Effects of Reteplase and Alteplase on Platelet Aggregation and Major Receptor Expression During the First 24 Hours of Acute Myocardial Infarction Treatment

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Objectives. We sought to compare platelet characteristics after reteplase and alteplase therapy in the setting of the Global Use of Strategies to Open Occluded Coronary Arteries (GUSTO-III) trial.

Background. Platelet function may be impaired during thrombolysis in patients with an acute myocardial infarction. The effects of reteplase and alteplase on platelet aggregation and major surface antigen expression during the first 24 h of infarction therapy are unknown.

Methods. Platelet aggregation and receptor expression by flow cytometry were determined in 23 patients before thrombolysis and thereafter at 3, 6, 12 and 24 h.

Results. Aggregation was higher after reteplase at 24 h when induced by 5 μmol/liter adenosine diphosphate (ADP) (p = 0.007), 10 μmol/liter ADP (p = 0.02), collagen (p = 0.003) and thrombin (p = 0.009) than after alteplase. Reteplase therapy exhibited greater glycoprotein (GP) IIb/IIIa (p = 0.04), very late antigen-2 (p = 0.04) and platelet/endothelial cell adhesion molecule-1 (p = 0.002) expression at 24 h. Trends toward decreased receptor expression early (3 to 6 h), followed by a progressive increase at 12 h and especially at 24 h occurred after both agents.

Conclusions. In this prospective clinical ex vivo platelet study, similar patterns of platelet aggregation and surface receptor expression occurred during the first 24 h of coronary thrombolysis with reteplase and alteplase. However, after reteplase, indicators of platelet activity were higher at 24 h after thrombolysis than after alteplase. These data suggest that GP IIb/IIIa inhibitors or other antiplatelet strategies may be particularly advantageous when used 12 to 24 h after thrombolysis, especially after reteplase therapy. It is at this time point during the first day of coronary thrombolysis that GP IIb/IIIa is markedly expressed and platelets are most active.

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Thrombolytic therapy is the standard treatment for patients with acute myocardial infarction (AMI). Whereas streptokinase and alteplase are established agents, the clinical efficacy of reteplase is evolving. Platelets play an important role in the natural history of AMI. Intravascular platelet activation may limit reperfusion or cause reocclusion of the recanalized infarct-related arteries, thus resulting in an overall decreased effectiveness of thrombolytic therapy (1,2). Glycoprotein (GP) IIb/IIIa receptor antagonists (3–8) and monoclonal antibodies (9–11), combined with thrombolytic agents, have been shown in animal studies and clinical trials to improve reperfusion.

The attempt to determine the platelet-related properties of thrombolytic agents is not new. There is substantial evidence from in vitro (12–14) and animal studies (15,16) that impaired platelet function occurs after thrombolytic therapy. Although there is agreement that concentrations of substances released by platelets are elevated after thrombolysis (17–20), there is no consensus about the dynamics of ex vivo platelet status in this setting (21–24). Such discrepancies could relate partly to the particular thrombolytic agent, limitations of the techniques to assess platelet function, the lack of a standard method to sample blood and no specified time course to handle samples. Indeed, the majority of human myocardial infarction (MI) studies lack a careful, precise description of the time course of platelet monitoring. The random measurement of platelet function, outside strict time intervals during MI, has revealed very limited information about arterial patency, thrombolytic success and possible platelet involvement in reocclusion, reinfarction and bleeding. Many clinical studies have examined platelets at unspecified times or even days after thrombolysis (17,20,22–25), when the...
effects of adjunctive therapy cannot be excluded (26). Moreover, our knowledge of the status of platelets during the first 24 h after thrombolysis is very limited, especially from controlled clinical trials.

The purpose of the present study was to define the immediate and early platelet-related effects of reteplase and alteplase in AMI. We assessed platelet aggregation in response to multiple agonists and determined major surface receptor expression with flow cytometry at specified times after thrombolysis in patients enrolled in the randomized Global Use of Strategies to Open Occluded Coronary Arteries (GUSTO-III) trial.

Methods

The study was approved by the Institutional Review Board of St. Agnes Hospital and Union Memorial Hospital (Baltimore, Maryland). Written informed consent was obtained from all study participants.

Patients. Twenty-three consecutive patients admitted to the emergency departments of St. Agnes Hospital or Union Memorial Hospital between July and December 1996 with a diagnosis of AMI were included. All patients were enrolled in the GUSTO-III trial of reteplase versus accelerated alteplase treatment for AMI. The inclusion criteria have been reported elsewhere (27). In summary, patients of any age who presented within 6 h of symptom onset with >30 min of continuous symptoms of AMI and had ≥1 mm ST segment elevation in two or more limb leads, ≥2 mm ST segment elevation in two or more contiguous precordial leads or bundle branch block on the 12-lead electrocardiogram were eligible for enrollment. Patients were excluded for bleeding diathesis; previous stroke, major operation or significant trauma in the past 6 weeks; or hypertension >200/110 mm Hg.

Treatments and clinical outcomes. Patients randomized to receive reteplase received two 10-MU boluses given 30 min apart. Patients assigned to alteplase received an accelerated regimen: a 15-mg bolus, then 0.75 mg/kg body weight over 30 min, then 0.50 mg/kg over 1 h. All patients in the current substudy had received 325 mg of aspirin and at least 5,000 U of intravenous heparin at baseline sampling. After administration of thrombolytic therapy, all patients received a continuous infusion of heparin for the first 24 h after thrombolysis, as recommended in the GUSTO-III protocol. Eighteen patients had successful reperfusion and remained free of recurrent ischemia in the first 24 h of their hospital stay. Three patients (two with reteplase, one with alteplase) had persistent chest pain and ST elevation and underwent immediate angiography, which revealed the absence of reperfusion. Two patients (one with reteplase, one with alteplase) developed recurrent ischemia in the first 24 h and also underwent emergency angiography.

Time course and exclusion of blood samples. The schedules for blood drawing, sample preparation and processing were critical issues of the study design and were monitored by an independent observer (A.R.S.). The actual timing of blood collection for the baseline sample was 9.5 ± 1.4 min (mean ± SD) before the start of thrombolytic therapy, 174.6 ± 21.8 min for the 3-h sample, 371.1 ± 24.2 min for the 6-h sample, 709.4 ± 17.8 min for the 12-h sample and 1,402.9 ± 18.8 min for the 24-h sample. Samples were processed within 1 h after blood drawing. Four patients (three in the reteplase group, one in the alteplase group) did not complete the protocol at all time points. The reasons for early termination were patient transfer for emergency angioplasty (n = 3) and inability to obtain a blood sample (n = 1). Twenty-three baseline samples, 22 samples collected at 3 h, 20 samples collected at 6 h, 20 samples collected at 12 h and 19 samples collected at 24 h were included in the analysis.

Platelet aggregation. Blood samples for platelet aggregation and flow cytometric studies were taken at specified intervals: in the emergency department immediately before administration of the thrombolytic therapy and in the coronary care unit at 3, 6, 12 and 24 h after thrombolytic therapy began.

Citrate and whole blood were immediately mixed in a 1:9 ratio and centrifuged at 1,200 × g for 2.5 min. The resulting platelet-rich plasma was kept at room temperature for use within 1 h. Platelet counts were determined for each plasma sample with a Coulter counter ZM. Platelet numbers were adjusted to 3.50 × 10^9/ml with homologous platelet-poor plasma. Platelet aggregation was induced by 5 and 10 μmol/liter adenosine diphosphate (ADP), 1 μg/ml of collagen, 1 mg/ml of thrombin and 1.25 mg/ml of ristocetin. All agonists were obtained from Chronolog Corporation. Aggregation studies were performed with a Chronolog Whole Blood Lumi-Aggregometer (Model 560-Ca). Aggregation was expressed as the maximal percent change in light transmittance from baseline, using platelet-poor plasma as a reference at the end of the recording time. Aggregation curves were recorded for 4 min and analyzed according to international standards (28).

Flow cytometry. Flow cytometric procedures have been described elsewhere (29,30) and were performed according to the manufacturer’s recommendations for sample preparation and processing. Briefly, venous blood (8 ml) was collected in a plastic tube containing 2 ml of acid-citrate-dextrose (7.3 g of citric acid, 22.0 g of sodium citrate × 2H₂O and 24.5 g of glucose in 1 liter of distilled water) and mixed well. The mixture was centrifuged at 1,000 rpm for 10 min at room
temperature. The upper two-thirds of the platelet-rich plasma was then collected and adjusted to a pH of 6.5 by adding acid-citrate-dextrose. It was then centrifuged at 1,600 × g for 10 min. The supernatant was removed, and the platelet pellet was gently resuspended in 4 ml of washing buffer (10 mmol/liter Tris/HCl, 0.15 mol/liter NaCl, 20 mmol/liter EDTA, pH 7.4). Platelets were washed in the washing buffer and then in Tris-buffered saline (TBS) (10 mmol/liter Tris, 0.15 mol/liter NaCl, pH 7.4). All cells were then divided into 10 plastic capped tubes. Nine portions of washed platelets were incubated with 5 μl of fluorescein isothiocyanate (FITC)-conjugated antibodies in darkness at 4°C for 30 min, and one part remained unstained and served as a negative control. Surface antigen expression was measured with monoclonal murine antihuman antibodies to CD9 (p24), CD41 (GP IIb/IIIa, αIIbβ3), CD42b (GP Ib), CD61 (GP IIIa) (DAKO Corporation), CD49b (very late antigen [VLA]-2, or αIIbβ3), CD62p (P-selectin), CD31 (platelet/endothelial cell adhesion molecule [PECAM]-1), CD 41b (gp Ib), and CD51/CD61 (αIIbβ3, or vitronectin receptor) (PharMingen). The antibody to the GP IIb/IIIa receptor has been demonstrated to react with the platelet population at rest and exhibits a dose-dependent increase of receptor binding after ADP or thrombin stimulation, or both. After incubation, the cells were washed in TBS and resuspended in 0.25 ml of 1% paraformaldehyde. Samples were stored in the refrigerator at 4°C and analyzed with a Becton Dickinson FACScan flow cytometer with laser output of 15 mW, excitation at 488 nm and emission detection at 530 ± 30 nm. The instrument was calibrated daily with fluorescence beads (CalIBRITE, Becton Dickinson) and measured FITC-conjugated fluorescence intensity. All variables were obtained using four-decade logarithmic amplification. The data were collected, stored in list mode and then analyzed using CELLQuest (version 1.2.2) software.

**Statistical analysis.** A post hoc comparison using the t test with the Bonferroni correction was performed to identify specific differences in platelet aggregation and receptor expression between time points within each treatment group. Between-treatment comparisons were made at each time point using t tests (platelet aggregation). A Mann-Whitney U test was used to analyze nonparametric data (flow cytometry). Normally distributed data are expressed as mean value ± SD. Data distributed otherwise are summarized as medians (range), and p < 0.05 was considered significant. Differences between individual flow cytometric histograms were assessed using the Smirnov-Kolmogorov test incorporated in the CELLQuest software.

**Results**

Baseline clinical and demographic characteristics did not differ substantially between the reteplase and alteplase groups (Table 1). Three more patients received aspirin daily in the reteplase group. Groups were also similar in location of infarction and other baseline clinical and laboratory characteristics before thrombolysis.

| Table 1. Clinical Characteristics of 23 Patients With an Acute Myocardial Infarction |
|-----------------------------------------------|-----------------|-----------------|
| Replatse  | (n=13) | Alteplase  | (n=10) |
|-----------------|-----------------|-----------------|
| Male/female  | 10/3  | 8/2  |
| Age (yr)  | 59.4 ± 9.5  | 62.7 ± 8.8  |
| Range  | 38–79  | 36–86  |
| Current smoker  | 6  | 5  |
| Alcohol use  | 3  | 3  |
| Hypertension  | 8  | 6  |
| Diabetes  | 3  | 2  |
| Prior MI  | 3  | 1  |
| Hypercholesterolemia  | 5  | 4  |
| Baseline medications  |  |  |
| CCBs  | 3  | 3  |
| BBs  | 2  | 2  |
| ACEIs  | 1  | 0  |
| Nitrates  | 2  | 1  |
| Diuretic drugs  | 1  | 2  |
| Aspirin  | 5  | 2  |
| Ant/inf MI  | 8/5  | 6/4  |
| Laboratory data  |  |  |
| WBCs (×10^9/ml)  | 8.2 ± 1.6  | 7.9 ± 1.9  |
| RBCs (×10^6/ml)  | 3.76 ± 0.5  | 3.59 ± 0.5  |
| Platelets (×10^9/ml)  | 225.1 ± 11.7  | 238.4 ± 10.5  |
| Hemoglobin (g/dl)  | 15.2 ± 0.6  | 15.4 ± 0.5  |
| Hematocrit (%)  | 40.9 ± 3.8  | 43.7 ± 3.9  |
| Creatinine (mg/dl)  | 1.0 ± 0.3  | 0.9 ± 0.4  |
| Time from symptom onset to thrombolysis (min)  | 214.5 ± 19.8  | 208.22 ± 21.0  |

Data presented are mean value ± SD, range or number of patients. ACEIs = angiotensin converting enzyme inhibitors; Ant = anterior; BBs = beta-blockers; CCBs = calcium blockers; inf = inferior; MI = myocardial infarction; RBCs = red blood cells; WBCs = white blood cells.

**Platelet aggregation.** Baseline platelet function did not differ between groups. In patients treated with alteplase, platelet aggregability did not differ significantly between the baseline sample and that at any other time point in response to all agonists except ristocetin (Fig. 1). Platelets aggregated significantly more in response to 5 μmol/liter ADP in the reteplase group at 12 h (p = 0.04) and 24 h (p = 0.007) than in the alteplase group. Compared with baseline values, platelet aggregability was significantly reduced at 3 h (p = 0.02) and 6 h (p = 0.02) after reteplase therapy. A similar pattern occurred in response to 10 μmol/liter ADP. Platelets were significantly more active in the reteplase group after 24 h (p = 0.02) than in the alteplase group, and platelet aggregation was significantly reduced 3 h (p = 0.03) and 6 h (p = 0.04) after reteplase treatment compared with baseline values. In response to the collagen agonist, platelet aggregation was again significantly increased in the reteplase group at 24 h (p = 0.003) compared with that in the alteplase group. Significant reduction of platelet aggregability occurred 6 h (p = 0.009) after reteplase therapy compared with baseline values. As with collagen stimulation, the only difference observed between groups after thrombin inducement was at 24 h, when platelets aggregated
significantly more in the reteplase group (p = 0.01). With ristocetin, platelet aggregation was consistently high, ranging between 92.6% and 94.1% for reteplase and between 92.7% and 93.1% for alteplase during the first 24 h after thrombolysis, with no significant differences between and within groups.

Flow cytometry. Baseline platelet receptor expression did not differ significantly between the reteplase and alteplase groups, but substantial changes in receptor expression resulted after thrombolysis in both groups (Table 2, Fig. 2). Expression of p24 did not differ significantly between groups at any time point. Significant decreases in receptor expression compared with baseline values were seen at 3 h (p = 0.004) and 6 h (p = 0.005), followed by an increase (p = 0.04) at 24 h, after reteplase therapy. Within the alteplase group, no early receptor inhibition was observed, but at 24 h receptor expression was higher (p = 0.01) than that at baseline. Similar receptor expression patterns were seen with GP Ib in both groups, with significant inhibition 12 h after either reteplase (p = 0.02) or alteplase (p = 0.007) therapy, but there were no significant differences between groups.

Although a slight early decrease, followed by an increase, in GP IIb occurred in both thrombolytic groups, there were no significant differences between or within the groups. A significant increase in GP IIIa expression was observed at 12 h (p = 0.01) and 24 h (p = 0.0003) after reteplase, but not alteplase, therapy. Again, these differences were not significant between groups.

Compared with baseline values, GP IIb/IIIa (αIIbβ3) expression was decreased significantly (p = 0.03) at 3 h after reteplase therapy. Its expression was markedly increased at 24 h for reteplase (p = 0.002) and alteplase (p = 0.035) treatment, but significantly more so in the reteplase group (p = 0.037), which exhibited a nearly threefold increase compared with baseline values and was twofold higher than that in the alteplase group. Representative overlapped histograms of platelets labeled with fluorescent antibodies to GP IIb/IIIa from patients treated with reteplase (circles) versus alteplase (squares) for AMI. *p < 0.05 between groups.

The only significant difference in platelet VLA-2 (α2β1) expression between and within groups was an almost threefold increase in fluorescent intensity among reteplase-treated patients at 24 h (p = 0.04) compared with alteplase-treated patients.

Very similar profiles in P-selectin expression were observed between groups: Early significant decreases (p = 0.001 for the reteplase group; p = 0.009 for the alteplase group) were followed by significant increases in its expression (p = 0.009 for the reteplase group; p = 0.02 for the alteplase group).

For PECAM-1, a significant increase (p = 0.002) in fluorescent intensity occurred in the reteplase group after 24 h compared with the alteplase group. A significant decrease in its expression (p = 0.01) was seen 3 h after reteplase treatment compared with baseline values.

Dynamic changes in platelet vitronectin receptor (αVβ3) expression were similar between groups; a significant increase
occurred at 12 h after thrombolysis with reteplase (p = 0.04) or alteplase (p = 0.04). At 24 h after thrombolysis, vitronectin receptor expression was even greater in the reteplase group (p = 0.008), whereas its expression in the alteplase group trended toward baseline levels.

Discussion

We determined the ex vivo platelet-related properties of two thrombolytic agents in patients with an AMI with the simultaneous use of flow cytometry and conventional aggregation techniques. We found that reteplase and alteplase, as used in the GUSTO-III trial, have similar effects on platelet aggregation in response to four agonists. The groups differed only late after administration (>12 h), in that the reteplase group showed enhanced aggregability in response to ADP, collagen and thrombin. In addition, the reteplase group showed mild early dysfunction (at 3 to 6 h) that was followed by late enhanced aggregation. We also observed significant changes in the expression of multiple surface antigens after treatment with both agents, particularly GP IIb/IIIa, VLA-2 and PECAM-1 at 24 h.

Platelets and thrombolytic therapy. Failure to reperfuse, acute reocclusion and severe bleeding complications are major limitations of systemically given thrombolytic therapy (31,32). Platelet thrombus formation has been implicated in all these events. Thus, the effect of thrombolytic therapy on platelet characterististics are important. Vasoconstriction affected by platelet release of thromboxane and other mediators, diminished heparin effects induced by platelet factor 4 and enhanced thrombin generation on the platelet surface all adversely affect reperfusion (33). Platelets also interact with both physiologic and drug-induced fibrinolysis. For example, platelet granules contain plasminogen activator inhibitor-1 (PAI-1) and alpha2-antiplasmin (34,35). Release of these proteins can theoretically result in reduced thrombolysis. Specific binding sites for plasminogen (36) and tissue-type plasminogen activator (37) have been described on the platelet surface. The localization of these mediators of fibrinolysis to platelets suggests that they may play an important role in the modulation of clot lysis.

The effect of thrombolytic therapy on platelets has been extensively debated, with evidence of both platelet activation and inhibition. Most studies have used in vitro models and indirect measures of activity, such as the determination of levels of various mediators and other constituents released from platelets. The recent use of flow cytometry has enhanced our knowledge of events that occur on the platelet surface during activation, allowing the precise measurement of receptors expressed at specified times after thrombolysis. However, there are few prospective studies of platelet flow cytometry in patients with an AMI.

Platelet aggregation. In the alteplase group, no significant changes in aggregation occurred after treatment. These data are similar to the results of Bertolino et al. (21), who observed no difference in aggregation in response to 5 μmol/liter ADP or collagen at 24 h after conventional dosing of alteplase. However, the significant reduction in aggregation (∼40%) at 1 to 3 and at 6 h after drug administration contrasts with our findings. Ristocetin-induced aggregation was not affected by alteplase in either study. To our knowledge, there are no published data on the time course of ex vivo platelet aggregation in patients with an AMI treated with reteplase.

Receptor expression. In the current study, we saw significant changes in the expression of multiple surface antigens after treatment with both agents. Early after thrombolysis, we observed only minor (<20%), antigen-specific reductions in receptor expression, which could reflect excessive plasmin-induced cleavage or occupancy of the receptor by ligands (38). The earliest decreases in antigen expression occurred with platelet antigen 24, GP IIb/IIIa, P-selectin and PECAM-1.

Table 2. Platelet Surface Antigen Expression in Patients With Acute Myocardial Infarction During First 24 Hours After Thrombolytic Therapy

<table>
<thead>
<tr>
<th>Group</th>
<th>CD9</th>
<th>GP IIb</th>
<th>GP IIIa</th>
<th>P-selectin</th>
<th>Vitronectin receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>53.31 ± 18.41</td>
<td>28.76 ± 3.08</td>
<td>318.29 ± 48.28</td>
<td>31.78 ± 4.64</td>
<td>59.41 ± 22.18</td>
</tr>
<tr>
<td>3 h</td>
<td>41.12 ± 13.68</td>
<td>26.92 ± 5.89</td>
<td>309.54 ± 65.01</td>
<td>29.90 ± 4.68</td>
<td>56.20 ± 30.49</td>
</tr>
<tr>
<td>6 h</td>
<td>43.69 ± 13.46</td>
<td>27.62 ± 5.75</td>
<td>339.92 ± 65.36</td>
<td>30.63 ± 3.48</td>
<td>56.04 ± 33.68</td>
</tr>
<tr>
<td>12 h</td>
<td>48.62 ± 19.73</td>
<td>29.77 ± 5.64</td>
<td>349.77 ± 64.52</td>
<td>32.54 ± 4.26</td>
<td>73.69 ± 44.05</td>
</tr>
<tr>
<td>24 h</td>
<td>83.39 ± 58.33</td>
<td>29.39 ± 5.46</td>
<td>385.23 ± 62.68</td>
<td>33.92 ± 4.05</td>
<td>92.23 ± 58.54</td>
</tr>
<tr>
<td>SD</td>
<td>9.61</td>
<td>1.62</td>
<td>8.69</td>
<td>1.87</td>
<td>7.89</td>
</tr>
</tbody>
</table>

*p < 0.05 versus corresponding baseline measurement. Data presented are mean value ± SD, expressed as log fluorescence intensity.
Later in thrombolysis (>12 h), we observed a marked increase in specific receptor expression, which was pronounced for VLA-2, PECAM-1 and GP IIb/IIIa. Bihor et al. (22) studied three patients treated with alteplase by platelet flow cytometry using monoclonal antibodies to P-selectin and fibrinogen bound to GP IIb/IIIa (22). They described GP IIb/IIIa activation within the first 72 h after treatment, which is in agreement with our findings.

A decrease in platelet GP Ib expression, observed after 12 h of reteplase or alteplase therapy, supports previous in vitro reports that thrombolysis attenuates its expression (39,40) and contrasts with other in vitro observations in which alteplase caused no change in its expression (41).

When we compared the effects of reteplase and alteplase, we found few striking differences in surface antigen expression. Indeed, from nine receptors studied, at five consecutive time points, the only significant differences between groups occurred at 24 h. At this time point, greater expression of GP IIb/IIIa, VLA-2 and PECAM-1 was shown in the reteplase group. This finding, perhaps the most important of our study, suggests that adjunctive GP IIb/IIIa inhibitors may be appropriately used at a later time point after thrombolysis, when platelets are most active. The heightened expression of GP IIb/IIIa, VLA-2 (GP Ia/IIa [a major receptor that binds collagen] [42]) and PECAM-1 (CD 31) (43) may play a role in rethrombosis and infarct-related vessel reocclusion.

Our data correlate with the Reteplase Versus Alteplase Patency Investigation During Myocardial Infarction (RAPID) trial (31), where reteplase exhibited early patency superior to that of alteplase. We speculate that the early decrease in platelet aggregability and receptor expression after reteplase could be relevant to early reperfusion. However, the GUSTO-III mortality data did not differ between agents (27). This finding may be explained in part by increased platelet activation observed in the reteplase group later at 24 h after thrombolysis. An intriguing postulate is that late enhanced platelet activation may be a consequence of early reperfusion occurring either directly as platelets course through the reperfused myocardium or through systemically released mediators.

Another implication of the present study is the failure of platelet aggregation techniques to detect mild but significant changes in the platelet status in the alteplase-treated group, as revealed by flow cytometry. The temporal pattern of platelet aggregation paralleled the dynamics of receptor expression more closely in the reteplase group, where the magnitude of changes was greater.

Limitations of the study. There are several limitations of the present study, which was designed as a pilot investigation and therefore has a small sample size. Statistical differences between groups might be revealed in a larger cohort of patients. The present report is primarily descriptive and does not lead to an understanding of the mechanisms of platelet activity changes after thrombolysis. Moreover, the role of most of the platelet receptors studied in the pathogenesis of AMI is still undefined. In addition to the observed differences in
and platelets are most active. Coronary thrombolysis that GP IIb/IIIa is markedly expressed over 12 to 24 h, especially after reteplase therapy. It is at this time point during the first day of thrombolysis, especially after alteplase treatment. A shift to the right indicates platelet activation.

**Figure 3.** Representative overlapped histograms of platelets labeled with fluorescent antibodies to GP IIb/IIIa after reteplase (A) or alteplase (B) treatment. A shift to the right indicates platelet activation.

reteplase and alteplase groups, clinical characteristics, such as use of antecedent aspirin and time of thrombolytic agent delivery, may have influenced the results. Finally, it is possible that we missed an early peak of platelet activation, which has been reported in in vitro and animal experiments.

**Conclusions.** In this prospective clinical ex vivo platelet study, similar patterns of platelet aggregation and surface receptor expression occurred during the first 24 h of coronary thrombolysis with reteplase and alteplase. However, in patients treated with reteplase, indicators of platelet activity were higher at 24 h after thrombolysis than in alteplase-treated patients. These data suggest that platelet GP IIb/IIIa inhibitors or other antiplatelet strategies may be particularly advantageous when used 12 to 24 h after thrombolysis, especially after reteplase therapy. It is at this time point during the first day of coronary thrombolysis that GP IIb/IIIa is markedly expressed and platelets are most active.

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