards" during the thrombolytic therapy in AMI patients (11). Nonetheless, other options remain open. For example, plasminogen activator inhibitor (PAI-1) which has been early recognized as a risk factor for recurrent myocardial infarction (12), is interlinked with thrombin (13) and prevents thrombolysis *in vivo* (14). The release of PAI-1 from platelets is selectively inhibited by NO-donors (15, 16) and therefore, SK (or plasmin) would induce the release of PAI-1 then NO-donors should abolish thrombogenesis by SK. However, it was not the case in our experiments (7). In reality, SK-induced thrombosis was influenced neither by NO-donors (SIN-1), nor by L-NNA nor by aspirin. As a matter of fact aspirin was reported to inhibit fibrinolysis — an effect opposed by a prostacyclin analogue, iloprost (17). In our experiments iloprost not only opposed thrombogenesis by SK but also it boosted thrombolysis by SK (7). A similar action to iloprost was manifested by camonagrel, a new thromboxane synthase inhibition (18).

An alternative explanation is that thrombolysis paradoxically results in the generation of thrombin or the release of thrombin trapped in the thrombus and that platelets activation is a results of thrombin activity. The major evidence indicating that thrombolysis generates thrombin is the markedly increased fibrinopeptide A levels found in treated patients, but the recent data showing direct cleavage of fibrinopeptide A from fibrinogen by t-PA leaves in doubt how much if any of the observed increases in fibrinopeptide A can be attributed to thrombin.

Fibrinolysis also may cause platelet activation by a mechanical mechanism, because if a narrow channel is opened in a blood vessel with substantial flow, the shear forces may be adequate to intiate platelet activation and aggregation (19). Since this process may occur immediately after thrombolytic reperfusion

begins, it could contribute to rapid reocclusion. Thus soon after initiating thrombolytic therapy, a series of events occur that tend to enhance platelet activation, perhaps leading to increased platelet deposition and the risk of rapid reocclusion of blood vessels that undergo reperfusion. Inhibition of platelet function by thrombolytic agents also has been identified, making it difficult to predict the net effect of thrombolytic therapy on platelet function (1). Low doses of plasmin inhibit arachidonic acid metabolism, especially in the presence of prostacyclin, and t-PA may selectively lyse fibrinogen bound to platelets, resulting in platelet disaggregation.

Since the role of fibrinogen in platelet thrombus formation *in vivo* remains an unsettled problem, it is difficult to assess the contributions of these phenomena to platelet function. Although few data are available, plasmin also may disrupt endothelial cell function, leading to a loss of normal vascular integrity. If this occurs, it may expose subendothelial structures and lead to thrombin generation. Finally, plasmin proteolysis of platelets may lead to shortened intravascular survival, but few data are available to assess whether this happens with current thrombolytic regimens.

EFFECTS OF ANTIPLATELET AGENTS ON THROMBOLYSIS

Caution is required in comparing the results of the various studies because of differences in the thrombotic models employed, with the major variables being the mechanism of thrombus formation, and the dose of thrombolytic agent used. Virtually all of the models *in vivo* using t-PA included simultaneous systemic heparinization.

Aspirin has been assessed in several of the animal models. In the Fitzgerald model a thrombus is induced in awake, closed-chest dogs by passing current through a needle electrode surgically implanted in a coronary artery 5 to 7 days before the experiment. Aspirin had a modest effect, reducing the time to achieve thrombolysis by t-PA and decreasing the percentage of reoccluded blood vessels at 1 hour (20). In the Gold model, which employs a severe, fixed stenosis in open-chest dogs and uses t-PA to achieve thrombolysis, aspirin also had only a modest effect; in the control group, the coronary arteries of seven of eight animals reoccluded rapidly (mean time = 7 minutes) and in the aspirin-treated group five of seven reoccluded rapidly (mean time = 8 minutes) (21). In our models of experimental thrombosis, both *in vitro* (22) and *in vivo* (5), aspirin as well as indomethacin significantly reduced the formation of thrombi on the surface of collagen strip superfused with flowing blood, however, it did not affect thrombogenesis and thrombolysis induced by streptokinase and t-PA (7, 23). Thus, we have concluded that pharmacological activity of streptokinase can not be influenced by endogenous unstimulated release of prostacyclin.

Moreover, since effects of streptokinase are not influenced by N^G-nitro-L-arginine (L-NNA) — the inhibitor of NO-synthase — it seems to us that its pharmacological activity is independent on endogenous NO as well (23, 24). It looks like endogenous PGI_2 (a platelet suppressant) has been designed to collaborate with endogenous t-PA (an activator of fibrinolysis). Indeed, both endothelial secretogogues seem to be released in a coupled manner (24, 25) whereas, a brutal pharmacological intervention with SK escapes this collaborative network. When endogenous t-PA, PGI_2 and NO act in a joined venture then small amounts of plasmin, which are generated within a clot adhering to vascular wall, digest its fibrin component, PGI_2 dissipates platelet component of a clot and prevents platelets from being reactivated by plasmin, while NO counteracts vasoconstriction by platelet-derived TXA_2. Thus, a collaboration between the endothelial secretagogues is ensured. Far from it, when high doses of streptokinase or t-PA are administered intravenously and evoke a solitary and sudden rise of plasmin in plasma with no "blunting effect" of endogenous PGI_2 on reactivation of platelets by plasmin.

Agents that increase platelet cyclic AMP have been also studied directly in several experimental models. Schumacher, Lee, and Lucchesi demonstrated that intracoronary PGI_2 improved blood flow after thrombolysis was achieved with streptokinase in a nonstenotic, open-chest dog model, despite PGI_2-induced decreases in blood pressure (26). Vaughan et al. noted that PGE_1, even at doses that did not alter blood pressure and only a modest effect on platelet aggregation, accelerated the rate and augmented the extent of fibrinolysis produced by t-PA in a nonstenotic rabbit jugular vein model (27). Fitzgerald et al. also studied the effect of PGI_2 in their coronary thrombosis model and found that it did not reduce the time to reperfusion but did reduce the frequency of reoccludion from 100% to 9% (20). To achieve this beneficial effect, the PGI_2 had to be administered at doses that inhibited platelet aggregation, and even the lowest of these doses produced significant hypotention. Golino et al. found that iloprost, a stable analog of PGI_2, decreased reperfusion time and prevented reocclusion without affecting blood pressure in a nonstenotic dog coronary model using t-PA (28).

Recently, we have reported on the *in vivo* genuine paradoxical pro-thrombotic action of streptokinase and its prevention by endogenous or exogenous prostacyclin (7, 23). In the above *in vivo* studies a selective induction of biosynthesis of prostacyclin was achieved by a pharmacological inhibition of thromboxane synthase, which was followed by a transfer of platelet prostaglandin endoperoxides to the endothelial prostacyclin synthase, where they were utilized for biosynthesis of prostacyclin (23, 24, 25). In subsequent investigations we have confirm this original observation by introducing our *in vitro* technique for studying effects of thrombolytic agents on thromboresistance of blood superfused aortic endothelium (22).

Agents that increase platelet cyclic GMP. We have found for the first time that nitric oxide donors may exhibit fibrinolytic activity through increased t-PA activity as a result of inhibition of PAI-1 release from platelets (15, 16, 29). While we have shown that NO-donors can induce fibrinolysis at lower concentrations than those required for inhibition of platelet aggregation (16, 30), prostacyclin exhibits fibrinolytic activity (31) at concentrations very similar to its anti-aggregatory concentrations (32). Also the time course of NO-induced fibrinolysis differs from that of prostacyclin (33, 34). Thus, the mechanism of fibrinolytic activity induced by activators of guanylate cyclase, such as sodium nitroprusside (NP), is different from that of activators of adenylate cyclase. Although various hypotheses have been proposed to explain the fibrinolytic activity induced by prostacyclin (33), it is accepted that activators of adenylate cyclase produce a long-lasting stimulation of fibrinolysis by an unknown mechanism. Furthermore, since the effect of NP on thrombolysis is observed *in vivo* but not on platelet aggregation *ex vivo*, these results indicate that NO-donors may induce thrombolysis while producing minimal changes in platelet cyclic GMP levels. Although our evidence is indirect fibrinolysis may be stimulated by small increases in cyclic GMP, as with platelet adhesion (35), whereas larger increases in cyclic GMP are required for inhibition of platelet aggregation. Our findings concerning inhibition of PAI-1 release from platelets by NO-donors, has been confirmed later by other group of investigators (36).

A thromboxane synthesis inhibitors. So far pharmacological consequences of inhibition of thromboxane A_2 (TXA_2) synthase by imidazole derivatives (e.g. camonagrel or dazoxiben were linked to suppression of platelet activity. Recently, we have reported that in patients with peripheral atherosclerosis or in cats with extracorporal thrombogenesis, treatment with camonagrel is associated with activation of fibrinolysis or thrombolysis (24). These phenomena seem to be related to the camonagrel-induced shift in metabolism of prostaglandin endoperoxides in platelets from TXA_2 to endothelial prostacyclin (25). Although in an *in vitro* model the involvement of L-arginine/NO pathway can not be excluded we conclude that in man and in cats camonagrel activates fibrinolysis and thrombolysis through the release of endogenous PGI_2 (24). It is also tempting to speculate from our experiments that the same mechanism is responsible for obliterating effect of camonagrel on SK-induced thrombogenesis (7). A joint administration of camonagrel with SK reconstitutes a situation close to that when endogenous prostacyclin and t-PA can collaborate each other in arterial thrombosis. Our data strongly support a conception that not only exogenous but also endogenous prostacyclin (released by camonagrel) presents a sufficient pharmacological measure against unwanted pro-thrombotic activity of SK. Moreover, the use of prostacyclin or camonagrel offers additional bonus since both of these agents not only abolish thrombogenesis by SK but also intensify thrombolysis.

The compound CGS 13080 was tested in combination with intracoronary steptokinase in a moderately stenotic, closed-chest dog model; it had no effect on the rate of thrombolysis but reduced the reocclusion rate (37). This same agent was tested in combination with t-PA in a nonstenotic rabbit femoral artery model; compared to intraarterial t-PA alone, combined therapy produced quicker thrombolysis and allowed the t-PA concentration to be reduced at least tenfold while retaining significant thrombolytic activity (38). In other studies various thromboxane A_2/endoperoxide antagonists, such as SQ 29548 (39), BM 13.177 (40), AH 23848 and L 636,499 (41), have been also found to protect from reocclusion or prolong the reocclusion time in animals treated with the combination of t-PA and antagonist. Recently, Golino et al (39) showed that an agent R 68070 which both blocked thromboxane A_2/endoperoxide receptor and inhibited thromboxane synthesis was much more potent then "pure" receptor antagonist SQ 29548 in enhancing the speed of thrombolysis and preventing reocclusion.

Antithrombins also have been tested as adjuncts to thrombolytic agents. Fitzgerald found that the highest concentration of argatroban tested (2.5 mg/kg/hour), which prolonged the activated partial thromboplastin time to more than three times the control value, shortened the time to reperfusion and decreased the frequency of complete reocclusion (41). The animals treated with argatroban, however, did have cycles of decreased blood flow after reperfusion, indicating that platelet thrombus formation was not completely abolished. Addition of a thromboxane A_2/cyclic endoperoxide receptor antagonist (GR32191) abolished the cyclical flow reduction (41). Yao et al studied the effect of heparin and the combination of heparin and hirugen, a synthetic hirudinbased peptide, on thrombolysis initiated by t-PA in a dog model using a copper coil to induce coronary artery thrombosis (42). With thrombi that were allowed to mature for 30 minutes before initiating therapy, the frequency of reperfusion with t-PA alone, with heparin plus t-PA, and with heparin plus hirugen and t-PA was similar, but the time to achieve reperfusion was shorter in the two groups receiving the antithrombin therapy. The reocclusion frequency was not significantly affected, but the time to subsequent reocclusion was increased by heparin, and even more so by heparin with hirugen. When thrombi were allowed to mature for 180 minutes before beginning therapy, only one of six animals achieved reperfusion with t-PA alone, whereas four of seven achieved reperfusion with t-PA plus heparin. The arteries of all four animals treated with heparin reoccluded after 43 ± 8 minutes. Hirugen was not tested in animals with thrombi allowed to mature for 180 minutes.

Thus although antithrombin therapy appears consistently to shorten the time to achieve reperfusion, current regimens are inadequate to prevent platelet thrombus formation completely after thrombolysis occurs.

CONCLUSION

The data presented here support the hypothesis that platelets play a vital role in mediating reocclusion of coronary arteries after successful thrombolysis. Thus what begins as a fibrin-platelet thrombus may be converted into an equally deadly platelet rich-fibrin poor thrombus. Histological evidence supports this view (21). In addition, it is possible that the thrombolytic agents paradoxically contribute to platelet-mediated reocclusion by their platelet activating effects. Moreover, if high shear rates are generated in the newly reperfused blood vessels, this also may contribute to platelet thrombus formation (19). Since reocclusion occurs so rapidly in these models, it raises the possibillity that at least some of the patients who currently are said to fail to reperfuse with thrombolytic agents really do obtain reperfusion, but the vessels reocclude so rapidly that the reperfusion is not recognized. It thus is possible that combining potent antiplatelet therapy with thrombolytic therapy will increase the rate of "reperfusion".

The studies also demonstrated that reperfusion occurred more rapidly when potent antithrombin or antiplatelet agents were used in combination with thrombolytic agents. This suggests that at the beginning of the thrombolytic process, there is a phase in which the lysis of an established fibrin-platelet thrombus is matched by platelet deposition mediated in large part by thrombin, and that reperfusion therefore is delayed.

Finally, the animal studies indicate that adjunctive therapy with antiplatelet agens may lower the dose of the thrombolytic agent required to attain reperfusion. Since current data indicate that the risk of hemorrhagic phenomena is related to the dose of thrombolytic agent used (1), it is possible that the increased risk of bleeding caused by antiplateled agent may be offset at least partly by using lower doses of the thrombolytic agents. The role of heparin and the newer antithrombins in adjective therapy remains controversial.

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Author's address: R. Korbut, Department of Pharmacology, Medical College of Jagiellonian University, 16 Grzegórzecka, 31-531 Cracow, Poland