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*Circulation*. 1998;97:5-9
doi: 10.1161/01.CIR.97.1.5

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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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Monitoring Platelet GP IIb/IIIa Antagonist Therapy

Barry S. Coller, MD

Platelet GP IIb/IIIa receptor antagonist therapy with abciximab (ReoPro), the Fab fragment of a mouse/human chimeric version of the murine 7E3 antibody, is currently used to prevent ischemic complications of percutaneous coronary interventions in select cases, and the efficacy and safety of abciximab for other related indications are under study.1-3 A number of low-molecular-weight GP IIb/IIIa antagonists patterned on the arginine-glycine-aspartic acid (RGD) cell recognition sequence have also shown benefit in the prevention and treatment of ischemic thrombotic coronary artery disease and currently are in advanced stages of development and approval.1,4

See Vol 96 p 3860

The ease with which samples of blood platelets can be obtained, the availability of well-characterized methods for assessing platelet function, and the development of a radiolabeled antibody assay to assess the percentage of GP IIb/IIIa receptors blocked by 7E3 permitted the incorporation into the preclinical and early clinical trials of 7E3 extensive correlative studies of the antithrombotic effects of 7E3 compared with its effects on bleeding time, platelet aggregation, and GP IIb/IIIa receptor blockade.5-8 These studies provided a framework for deciding on a dosing schedule for the first phase III study (EPIC).9 Similar studies have been reported with some of the other GP IIb/IIIa antagonists.10-12 The more rapid GP IIb/IIIa off-rates of many of the low-molecular-weight compounds compared with 7E3 have made it technically more difficult to directly assess GP IIb/IIIa receptor blockade with these agents, but binding studies have been reported that used fluorescent compounds in conjunction with flow cytometry, radiolabeled compounds, or the expression of ligand-induced binding sites on GP IIb/IIIa induced by the binding of the compounds.13-16

The introduction of GP IIb/IIIa antagonists as a new class of therapeutic agents raises several important questions, especially because oral, and perhaps even transdermal and intranasal, agents may eventually be available for long-term therapy. Several questions must be addressed. First, would dose monitoring and patient-specific dose adjustment improve safety and/or efficacy of the therapy? Second, if monitoring is desirable, would it be preferable to measure plasma levels of the drug, GP IIb/IIIa receptor blockade by the drug, or the effect of the drug on platelet function? Third, can an assay be developed that will be simple, rapid, robust, and inexpensive enough to be of value in clinical practice?

Conceptually, the first question can be subdivided into three separate questions. The first is whether there is significant interindividual variations in assay responses when the currently recommended doses of the agents are used. The second is whether the observed variations in response correlate with clinical outcome. For example, do patients who have the least inhibition of platelet function or the lowest percentage of blocked GP IIb/IIIa receptors have more hemorrhagic complications? If such correlations are established, the third question is whether modifying drug dose on the basis of the results of the monitoring assay actually improves the efficacy or safety.

Theoretical arguments can be advanced to support the view that monitoring may not be necessary or desirable. Thus, low-molecular-weight heparins appear to be safe and efficacious without monitoring or dose adjustment, as are many other medications. On the other hand, there are reasons to believe that dose monitoring may be beneficial, especially since the dose-response curves of GP IIb/IIIa antagonists are generally considered “steep.” Thus, there is relatively little inhibition of turbidimetric platelet aggregation initiated by ADP and similar agonists until ≈50% of the receptors are blocked.6,17 Because carriers of Glanzmann thrombasthenia, who have ≈50% to 60% of the normal number of platelet GP IIb/IIIa receptors, rarely have a hemorrhagic diathesis, it is unlikely that doses of drugs that produce 40% to 50% receptor blockade will produce significant impairment of hemostasis.18 At ≥80% GP IIb/IIIa receptor blockade, turbidimetric platelet aggregation with conventional agonists is usually nearly abolished.6,9,17 One should not, however, conclude from this last datum that all GP IIb/IIIa-dependent platelet function is maximally inhibited when turbidimetric aggregation is abolished. For example, we found that in nonhuman primates, giving an additional dose of either antibody 10E5 or 7E3, both of which react with GP IIb/IIIa, to animals who already had complete inhibition of platelet aggregation resulted in further prolongation of the bleeding time, demonstrating that the additional antibody had functional consequences that could not be assessed by turbidimetric aggregometry.7

The rationale for the current dosing of abciximab as an initial bolus followed by a 12-hour infusion was based on studies demonstrating that this dose was required to achieve and sustain ≥80% receptor blockade in most patients8 and that such high-grade receptor blockade was likely to be required to...
Probability of no urgent repeated percutaneous revascularization procedures in the three treatment groups (Kaplan-Meier plots) during the first 48 hours. Events began to occur shortly after the index procedure in the placebo group, between 6 and 12 hours after the procedure in the group given the bolus of c7E3 Fab, and even later in the group given both the bolus and the infusion. The y axis is truncated at 97% to demonstrate the difference in this end point, which occurred with low frequency. Reprinted with permission (N Engl J Med. 1994;330:956-961).

Monitoring GP IIb/IIIa Antagonist Therapy

prevent ischemic complications in response to severe thrombotic provocations such as balloon angioplasty-induced damage to a stenotic, atherosclerotic blood vessel.5,6 The most compelling evidence to support the need for high-grade GP IIb/IIIa receptor blockade to prevent ischemic complications of percutaneous coronary interventions (PCI) comes from the EPIC study in which patients receiving only a bolus of abciximab appeared to be fully protected from the need for urgent repeated interventions for a 4- to 6-hour period,9 during which time it was likely that GP IIb/IIIa receptor blockade was ≥80% (Figure).8 In contrast, patients in the placebo group showed no protection in the first 4 to 6 hours, and patients receiving the bolus and 12-hour infusion were protected for nearly the entire time of the infusion.7 Thus, the goal with abciximab treatment for PCI was to achieve and sustain a threshold of receptor blockade ≥80% until the damaged blood vessel was no longer highly reactive to platelets (ie, until the surface was passivated). Whether this same extent of receptor blockade will be necessary or desirable for the other abciximab indications under investigation remains to be established. No upper limit on GP IIb/IIIa receptor blockade by abciximab for PCI was set because it was hoped that by limiting the therapy to only a relatively brief period of time, the risk of serious hemorrhage would be acceptable. The recent data from the EPILOG study appear to support this strategy, because with reductions in heparin dosing and careful attention to the access site wound, major bleeding events were not increased in patients treated with abciximab.9 However, it remains possible that bleeding complications could be reduced further by avoiding GP IIb/IIIa blockade values above a certain level.

Thus, for acute GP IIb/IIIa blockade therapy for PCI, monitoring may not be necessary if a dosing regimen can be identified that consistently achieves high-grade receptor blockade in all patients and if clinical data do not suggest an increase in clinical hemorrhage in patients having the greatest receptor blockade. Previous studies of the pharmacokinetics of abciximab,8,20,21 and the data from Mascelli et al22 support the hypothesis that most patients appear to achieve and sustain the 80% threshold level with the current dose of 0.25 mg/kg bolus and infusions of either 10 μg/min or 0.125 μg·kg⁻¹·min⁻¹. However, some interindividual variations in receptor blockade have been observed,20,21 and as seen in Fig 1 of the study by Mascelli et al,22 mean GP IIb/IIIa receptor blockade was ≈80% at 6 and 12 hours after therapy was initiated, so some patients may have been below this level. Moreover, because the theoretical peak whole-blood level of abciximab exceeds the amount of antibody required to fully saturate the GP IIb/IIIa receptors on a normal number of circulating platelets by only approximately twofold, it is predictable that patients with severe thrombocytosis will not achieve as high a degree of receptor blockade as patients with normal platelet counts, and one published case has documented this phenomenon.20 The platelet counts of the patients in the first stage of the study by Mascelli et al22 were not provided, but the highest count in the second stage was only 337 000 μL. Thus, a systematic assessment of the effect of elevated platelet counts on the efficacy of abciximab would be desirable, and dose adjustments in this subgroup have the potential to improve efficacy.

Long-term GP IIb/IIIa receptor blockade for primary or secondary prophylaxis of ischemic vascular disease will likely require a different strategy from that used for short-term therapy. The ongoing mucocutaneous bleeding suffered by patients with Glanzmann thrombasthenia, including variable degrees of excess bruising, epistaxis, and gingival bleeding,23 is likely to be unacceptable to patients treated with GP IIb/IIIa antagonists for prophylaxis. Therefore, a targeted upper limit of GP IIb/IIIa receptor blockade is likely to be desirable. Similarly, a lower limit will almost certainly be required to achieve efficacy. Thus, a therapeutic window will most likely need to be defined, as has been done for warfarin therapy with the international normalized ratio. Maintaining patients within such a window by use of a single dosing regimen may be difficult because the pharmacokinetics of the low-molecular-weight agents are likely to be sensitive to interindividual differences in renal function and/or hepatic metabolism, and these differences may be considerable in a large population of patients with vascular disease.

Assessment of receptor blockade after discontinuing GP IIb/IIIa antagonist therapy may also be valuable in determining the return of platelet function toward normal. This information may help physicians decide whether platelet transfusion therapy is necessary or desirable before surgery or an invasive procedure. As noted above, in general, there is little or no hemostatic compromise at receptor blockade levels <50%.

If GP IIb/IIIa dose monitoring is desirable, which parameter should be monitored? Traditionally, drug blood levels are taken as surrogates for drug effects, but in the case of GP IIb/IIIa antagonists, interindividual variations in platelet count, density of GP IIb/IIIa receptors, intrinsic platelet functional competence, tendency to sustain hemorrhage or thrombosis, plasma levels of platelet cofactors, and other unknown factors may affect the functional response to a given plasma level of a GP IIb/IIIa antagonist. It is enormously tempting, therefore, to take advantage of the ready access to blood by simple venipuncture and study the impact of the therapy directly on the end organ itself, the platelet. It is unclear, however, whether the most valuable parameter is the percentage (or number) of GP IIb/IIIa receptors blocked (or free) or the effect of the receptor blockade on platelet function. Expressing GP IIb/IIIa doses in relationship to receptor blockade has the advantage of...
avoiding consideration of the many variables that may affect the impact of a GP IIb/IIIa antagonist on any single platelet function such as the anticoagulant, platelet preparation, choice and dose of agonist, end point measured, and equipment used. Conceptually, therefore, expressing GP IIb/IIIa antagonist doses in relation to their blockade of GP IIb/IIIa receptors has the distinct advantage of normalizing the information.

There are significant drawbacks, however, to devising a monitoring system based on receptor blockade. These include (1) the need for different reagents and perhaps assay techniques for each drug; (2) theoretical concerns about the impact of variability in drug uptake by platelets on the results of binding studies; (3) the need for both expensive equipment (flow cytometer or radiation counter) and technical expertise, which essentially preclude widespread point-of-care, office-based, or home testing; and (4) the difficulty in performing and interpreting receptor blockade studies during transition periods when two different GP IIb/IIIa antagonists are present simultaneously, as when switching from an intravenous to an oral agent. Moreover, the original correlations between receptor blockade and inhibition of platelet function were based on studies of apparently normal animals and humans who met rigorous inclusion and exclusion criteria for entry into clinical trials; extrapolation of these data to a much wider population of patients with chronic illnesses that may affect platelet function (such as renal or hepatic insufficiency), greater variations in platelet count, and more intercurrent medications may not be justified.

Using platelet function testing to monitor GP IIb/IIIa antagonist therapy has the theoretical advantage of directly assessing the goal of therapy as well as integrating the effects of nearly all of the variables listed above. For example, a patient may be receiving a dose of a GP IIb/IIIa antagonist that produces GP IIb/IIIa receptor blockade within the desired range, but if that patient has a borderline low platelet count, is taking other medications that affect platelet function, or has illnesses that affect platelet function, the inhibitory impact may be excessive. Another potential advantage of using a functional assay is that a single assay may be applicable to monitor all of the available agents. There are, however, an enormous number of different tests of platelet function, and variations in technique and instrumentation from laboratory to laboratory present significant problems in standardization. Thus, we arrive at our third question, whether an assay exists or can be developed that will meet the exacting requirements for clinical utility.

The bleeding time is considered an important test of platelet function, but it is poorly standardized and labor intensive; moreover, operators need extensive training in performing the test and judging the subjective end point, making it impractical and undesirable as a monitoring assay. In addition, bleeding time prolongation has not been helpful in identifying patients treated with abciximab who were most likely to have bleeding complications, an observation consistent with older data demonstrating that the bleeding time is a very poor predictor of clinical hemorrhage in other clinical settings. There is also considerable confusion about the interpretation of the bleeding time in relation to GP IIb/IIIa antagonist therapy. Patients with Glanzmann thrombasthenia have markedly prolonged bleeding times, so one could argue that any agent that blocks nearly all of the GP IIb/IIIa receptors should produce a long bleeding time. Thus, failure to prolong the bleeding time may indicate a lack of therapeutic potency. Some have implied, however, that prolongation of the bleeding time by GP IIb/IIIa antagonists is to be avoided because this indicates a predisposition to clinical hemorrhage. Claims have been made that some agents can inhibit platelet function without significantly prolonging the bleeding time, leaving the impression of perhaps a greater therapeutic index for these drugs. As noted above, however, it is possible to essentially eliminate turbidimetric platelet aggregation while having only a modest effect on the bleeding time if receptor blockade does not reach the highest levels, so it is not clear that the agents claimed to have little effect on the bleeding time are not just limited in their ability to inhibit GP IIb/IIIa receptors. To date, no human clinical trials have substantiated claims of clinical efficacy without prolongation of the bleeding time. Moreover, as shown in the EPILOG study, marked prolongation of the bleeding, as with abciximab, does not necessarily translate into increased major bleeding.

Conventional turbidimetric platelet aggregometry using citrated platelet-rich plasma is the most widespread and accepted method of testing platelet function, but it requires sample preparation, extensive quality control, operator expertise, and expensive equipment. Moreover, it appears that the calcium chelation caused by citrate anticoagulation may artifactualy enhance the inhibition observed with some GP IIb/IIIa antagonists such as eptifibatide (Integrilin). Recognizing the practical limitations of turbidimetric aggregometry, used whole-blood aggregometry (modified to include automated calibration and readout functions), which relies on impedance measurements and requires only dilution of whole blood. It does, however, require relatively expensive equipment, the need to prepare and pipette reagents, proper care of the electrode, and appropriate quality control procedures. Although it appears to be suitable for use in catheterization laboratories with a high volume of patients treated with abciximab, it is less likely to be useful in monitoring long-term therapy in large numbers of patients as a point-of-care assay. Moreover, the whole-blood dilution step may limit its utility in monitoring low-molecular-weight agents with rapid GP IIb/IIIa off-rates, because even modest dilutions of blood for brief periods of time may decrease the observed inhibition. Although Mascelli et al recognized the practical limitations of turbidimetric aggregometry, used whole-blood aggregometry (modified to include automated calibration and readout functions), which relies on impedance measurements and requires only dilution of whole blood. It does, however, require relatively expensive equipment, the need to prepare and pipette reagents, proper care of the electrode, and appropriate quality control procedures. Although it appears to be suitable for use in catheterization laboratories with a high volume of patients treated with abciximab, it is less likely to be useful in monitoring long-term therapy in large numbers of patients as a point-of-care assay. Moreover, the whole-blood dilution step may limit its utility in monitoring low-molecular-weight agents with rapid GP IIb/IIIa off-rates, because even modest dilutions of blood for brief periods of time may decrease the observed inhibition. Although Mascelli et al used a citrate anticoagulant, the assay can be performed using other anticoagulants.

Mascelli et al made the important new observation that the inhibition of platelet function produced by abciximab in patients receiving aspirin was greater and lasted longer when assessed by whole-blood aggregometry rather than turbidimetric aggregometry (see their Figs 1 and 2). This raises the possibility that previous studies using turbidimetric aggregometry underestimated both the platelet inhibitory effect of abciximab and the duration of its effect. A similar trend was found in patients treated with aspirin and heparin (see their Fig 4), but the differences in results with the two techniques were not as dramatic. The authors propose several possible explanations for the observed differences in results, to which might be added the transcellular metabolism between platelets and
erythrocytes and the possibility that erythrocyte hemoglobin binds nitric oxide produced by platelets. Mascelli et al also found that impedance aggregometry using 5 μg/mL collagen correlated better with GP IIb/IIIa receptor blockade than did turbidimetric platelet aggregation with any of the agents they tested. The choice of the collagen dose used in the study was based, however, on a careful titration experiment; because collagen is prepared by extraction from biologic material, it is not clear whether lot-to-lot variability would require repeated titrations.

A number of other functional assays are currently being studied for monitoring GP IIb/IIIa antagonist therapy, including thromboelastography; assays based on the occlusion of apertures in membranes or tubing by platelet thrombi; and assays of shear-induced platelet deposition and platelet-supported thrombin generation. Our own attempt involves an assay based on the ability of activated platelets in anticoagulated whole blood to agglutinate fibrinogen-coated beads. The original assay has been reconfigured into a cartridge-based, microprocessor-controlled system that is compatible with a variety of anticoagulants, requires no blood preparation or dilution, and has a digital printout.

In conclusion, although there are excellent theoretical reasons to believe that dose monitoring of GP IIb/IIIa antagonist therapy, coupled with dose adjustments, will improve the safety and efficacy of these agents, especially if low-molecular-weight agents are used for long-term therapy, the difficulties in developing an ideal assay are considerable, and ultimately it remains to be proved through clinical trials that the expense and effort are justified on the basis of improved outcomes.

Acknowledgments

This work was supported in part by grant 19278 from the NHLBI. I wish to thank Suzanne Rivera and Jennifer Berrucca for outstanding secretarial assistance. Dr. Coller is a coinventor of an inventor of the technology underlying the rapid platelet function assay being developed by Accumetrics, San Diego, CA, a member of the scientific advisory board of Accumetrics, and a stockholder in Accumetrics.

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KEY WORDS: angioplasty thrombosis glycoproteins receptors platelet aggregation inhibitors