The glycoprotein IIb/IIIa antagonist c7E3 inhibits platelet aggregation in the presence of heparin-associated antibodies

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Purpose: **Heparin-associated antibodies (HAAb), in the presence of heparin, can cause platelet activation and aggregation. The purpose of this study was to assess whether a platelet glycoprotein (GP) IIb/IIIa receptor antagonist, c7E3, would inhibit platelet aggregation in the presence of HAAb. If aggregation is inhibited by c7E3, enzyme-linked immunosorbent assays (ELISA) would be done to determine whether c7E3 interfered with the binding of heparin and the HAAb.**

Methods: **HAAb-positive plasmas from 21 patients (determined by platelet aggregation assays) were studied. Normal donor platelet-rich plasmas (PRP) were incubated (1 minute) with either saline solution or 3 μg/ml of c7E3. Platelet-poor plasma from patients with HAAb and one of three sources of heparin (25 μl, 10 U/ml; porcine heparin, bovine heparin, and low molecular weight heparin [enoxaparin]) were added to the PRP mixture. Aggregation was determined using a platelet aggregometer by measuring time to aggregation, the slope of the aggregation curve, and the percent change in optical density.**

Results: **Platelet aggregation occured in 100%, 100%, and 95% of the saline solution incubations exposed to porcine heparin, bovine heparin, and enoxaparin, respectively. Incubation with c7E3 caused 100% inhibition of platelet aggregation in plasma exposed to porcine heparin, bovine heparin, and enoxaparin. The optical density curves obtained from the ELISA, which were dependent on the binding of HAAb to heparin, were not significantly different when c7E3 was compared to buffer alone.**

Conclusions: **The GP IIb/IIIa receptor antagonist, c7E3, inhibits HAAb-induced platelet aggregation via a mechanism that does not appear to interfere with the binding between heparin and HAAb. Clinical trials are warranted to assess whether GP IIb/IIIa antagonists may allow patients with HAAb to safely receive heparin. (J Vasc Surg 1997;25:124-30.)**

Heparin-induced thrombocytopenia (HIT) occurs as a complication in 5% to 6% of patients who receive heparin therapy.[1,2] As many as 61% of patients with HIT develop arterial or venous thrombotic complications related to platelet activation and aggregation.[3] To date, the only completely effective treatment for HIT is cessation of heparin therapy. Attempts to treat HIT with platelet function–inhibiting agents such as aspirin, dipyridamole, and iloprost have met with variable success.

Recently, the central role of the platelet glycoprotein IIb/IIIa (GP IIb/IIIa) receptor in mediating platelet aggregation has been elucidated. Binding of the GP IIb/IIIa receptor to fibrinogen and other adhesive glycoproteins (von Willebrand factor, vitronectin, and fibronectin) is one of the last steps in the pathway leading to platelet aggregation, regardless of the platelet agonist.[4]

Agents have been developed that bind to the GP IIb/IIIa receptor on human platelets and effectively inhibit aggregation.[4] The Fab (fragment antigen binding) fragment of a chimeric murine monoclonal antibody (c7E3) that binds to the GP IIb/IIIa receptor has undergone extensive evaluation in clinical trials. The purpose of this study was to determine whether the c7E3 Fab fragment could inhibit platelet aggregation in the presence of heparin and hepa-

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rin-associated antibodies (HAAb), and whether the c7E3 interfered with the binding of heparin to the HAAb.

METHODS

The study was approved by the Institutional Review Board at the University of Missouri-Columbia. Plasma from 21 patients with known HAAb, as determined by platelet aggregation testing and patient clinical course, was obtained from the Thromboembolic Laboratory at the University of Missouri-Columbia. Platelet-rich plasma from normal donors and platelet-poor plasma from affected patients were prepared by differential centrifugation of whole blood collected in a 3.8% citrate solution.

The platelet-rich plasmas (0.1 ml) were incubated with either 10 μl (3 μg/ml) of the c7E3 Fab fragment (Abciximab, Eli Lilly and Co., Indianapolis) or 10 μl of normal saline for 1 minute at room temperature. Platelet-poor plasmas (0.15 ml) from patients with HAAb were then added and the mixtures incubated for 3 minutes at 37° C. Each mixture was then incubated in an aggregometer with one of three sources of heparin (25 μl, 10 U/ml) for an additional 15 minutes: porcine intestinal mucosa heparin (Solopak Laboratories, Elk Grove, Ill.), bovine lung heparin (Upjohn Company, Kalamazoo, Mich.), and low molecular weight heparin (enoxaparin) (Lovenox, Rhone-poulenc Rorer Pharmaceuticals, Inc., Collegeville, Pa.). Platelet aggregation was measured using a dual channel aggregometer (Chrono-log Corporation, Havertown, Pa.).

An aggregometer transmits light through suspended platelets. As aggregation occurs, the optical density (OD) of the column of plasma decreases and is associated with an increased percent light transmittance. Although any deflection of the OD curve indicates that some platelet aggregation is occuring, a more accepted indication for aggregation is a greater than 20% change in OD. Aggregation parameters measured included the slope of the aggregation curve, maximum percent change in OD, and lag time until the start of aggregation. If aggregation did not occur, 25 μl of adenosine diphosphate (ADP) (10^{-5} mol/L solution) was added to assess whether ADP-associated platelet aggregation was also inhibited by the c7E3 Fab fragment.

The proportion of HAAb-positive plasmas that demonstrated platelet aggregation in the presence of saline solution was compared with the proportion in the presence of c7E3. A positive result of an aggregation study was defined in two ways. The first definition was any upward deflection in the aggregation curve; the second definition was a greater than 20% change in optical density. The results were compared using a McNemar's test, considering a two-sided alternative. Significant results are those with a p value less than 0.01.

An enzyme-linked immunosorbant assay (ELISA) was developed to assess whether the c7E3 Fab fragment interferes with the binding of heparin to the heparin-associated antibodies. Microtiter plates were coated with porcine heparin (1 Unit). Nonspecific binding sites on the microtiter plates were blocked with 3% bovine salt albumin (BSA) in phosphate-buffered saline solution (PBS). Platelet-poor pooled plasma from 40 patients with HAAb (100 μl) was incubated in the microtiter wells (n = 5) with either PBS or 30 μg/ml c7E3 Fab fragment. A second set of five incubations were performed in separate wells with normal platelet-poor plasma (100 μl). Goat antihuman-IgG (100 μl, 1:500 concentration) (Sigma Chemical Co., St. Louis), conjugated with alkaline phosphatase, was added to all wells. To detect the presence of heparin-associated antibody and antihuman-IgG, p-nitrophenyl phosphate (1 mg/ml) (Sigma Chemical Co.) was added as a colorimetric substrate for the alkaline phosphatase. The reaction was stopped after 60 minutes with 2 N NaOH, and the plates were read at 405 nm wavelength with a microplate reader (Anthos HTII, Anthos Labtec Instruments, Salzburg, Austria).

The ELISA, when performed using undiluted plasma, resulted in spectrophotometric absorbances outside of the practical range. More usable measurements were obtained when the HAAb-positive and HAAb-negative plasmas were serial diluted, with a range from 1:8 to 1:1024. This resulted in dilution curves, expressed as OD, which were characteristic for each plasma sample and for each incubation with either c7E3 or PBS. The OD from incubations containing PBS were compared with the OD from c7E3 incubations. In addition, the OD from the ELISA performed with HAAb-positive plasma was compared with the OD from HAAb-negative plasma. The dilution curves were fit to a quadratic equation, and a multivariate ANOVA was used for the statistical analysis. Significant results are those with a p value less than 0.01. In addition, 95% confidence intervals were determined when comparisons were made between various incubation curves.

RESULTS

The aggregation results were expressed as the average ± standard deviation of the 21 patient samples. If positive aggregation is defined as any upward

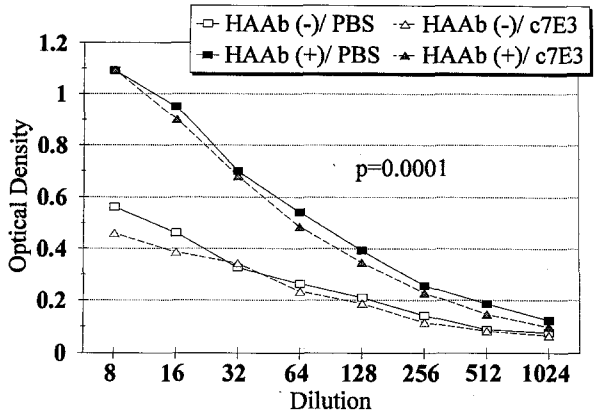

Fig. 1. Dilution versus optical density for HAAb-positive and HAAb-negative plasmas incubated either with c7E3 or phosphate buffered saline (PBS).

deflection in the OD curve, platelet aggregation occured in 100%, 100%, and 95% of the plasma-saline incubations exposed to porcine heparin, bovine heparin, and enoxaparin, respectively. Normal donor platelets aggregated in 20 out of 21 plasma samples containing HAAb when they were exposed to enoxaparin. The one HAAb plasma-saline incubation that did not aggregate with enoxaparin demonstrated strong platelet aggregation when 10^{-5} mol/L ADP was added.

If positive aggregation is defined as a greater than 20% change in OD, platelet aggregation occured in 100%, 100%, and 71% of plasma-saline incubations exposed to porcine heparin, bovine heparin, and enoxaparin, respectively. All HAAb-positive plasma-saline incubations demonstrated a greater than 20% change in OD when exposed to bovine and porcine heparin. Six of the 21 HAAb-positive plasmas had a less than 21% change in OD when exposed to enoxaparin. This group of six includes the one HAAb plasma-saline incubation that had no upward deflection in the aggregation curve whatsoever.

Complete (100%) inhibition of platelet aggregation occurred when donor platelets were incubated with the c7E3 Fab fragment. This was true for porcine heparin, bovine heparin, and enoxaparin. When 10^{-5} mol/L ADP was subsequently added, aggregation occured in none of the 21 plasma test samples. The proportion of HAAb-positive plasmas that exhibited aggregation in the presence of saline solution versus c7E3 Fab fragment was significantly different. The data and p values are summarized in Table I.

The OD results for the ELISA assays are presented in Table II and are expressed as the average ± standard deviation. Fig. 1 is a graphical presentation

Table I. Proportion of HAAb-positive plasmas with positive aggregation

	Porcine heparin	*Bovine heparin*	*Enoxaparin*
Saline*	21/21	21/21	20/21
c7E3 Fab*	0/21†	0/21†	0/21†
Saline‡	21/21	21/21	15/21
c7E3 Fab‡	0/21†	0/21†	0/21§

*Definition of (+) aggregation is an upward deflection in optical density.
†Significant difference between saline solution and c7E3 Fab ($p < 0.0001$).
‡Definition of (+) aggregation is >20% change in optical density.
§Significant difference between saline and c7E3 ($p < 0.0005$).

of the OD for HAAb-positive and HAAb-negative plasmas incubated either with c7E3 or phosphate buffer. The HAAb-positive plasma ELISA with phosphate buffer alone (n = 5) was compared with the ELISA assays incubated with c7E3 (n = 5). The incubation with c7E3 was not significantly different from the incubation with PBS ($p = 0.225$). However, the ELISA with HAAb-positive plasma was significantly different than the ELISA with HAAb-negative plasma using a multivariate analysis of variance ($p = 0.0001$).

Ninety-five percent confidence intervals (CI) were determined for the difference in OD between incubations with c7E3 and PBS. The 95% CI were narrow, ranging from −0.099 to +0.034 at the 1:8 dilution, to −0.088 to +0.045 at the 1:1024 dilution. These narrow intervals suggests that c7E3 caused no significant interference in the binding of HAAb by heparin.

Ninety-five percent CI also were determined for the difference between ELISA dilution curves with HAAb-negative pooled plasma and HAAb-positive pooled plasma. The 95% CI demonstrated the greatest difference at the 1:8 dilution (+0.547 to +0.651), and the least difference at the 1:1024 dilution (−0.013 to +0.091). At dilutions of 1:128 or less, the 95% CI indicates a clear separation with a difference in OD of at least 0.1 (Fig. 1).

DISCUSSION

Platelet function inhibiting agents have been used with variable success in the management of HAAb-positive patients who need continued anticoagulation with heparin. Included among these agents have been aspirin, dipyridamole, and iloprost (a prostacyclin analog).[5-7] Aspirin, an inhibitor of cyclooxygenase and thromboxane A2 production, does not reliably inhibit platelet aggregation in all patients. Although iloprost may increase the protection

Table II. ELISA, optical density at various dilutions

Dilution	*8*	*16*	*32*	*64*	*128*	*256*	*512*	*1024*
HAAb (+) buffer	1.091 ± 0.03	0.945 ± 0.11	0.699 ± 0.06	0.541 ± 0.06	0.393 ± 0.05	0.256 ± 0.03	0.187 ± 0.01	0.123 ± 0.01
HAAb (+)* c7E3	1.093 ± 0.17	0.900 ± 0.09	0.680 ± 0.02	0.485 ± 0.04	0.346 ± 0.03	0.228 ± 0.02	0.146 ± 0.01	0.098 ± 0.01
HAAb (−)† buffer	0.562 ± 0.01	0.462 ± 0.02	0.326 ± 0.03	0.263 ± 0.04	0.210 ± 0.01	0.142 ± 0.02	0.088 ± 0.01	0.077 ± 0.01
HAAb (−) c7E3	0.460 ± 0.04	0.386 ± 0.03	0.344 ± 0.03	0.234 ± 0.06	0.188 ± 0.03	0.116 ± 0.01	0.084 ± 0.02	0.066 ± 0.01

*In HAAb (+) plasma, c7E3 caused no significant change in the optical density curve when compared with buffer alone using ANOVA ($p = 0.225$).
†Optical density with HAAb (+) plasma was significantly greater than the optical density with HAAb (−) plasma using ANOVA ($p = 0.0001$).

HAAb-positive patients during reexposure to heparin, it causes significant hypotension in some patients[8,9] and is not recommended at present.

Alternative anticoagulants (low molecular weight heparins and heparinoids, ancrod, and hirudin) have been used in patients with HIT.[10-13] Low molecular weight heparins and heparinoids may be used if the platelet aggregation assays demonstrate no cross-reactivity with heparin.[14-16] However, cross-reactivity rates vary from 20% to 88%. This variable cross-reactivity explains why enoxaparin did not cause platelet aggregation in up to five of 21 (29%) HAAb-positive patients.

Ancrod is a defibrinogenating agent that has been used as an alternative anticoagulant in HAAb-positive patients with deep vein thromboses. It also has been used successfully as a perioperative anticoagulant in patients undergoing cardiovascular procedures.[12,17] Hirudin, a direct inactivator of thrombin, is another alternative to heparin that has been used in a small number of HAAb-positive patients.[13] At present, neither ancrod nor hirudin is approved by the Food and Drug Administration for clinical use. In addition, potential problems with ancrod include the development of antibodies and resistance to anticoagulation.[15]

A monoclonal antibody against the glycoprotein IIb/IIIa receptor was developed by Coller et al. in 1983.[18] Platelet aggregation in response to ADP, epinephrine, and thrombin was inhibited in a dose-dependent fashion. c7E3 is the Fab fragment (variable region) of an IgG antibody produced via mammalian cell culture, and is directed against the platelet GP IIb/IIIa receptor. The constant region of the intact antibody has been removed, via the restriction enzyme papain to decrease the antigenicity of the molecule.

After intravenous bolus administration, free c7E3 has an initial half-life of 10 minutes and a second-phase half-life of 30 minutes. This 10 minute half-life is most likely a result of rapid binding to the platelet GP IIb/IIIa receptor. Platelet function recovers within approximately 48 hours after a bolus but can be inhibited for longer periods with a continuous infusion of c7E3. Greater than 80% GP IIb/IIIa receptor blockade is achieved in vivo when c7E3 is administered with an intravenous bolus dose of 0.25 mg/kg followed by a continuous infusion of 10 μg/min.

More recently, monoclonal GP IIb/IIIa antibodies have been used to treat patients after coronary angioplasty and thrombolysis to reduce the risk for reocclusion, and to treat patients with unstable angina.[19-21] These studies demonstrated a decreased risk of ischemic complications in all groups. The largest of these studies also demonstrated a significantly increased risk of bleeding complications in patients who received a c7E3 bolus and infusion, when compared with placebo (14% vs 7%).[19] Allergic or hypersensitive reactions are extremely rare. Hypotension, nausea, vomiting, bradycardia, and fever have been recorded in few patients.

This report demonstrates that the GP IIb/IIIa, c7E3 Fab fragment, effectively inhibits in vitro platelet aggregation when HAAb-positive plasma is incubated with bovine, porcine, and the low molecular weight heparin, enoxaparin. It has been demonstrated that the monoclonal antibody binds to the GP IIb/IIIa platelet receptor and blocks the binding to platelets of fibrinogen and other glycoproteins (fibronectin, von Willebrand factor, and vitronectin).[18,22] The c7E3 Fab fragment may be the strongest platelet function–inhibiting agent available for use in HAAb-positive patients who require continued heparin anticoagulation for acute thrombotic disorders or cardiovascular operations. We performed ELISAs to evaluate whether c7E3 also inter-

fered with the binding of heparin to HAAb. The ELISAs indicated that c7E3 does not interfere with the binding between heparin and HAAb. It is possible that the number of assays (five in each group) were too few to identify differences in the binding of heparin and HAAb. Additional studies are underway to determine whether a small difference exists. Nevertheless, any interference caused by c7E3 in the binding between heparin and HAAb appears to be small. The major mechanism by which c7E3 prevents platelet aggregation appears to be the inhibition of binding of fibrinogen and other glycoproteins to the GP IIb/IIIa receptor.

Although this study used donor platelets to detect the presence or absence of aggregation, it is likely that c7E3 also would inhibit aggregation when platelets from HAAb-positive patients are tested. Regardless of the platelet source and the agonist causing activation, c7E3 blocks the final common pathway of platelet aggregation, the binding of fibrinogen and fibrin to the platelet GP IIb/IIIa receptor. Assays to detect platelet activation (e.g.,^{14}C serotonin release) were not performed because there was little reason to doubt that platelet activation had occurred. The saline control platelet aggregation assays clearly demonstrated the presence of significant activation and aggregation.

Clinical trials using c7E3 in HAAb-positive patients who require continued heparin-like anticoagulation (especially vascular surgery, cardiac surgery, and vascular radiology patients) are planned. Some questions that remain to be answered about c7E3 in patients with HIT include: Does c7E3 prevent or aggravate thrombocytopenia in vivo in patients with HIT? What is the fate of platelets activated but not aggregated by the HAAb? What is the adequate dose of c7E3 for patients with HIT? Besides the risk of bleeding, will there be other complications from the use of c7E3?

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DISCUSSION

Dr. William C. Krupski (Denver, Colo.). I would like to congratulate Dr. Liem and his colleagues for performing an interesting, well-conducted study and for presenting it so clearly here today. I would also like to congratulate all of you in the audience for sticking around until the bitter end, because I think that this is one of the most important papers of the entire program. Dr. Silver and his associates at the University of Missouri have led the way in the recognition, diagnosis, and management of the formidable problem of heparin-induced or heparin-associated thrombocytopenia, which is also called HIT or HAT.

HAAb binds with platelet receptors that initiate intracellular signaling pathways, which gives rise to conformational change in the membrane GP IIb/IIIa, whereby these sites bind avidly with fibrinogen or other bivalent adhesive proteins, which in turn leads to platelet-platelet interaction that we call aggregation. GP IIb/IIIa is a calcium-dependent heterodimer that belongs to a general class of structurally similar adhesion receptors that are designated as integrins. These receptors interact with the amino acid sequence arg-gly-asp, or RGD, common to several proteins that mediate cell adhesion and cohesion. Expression of functional fibrinogen receptors is the final common pathway in the process of platelet recruitment. Therefore, GP IIb/IIIa is an important target for interventions aimed at interrupting clinically significant, pathologic thrombosis.

Directly blocking GP IIb/IIIa receptor on platelets is an attractive strategy for preventing pathologic thrombosis. There are three categories of molecules that have been evaluated for this purpose. The first category include naturally occurring RGD-containing proteins, such as echistatin, bitistatin, and applagin, that are isolated from the venom of various species of vipers. The second category comprises synthetic peptides with one or more RGD sequences; several are currently under evaluation, but none has yet been approved for clinical use. Monoclonal antibodies that specifically bind and inhibit GP IIb/IIIa make up the third category. In a sense, the administration of such antibodies creates a physiologic state that is comparable with that seen in patients with Glanzmann thrombasthenia who are deficient in membrane GPs IIb and IIIa. These patients have prolonged bleeding and an abnormal aggregation response.

The in vitro study reported today demonstrates that murine monoclonal antibody, c7E3-a fibrinogen receptor antagonist-prevents platelet aggregation induced by HAAb without blocking the binding of HAAb to platelets. This result is predictable because HAAb binds with the Fc-like receptors separate from membrane GP IIb/IIIa. Accordingly, HAAb will bind without interfering with the fibrinogen receptor, and vice versa. Because in vitro aggregation absolutely depends on fibrinogen receptors to mediate platelet aggregation, platelet activation by HAAb will induce the expression of functional fibrinogen receptors, but if the fibrinogen receptors are blocked by their binding with c7E3, aggregation cannot proceed.

The potential usefulness of administering c7E3 in vivo depends on the relative importance of three unknown factors, and my questions to the authors relate to their speculation on what might occur.

The first unknown is the extent to which platelets are destroyed by immune-mediated mononuclear phagocytic removal that results from the binding of HAAb to platelets, similar to other immune-mediated destructive processes. If this process predominates, c7E3 will have no significant protective effect on the platelet count.

The second unknown is the extent to which platelets are removed because of HAAb-dependent activation of platelets. As the authors themselves ask, what is the fate of platelets that are activated but not aggregated by c7E3? If platelets are removed by the consequent formation of aggregates/microemboli and microvascular occlusion, c7E3 will be protective if the microembolic mechanism impairs the primary process.

The third unknown is the extent to which c7E3 impairs critical hemostasis. It is well established that the dose of c7E3 that is needed to protect the platelet count will severely interrupt platelet hemostatic function, with bleeding times greater than 30 minutes. The combination of heparin anticoagulation and c7E3-mediated paralysis of platelet hemostasis may not be compatible with performing cardiac or vascular surgical procedures without unacceptable risk. The relative importance of each of these variables may be quite different in individual patients, thus making it impossible to predict the outcome in different patients.

Finally, it will be some time before human clinical trials are completed to ascertain the efficacy of c7E3 in patients with HIT. In the meantime, what advice would you give us when we encounter a patient with this disorder now?

Dr. Timothy K. Liem (Columbia, Mo.). Regarding the question of whether the heparin-activated platelets are eliminated by immune-mediated phagocytosis, we have no clear answer, but I would speculate that this elimination occurs to some extent. There is one difference between the heparin thrombocytopenia syndrome and other drug-induced or immune-mediated processes. In the latter conditions, the antibody is directed against the platelet, thereby exposing the Fc portion for efficient opsonization. In heparin thrombocytopenia, the antibody is directed against heparin, possibly in combination with platelet factor 4. The Fc portion of the antibody attaches to the platelet Fc receptor that causes platelet activation.

This leads to the second and related question of whether the thrombocytopenia is caused by the formation of platelet aggregates. There is some evidence that this occurs. A few studies have evaluated thrombi removed from patients with the heparin thrombocytopenia syndrome. These are often white and rich with platelets, so called "white clot."

Use of c7E3 is associated with an increased incidence of bleeding in patients who undergo percutaneous coronary angioplasty in phase 3 trials. The risk of bleeding during cardiac and vascular procedures is most likely increased as well when concomitant c7E3 is given.

Our current approach to patients with heparin thrombocytopenia includes, first and foremost, discontinuation of the heparin. If the patient requires further anticoagulation therapy, low molecular weight heparins are used if platelet aggregation studies demonstrate no cross-reactivity with the unfractionated heparin. Long-term anticoagulation with warfarin is started as well. If the low molecular weight heparin does cross-react, our next choice for anticoagulation therapy would be ancrod possibly obtained through a compassionate use protocol.

Dr. Jawed Fareed (Maywood, Ill.). I would like to make three comments on your presentation.

The first is that these results you presented with the antibodies are consistent with what we presented in February at a Society meeting. Not only the antibodies, the ReoPro, or c7E3 antibody you described, but also a chimeric antibody from Japan, YM337, and the synthetic inhibitors, which were described by our discussant, produced the blunting of the heparin-induced thrombocytopenia.

There are synthetic agents that are currently being developed for oral usage that are much more active in subnanogram amount in blunting this effect. So the concern that is expressed by our discussant that the primary hemostatic function that may be blunted by the c7E3 could be answered that we could use these newer agents, the synthetic inhibitors, at a very low concentration will not affect the primary hemostasis and they are readily more reversible than the antibody.

The second comment is the agent you described, hirudin, and some of the other antithrombin agents, are in clinical trial at the present time for heparin-induced thrombocytopenia both in Europe and in the United States. I wish to ask you whether you tried hirudin or some of the antithrombin agents in your studies or in the in vitro testing of these agents?

I do not completely agree with the conclusion you have on the comparison of heparin and low molecular weight heparins. I am also not clear on what concentration you used for the low molecular weight heparin. If you increase the concentration of these agents, low molecular weight heparin also produces a similar degree of heparin-induced thrombocytopenic effect in vitro as well as in vivo. So when we use this low molecular weight heparin at the very high concentration or dosage, there is no practical difference; however, there is a synthetic pentasaccharide, which has recently been developed and will go in clinical trial in this country, that is devoid of the heparin-induced thrombocytopenic potential, at least in vitro conditions. So I am pleased that this forum included this very important paper, which has practical implications, and your findings are very clear. I hope we can provide the GP IIb/IIIa inhibitors that can be safe in addition to producing the platelet inhibition that could also be given simultaneously with heparin.

So the final question I wish to ask is whether you have used any of the hirudin or thrombin inhibitors, such as argatroban and some of the newer synthetic inhibitors, in your assay for the effects you have observed with the GP IIb/IIIa antibody.

Dr. Liem. First, regarding the "hemostatic effects" of c7E3. In the largest clinical trial, patients received a bolus injection followed by an infusion of c7E3. These patients did have prolonged bleeding times greater than 30 minutes. After about 48 hours the bleeding time decreased to less than 12 minutes.

I am unfamiliar with clinical trials that are using the synthetic GP IIb/IIIa inhibitors in heparin thrombocytopenia patients.

We have not used hirudin or argatroban or any of the numerous other direct thrombin inhibitors in HIT patients, but we would look forward to using those agents in the future.

We have used low molecular weight heparin in three or four patients with heparin thrombocytopenia who had no cross-reactivity with unfractionated heparin. We found that low molecular weight heparin can be used successfully with prevention of the thrombotic processes.

Dr. G. Patrick Clagett (Dallas, Tex.). I am very curious as to what specific type of patient and what clinical scenario you are going to treat with this drug. Short-term use, as during a vascular operation, might be disastrous because of the bleeding complications. And it seems to me, if I recall correctly, that the antibody itself is immunogenic and so a more prolonged use would have limited effectiveness. So I'm trying to think of a patient in whom you're going to use this, and I am not sure exactly what that would be. I'd like your comments.

Dr. Liem. We would first try c7E3 in patients with deep vein thromboses who require further anticoagulation therapy. It could be given in the interim period until the warfarin is adequately effective as an antithrombotic agent.

Regarding the question about whether it causes antigenicity, so far the clinical trials have used bolus injections with less than 72 hours of infusion, and we would hope that in that period of time the rate of developing allergic reactions, or antigenicity, would be quite low.