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# Comparison of the antiplatelet effect of YM337 and abciximab in rhesus monkeys

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**Abstract**

We directly compared the effects of YM337, the Fab fragment of the humanized monoclonal antibody C4G1, on platelet aggregation and template bleeding time with those of abciximab, the Fab fragment of the human/murine chimeric monoclonal antibody 7E3, in rhesus monkeys. The duration of inhibition of platelet aggregation by abciximab after i.v. bolus injection was much longer than that by YM337. Although YM337 significantly prolonged template bleeding time at 5 min after i.v. bolus injection, this action recovered within 1 h after injection. In contrast, although abciximab also prolonged template bleeding time, the duration of this effect was sustained. In a dose-escalating continuous infusion study, we evaluated the relationship between inhibition of platelet aggregation and prolongation of template bleeding time. Platelet aggregation was inhibited by over 80% by both agents at 3 μg/kg per min, and template bleeding time was prolonged to about 30 min at 30 μg/kg per min for YM337 and 10 μg/kg per min for abciximab. Interestingly, plasma concentrations between inhibition of platelet aggregation and prolongation of template bleeding time did not overlap with YM337, but did overlap with abciximab. These results suggest that YM337 allows easier control of antiplatelet activity with less effect on bleeding time than abciximab, and has a wider therapeutic window than abciximab. © 1997 Elsevier Science B.V.

*Keywords:* Glycoprotein IIb/IIIa; Monoclonal antibody; Bleeding time; Platelet aggregation

## 1. Introduction

Platelets play a crucial role in thromboembolic disorders such as stroke, myocardial infarction and peripheral arterial occlusive diseases. Antiplatelet drugs such as aspirin and ticlopidine inhibit platelet activation and may be useful in the treatment of these disorders (Coller, 1992; Antiplatelet Trialists' Collaboration, 1994; Schrör, 1995).

A new class of antiplatelet drugs is known as platelet glycoprotein (GP) IIb/IIIa receptor antagonists (Cook et al., 1994; Zablocki et al., 1994). The binding of fibrinogen to GPIIb/IIIa via the Arg–Gly–Asp (RGD) sequence of fibrinogen is the final common pathway for all agonist-induced platelet aggregation (Pytela et al., 1986; Phillips et al., 1988; Hynes, 1992). On this basis, GPIIb/IIIa receptor antagonists strongly inhibit platelet aggregation, and prevented thrombus formation more potently than any other antiplatelet agents (Frishman et al., 1995). Recently, RGD-containing peptides, RGD-mimetic compounds and monoclonal antibodies have been developed (Cook et al., 1994; Cox et al., 1994).

One such antagonist is abciximab, the Fab fragment of a human/murine chimera monoclonal antibody (c7E3) to GPIIb/IIIa (Tcheng et al., 1994). In clinical trials, bolus injection of 0.25 mg/kg of abciximab followed by 12 h infusion at 10 μg/min in high risk patients undergoing percutaneous transluminal coronary angioplasty decreased the incidence of ischemic complications within 30 days by 35% (The EPIC Investigators, 1994) and provided relatively long-term benefit by reducing the incidence of clinical restenosis during the 6 months following percutaneous transluminal coronary angioplasty (Topol et al., 1994). This agent has been approved by the Food and Drug Administration for use in high risk patients who are undergoing coronary angioplasty. While abciximab is clinically accepted in the United States and European countries,

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bleeding complications associated with this drug have been reported (Bernardi et al., 1993; Aguirre et al., 1995).

Recently, we have developed YM337, the Fab fragment of a humanized monoclonal antibody (hC4G1) to GPIIb/IIIa (Co et al., 1994; Kaku et al., 1996). This antibody specifically binds to GPIIb/IIIa and inhibits various agonist-induced platelet aggregation completely in humans and monkeys (Yano et al., 1994). YM337 immediately inhibited platelet aggregation after i.v. bolus injection to rhesus monkeys, an effect which rapidly disappeared after the end of administration, and inhibited occlusive thrombus formation in a photochemically-induced thrombosis model in squirrel monkeys (Kaku et al., 1996). Furthermore, YM337 inhibited platelet aggregation at doses which did not prolong template bleeding time (Kaku et al., 1996). Since the effect of GPIIb/IIIa receptor antagonists on bleeding time depends on both the animal and method used, conclusive results required comparison with these conditions standardized.

In the present study, we directly compared the effects of YM337 on platelet aggregation and template bleeding time with abciximab in an ex vivo study in rhesus monkeys, and evaluated the relationship between the inhibition of platelet aggregation and prolongation of template bleeding time of both agents.

## 2. Materials and methods

### 2.1. Drugs

The production and humanization of monoclonal antibody C4G1 have been described in detail elsewhere (Yano et al., 1994; Co et al., 1994). The Fab fragment of the hC4G1, YM337, was prepared using papain according to established methods (Harlow and Lane, 1988). Abciximab was purchased from Eli Lilly (Indianapolis, IN, USA).

### 2.2. Intravenous bolus administration study in monkeys

Three rhesus monkeys were used for each treatment group. The animals were anesthetized with ketamine hydrochloride injected into the femoral muscle (5 mg/kg). YM337 and abciximab were intravenously administered at doses of 0.25, 0.5 and 1.0 mg/kg. Blood samples were withdrawn and template bleeding time was measured just before administration and at 5 min, and 1, 3, 6, 12, and 24 h after administration of drugs. ADP (20 μM)-induced platelet aggregation in Platelet-rich plasma, hematological parameters and coagulation parameters were measured. Blood pressure and heart rate were measured with a blood monitoring system (BP-8800, Colin, Komaki, Japan). Body temperature was measured at baseline and 24 h after administration. Results are expressed as mean ± S.E.M. for each group of 3 animals.

### 2.3. Dose-escalation intravenous continuous infusion study in monkeys

Rhesus monkeys were administered with a continuous intravenous infusion of YM337 or abciximab at doses which increased in a stepwise manner from 0.1 μg/kg per min. At 30 min after the start of infusion, blood samples were collected, and template bleeding time was measured. After template bleeding time was measured, the next dose was started. Infusion doses were increased until template bleeding time was more than 30 min.

### 2.4. Platelet aggregation

Platelet-rich plasma and platelet-poor plasma were prepared by centrifugation of citrate-anticoagulated blood (3.8% sodium citrate:blood = 1:9) from healthy human volunteers and rhesus monkeys. Platelet counts in the Platelet-rich plasma were measured with an automatic cell counter (MEK-5158, Nihon Kohden, Tokyo, Japan), and were adjusted to a count of $3 \times 10^5/\mu l$ with Platelet-poor plasma. Platelet aggregation was measured using an aggregometer (Hema Tracer 801, MC Medical, Tokyo, Japan) by recording the increase in light transmission through a stirred suspension maintained at 37°C for 5 min. Aggregation in Platelet-rich plasma was induced with 20 μM of ADP. $IC_{50}$ values were calculated from the dose–response curve of at least 3 separate experiments.

### 2.5. Template bleeding time

Template bleeding time was measured in the forearm with a spring-loaded blade system (Simplate® R, Organon Teknika, Durham, NC, USA). Blood coming from the incision was blotted with filter paper every 30 s until blood no longer stained the filter paper. If the template bleeding time did not stop within 30 min, measurement was terminated and the result was recorded as > 30 min.

### 2.6. Hematological and coagulation parameters

Blood cell counts, hematocrit and hemoglobin of EDTA-anticoagulated whole blood were measured with an automatic cell counter. Prothrombin time and activated partial thromboplastin time were measured in citrate-anticoagulated plasma at 37°C using a coagulometer (KC4A, Amelung, Lehbrinksweg, Germany) with appropriate reagents (Ortho Diagnostic Systems, Tokyo, Japan).

### 2.7. Plasma concentration of unbound YM337 and abciximab

Concentration of unbound YM337 and abciximab in plasma were measured by sandwich enzyme-linked immunosorbent assay (ELISA) with rabbit polyclonal anti-

bodies against YM337 and abciximab, respectively (Kaku et al., 1996). Briefly, 96-well microtiter plates were coated with antibodies at 1 μg/ml. The plates were then blocked with 1% bovine serum albumin. Plasma samples were poured into the wells and incubated at room temperature for 1 h. After washing several times, the plates were incubated with 1 μg/ml of biotinylated antibodies at room temperature for 1 h and subsequently washed again several times. 0.1 ml of 1000-fold diluted streptavidin biotinylated peroxidase complex (Amersham Japan, Tokyo, Japan) was poured into each well, and the plates were incubated for 30 min at room temperature, followed by several washings. The plates were developed by adding 100 μl of 2,2′-azino-di-[3-ethylbenzthiazoline-6-sulfonic acid] (ABTS) solution (Bio-Rad, Richmond, CA, USA) to each well for 20 min before stopping the reaction by the addition of 50 μl of 2% oxalic acid. The absorbance at 414 nm was measured using a microtiter plate reader (Thermo Max; Molecular Devices, Menlo Park, CA, USA).

### 2.8. Platelet-bound YM337 and abciximab

Platelet-bound YM337 and abciximab were measured by capture ELISA. Briefly, 96-well microtiter plates coated with 2 μg/ml of murine monoclonal antibody B6A3 (Yano et al., 1994) against $\beta_3$ integrin, which does not competed with YM337 and abciximab in binding to GPIIb/IIIa. The plates were then blocked with 1% bovine serum albumin. 0.1 ml of Platelet-rich plasma ($3 \times 10^5/\mu l$) were solubilized by the addition of 10 μl of 10% Triton X-100, and 10 μg/ml of YM337 and abciximab were added to pre-dose samples for a 100% value before solubilization. The 10-fold diluted lysate was poured into the wells and incubated at room temperature for 2 h. After washing several times, the plates were incubated with 5 μg/ml of biotinylated anti-YM337 or abciximab antibodies at room temperature for 1 h and then treated as for measurement of plasma concentration. Platelet-bound antibodies were calculated as a percentage of maximal binding to platelets.

## 3. Results

### 3.1. In vitro platelet aggregation study

Both agents dose-dependently inhibited ADP-induced platelet aggregation in human and rhesus monkey Platelet-rich plasma in vitro. $IC_{50}$ values against ADP-induced in vitro platelet aggregation was 0.8 μg/ml for YM337 and 1.7 μg/ml for abciximab in humans, and 1.1 μg/ml for YM337 and 2.6 μg/ml for abciximab in rhesus monkeys (data not shown).

### 3.2. Intravenous administration study in monkeys

Figs. 1 and 2 show the inhibitory effects of both agents on ADP-induced platelet aggregation and template bleeding time in rhesus monkeys after i.v. bolus injection, respectively. YM337 completely inhibited platelet aggregation at 5 min after i.v. bolus injection of all doses and at 1 h after that of 1.0 mg/kg. Even at 1.0 mg/kg of YM337, the inhibition activity of platelet aggregation rapidly decreased to 50% after 3 h. Although template bleeding time at 1.0 mg/kg was prolonged for more than 20 min at 5 min after administration, it returned to less than 10 min within 1 h.

In contrast, abciximab completely inhibited platelet ag-

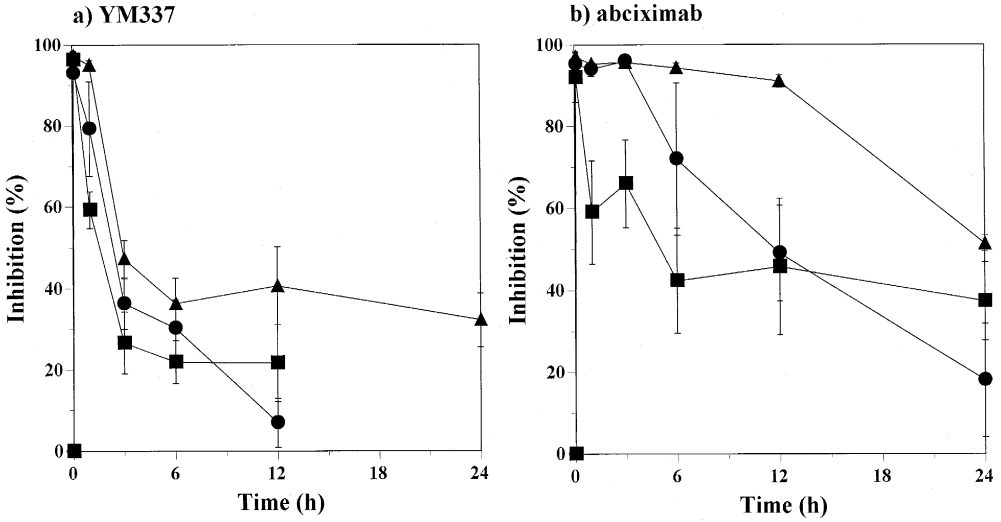


Fig. 1. Effect of YM337 (a) and abciximab (b) on ADP-induced platelet aggregation in rhesus monkeys after i.v. bolus injection of drugs at doses of 0.25 (■), 0.5 (●) and 1.0 mg/kg (▲). Data are presented as mean ± S.E.M. ($n = 3$).

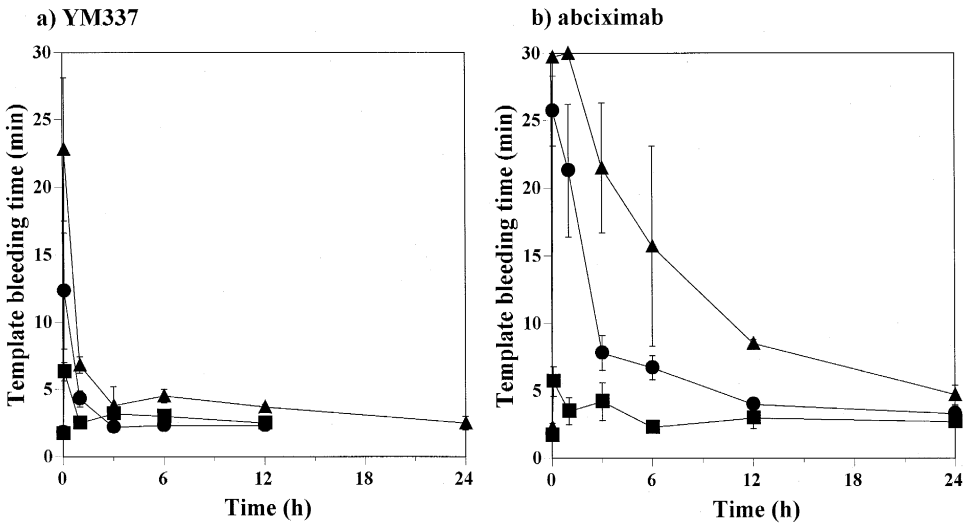


Fig. 2. Effect of YM337 (a) and abciximab (b) on template bleeding time in rhesus monkeys after i.v. bolus injection of drugs at doses of 0.25 (■), 0.5 (●) and 1.0 mg/kg (▲). Data are presented as mean ± S.E.M. ($n = 3$).

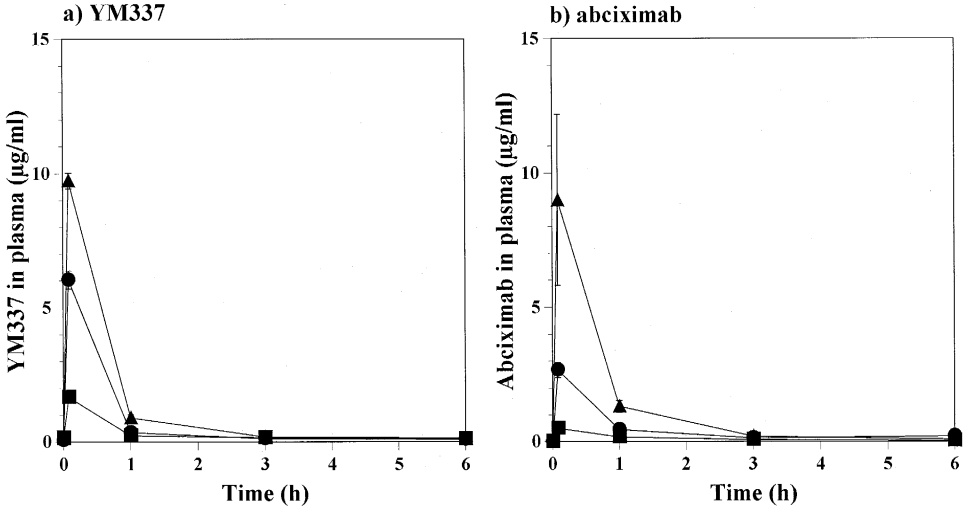


Fig. 3. Time course of plasma concentration of YM337 (a) and abciximab (b) in rhesus monkeys after i.v. bolus injection of drugs at doses of 0.25 (■), 0.5 (●) and 1.0 mg/kg (▲). Data are presented as mean ± S.E.M. ($n = 3$).

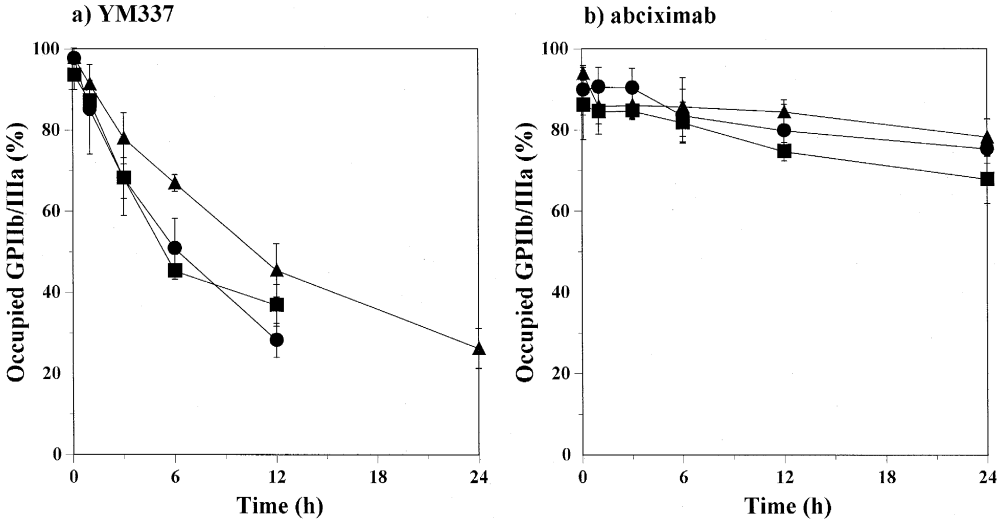


Fig. 4. Time course of platelet-bound YM337 (a) and abciximab (b) in rhesus monkeys after i.v. bolus injection of drugs at doses of 0.25 (■), 0.5 (●) and 1.0 mg/kg (▲). Platelet-bound antibodies were calculated as a percentage of maximal binding to platelets. Data are presented as mean ± S.E.M. ($n = 3$).

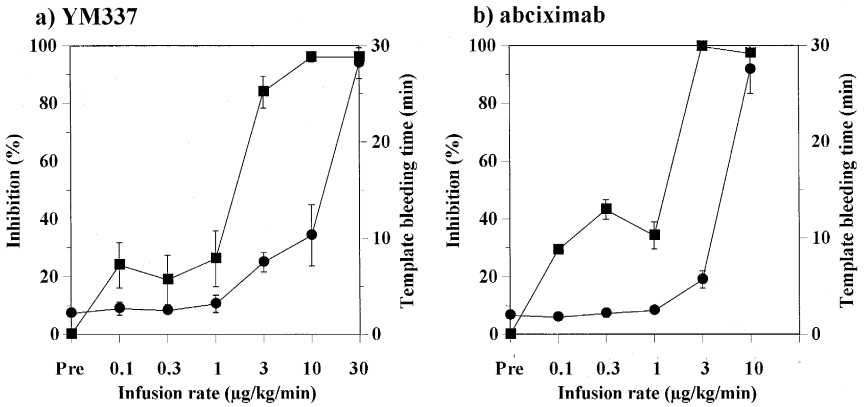


Fig. 5. Prolongation of template bleeding time (●) and inhibition of ADP-induced platelet aggregation (■) of YM337 (a) and abciximab (b) during continuous infusion in rhesus monkeys. Data are presented as mean ± S.E.M. ($n = 3$).

gregation for 3 h after injection of 0.5 mg/kg, and for 12 h after that of 1.0 mg/kg. Recovery of inhibitory activity of abciximab to baseline level was much slower than that of YM337. Template bleeding time was prolonged over 20 min at 1 h after injection of 0.5 mg/kg, and at 3 h after that of 1.0 mg/kg. Moreover, template bleeding time was prolonged to about 15 min even at 6 h after injection of abciximab at 1.0 mg/kg.

Figs. 3 and 4 show the time course of plasma concentrations and platelet-bound antibodies of both agents in rhesus monkeys after i.v. bolus injection, respectively. The plasma free concentrations of both agents dose-dependently increased at 5 min after i.v. bolus injection and decreased to near-baseline level at 3 h after injection. Platelet-bound YM337 at 12 h after i.v. bolus injection of 0.25, 0.5 and 1 mg/kg was 37%, 28% and 45%, respectively. In contrast, platelet-bound abciximab after i.v. bolus injection of 0.25, 0.5 and 1 mg/kg as long as 24 h after administration was 74%, 80% and 84%, respectively.

There were no significant changes in hematological parameters such as red and white blood cell counts, hematocrit and hemoglobin, and blood pressure and heart rate compared with the saline group (data not shown). No significant change in prothrombin time or activated partial thromboplastin time was seen in any group (data not shown).

### *3.3. Dose-escalation intravenous continuous infusion study in monkeys*

Fig. 5 shows the prolongation of template bleeding time and inhibition of ADP-induced platelet aggregation of YM337 (a) and abciximab (b) during continuous infusion in rhesus monkeys. Both agents dose-dependently inhibited platelet aggregation during continuous infusion, with complete inhibition achieved at an infusion rate of 0.3 μg/kg per min. The infusion rate for which template bleeding time was prolonged to about 30 min was 30 μg/kg per min for YM337 but only 10 μg/kg per min for abciximab. The difference between the dose that prolonged template bleeding time and that which inhibited platelet

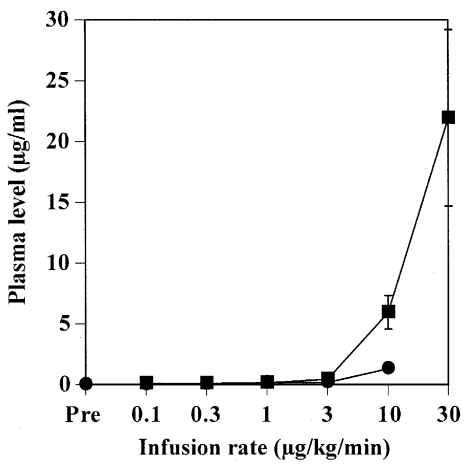


Fig. 6. Plasma concentration of YM337 (■) and abciximab (●) during continuous infusion in rhesus monkeys. Data are presented as mean ± S.E.M. ($n = 3$).

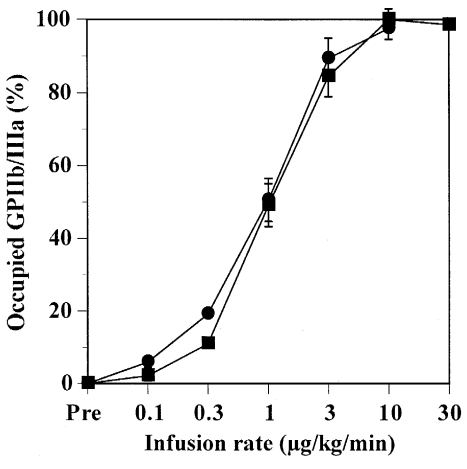


Fig. 7. Blockade of GPIIb/IIIa by YM337 (■) and abciximab (●) during continuous infusion in rhesus monkeys. Platelet-bound antibodies were calculated as a percentage of maximal binding to platelets. Data are presented as mean ± S.E.M. ($n = 3$).

aggregation was 10 times for YM337 and 3 times for abciximab.

Figs. 6 and 7 show the plasma concentrations and platelet-bound antibodies of both agents, respectively. Plasma concentration was not detected at a dose of 3 μg/kg per min for either agent, and plasma concentration of YM337 at 10 mg/kg per min was higher than that of abciximab at this dose. The change in platelet-bound antibodies correlated well to the change in inhibition of ADP-induced platelet aggregation.

### *3.4. Relationship between plasma concentration, inhibition of platelet aggregation and prolongation of template bleeding time*

Fig. 8 shows the relationship between the inhibition of platelet aggregation and prolongation of template bleeding time of both agents in this study. Template bleeding time prolongation and inhibition of platelet aggregation at all doses and time points were plotted against plasma levels of YM337 and abciximab. YM337 inhibited platelet aggregation at plasma levels of YM337 between 0.1 and 0.3 μg/ml, and prolonged template bleeding time at plasma levels between 3 and 10 μg/ml. Thus, the dose at which YM337 inhibited platelet aggregation and that at which it prolonged template bleeding time did not overlap. In contrast, abciximab inhibited platelet aggregation at plasma levels between 0.04 and 0.5 μg/ml, and prolonged template bleeding time at plasma levels between 0.06 and 0.8 μg/ml, and thus the doses at which it inhibited platelet aggregation and prolonged template bleeding time overlapped.

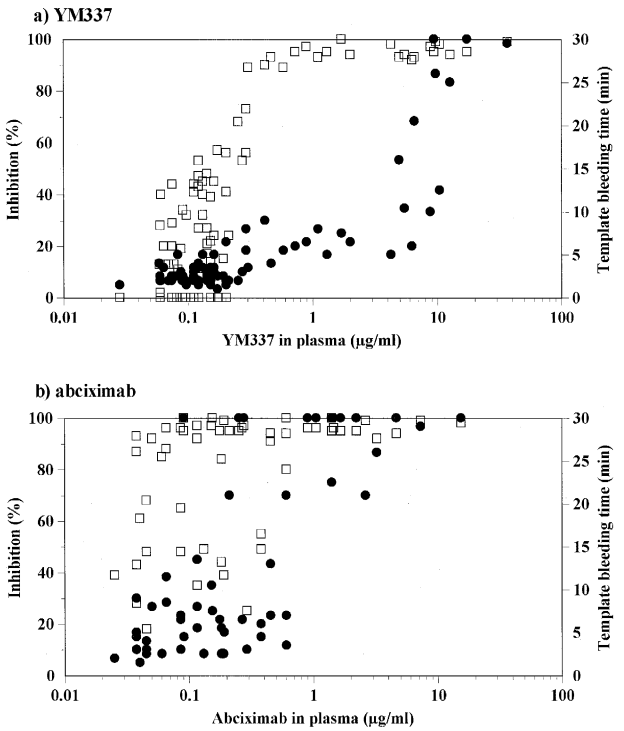


Fig. 8. Inhibition of platelet aggregation (□) and prolongation of template bleeding time (●) as a function of plasma levels of YM337 (a) and abciximab (b) at all doses and time points measured in this study.

## 4. Discussion

In the present study, YM337 immediately and strongly inhibited platelet aggregation after i.v. bolus injection in rhesus monkeys, but with a duration of action which was much shorter than that of abciximab. Moreover, YM337 shows a separation of plasma concentrations between that at which it inhibited platelet aggregation and that at which it prolonged template bleeding time was observed in YM337, whereas abciximab showed no such separation.

Bleeding time is widely used as a clinical parameter and is a common test in the study of primary hemostasis. A recent review of the literature involving 13 studies concluded, however, that the use of bleeding time does not predict excessive surgical bleeding in patients without a history of bleeding, and should not be used as a general preoperative screening (Lind, 1991). Moreover, in clinical trials of abciximab the prolongation of bleeding time was not predictive of hemorrhagic events in patients (Bernardi et al., 1993). However, bleeding time reflects important aspects in the investigation of patients with a history of bleeding episodes, namely in identifying such disorders of primary hemostasis as thrombocytopenia, platelet dysfunction or von Willebrand's disease, and can also be used for evaluating the effects of treatment in such patients. Moreover, bleeding time has been used as a hemostatic index in a wide range of clinical trials, especially with antiplatelet and antithrombotic agents.

In i.v. administration studies maximum inhibition of platelet aggregation by both agents was observed at 0.25 mg/kg, consistent with $IC_{50}$ values of both agents in in vitro platelet aggregation study. The duration of both inhibition of platelet aggregation and prolongation of bleeding time by YM337 was much shorter than that of abciximab. These sustained effects by abciximab were consistent with the sustained occupancy of GPIIb/IIIa receptors on platelets. Since plasma concentrations of these agents was similarly and rapidly decreased after i.v. bolus injection, the mechanism of the short duration of action of YM337 on platelet aggregation and template bleeding time may be explained by ease of displacement from platelet GPIIb/IIIa receptors of once platelet-bound agents. Namely, in our previous study, although the $K_d$ value of YM337 was closely similar to that of abciximab, the dissociation of YM337 from platelets was much faster than that of abciximab (Yano et al., 1995). These results strongly suggest that the effects of YM337 on platelet aggregation and template bleeding time are more controllable than those of abciximab. Further, this shorter duration of action

of YM337 may be a useful characteristic in the acute treatment of thrombotic disorders: in the event of hemorrhage, platelet function would be rapidly restored after the end of administration.

In a dose-escalation continuous infusion study, YM337 shows a 3-fold greater difference than abciximab in the doses at which it inhibited platelet aggregation and that which prolonged template bleeding time. Interestingly, when prolongation of template bleeding time and inhibition of platelet aggregation at all doses and time points were plotted against plasma free concentrations of both agents, plasma YM337 concentrations which produced complete inhibition of platelet aggregation were clearly separated from those which maximally prolonged template bleeding time. In contrast, plasma abciximab concentrations at which it inhibited platelet aggregation and prolonged bleeding time were overlapping. The relationship between prolongation of bleeding time and inhibition of platelet aggregation or thrombus formation has been discussed in many recent reports on GPIIb/IIIa receptor antagonists. Some agents were shown to prevent thrombus formation at doses that did not prolong bleeding time. However, thrombus formation was prevented at doses at which partial inhibition of ex vivo platelet aggregation was produced, and bleeding time was significantly prolonged at doses which inhibited ex vivo platelet aggregation completely (Aoki et al., 1996; Bostwick et al., 1996; Kawamura et al., 1996). Certain compounds completely inhibited ex vivo platelet aggregation and thrombus formation at doses which caused no change in bleeding time (Tschopp et al., 1993, 1994; Collen et al., 1994; Mazur et al., 1994). However, bleeding time strongly depends on both the experimental animal and method used. On this basis we consider the results of the present study to be reliable because the two agents were directly compared using a standardized method in monkeys. It remains to be demonstrated in a clinical study whether easier controllable GPIIb/IIIa receptor antagonists such as YM337 are safer in hemorrhagic complications than abciximab.

To our knowledge, this is the first report to describe a direct comparison between GPIIb/IIIa receptor antagonists in terms of the relationship of inhibition of platelet aggregation to prolongation of bleeding time. The mechanism of this dissociation between the inhibition of platelet aggregation and prolongation of bleeding time remain unknown in detail. However, we can propose the following hypothesis to help explain this perceived separation. First, YM337 specifically bound to the GPIIb/IIIa complex, whereas abciximab bound not only to GPIIb/IIIa but also cross-reacted with $\alpha_v\beta_3$ integrin (Coller et al., 1991). Abciximab was shown to block $\alpha_v\beta_3$ integrin-mediated cell adhesion to extracellular matrix (Charo et al., 1987). Moreover, abciximab recognized an activation-dependent neo-antigenic epitope on $\alpha_M\beta_2$ integrin (CD11b/CD18 or Mac-1), and bound recombinant $\alpha_M$ I-domain (Altieri and Edgington, 1988; Zhou et al., 1994). Second, abciximab binds to platelets of humans, monkeys and dogs, whereas YM337 binds only to platelets of humans, and rhesus and squirrel but not cynomolgus monkeys. That is, YM337 has a higher species specificity than abciximab. The specificity of antibodies may have different effects. Third, the binding of YM337 to purified GPIIb/IIIa was not blocked by RGDS at all, suggesting that the binding site of YM337 is different from that of abciximab and other GPIIb/IIIa receptor antagonists (Yano et al., 1995). In any case, the results of the present study suggest that YM337 has a wider therapeutic window than abciximab.

In conclusion, YM337 is an easy controllable GPIIb/IIIa receptor antagonist with a short half-life. This agent has less effect on template bleeding time than abciximab. YM337 may be a safe antiplatelet agent for the acute treatment of patients with thromboembolic diseases.

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## References

The EPIC Investigators, Aguirre, F.V., Topol, E.J., Ferguson, J.J., Anderson, K., Blankenship, J.C., Heuser, R.R., Sigmon, K., Taylor, M., Gottlieb, R., Hanovich, G., Rosenberg, M., Donohue, T.J., Weisman, H.F., Califf, R.M., 1995. Bleeding complications with the chimeric antibody to platelet glycoprotein IIb/IIIa integrin in patients undergoing percutaneous coronary intervention. Circulation 91, 2882–2890.

Altieri, D.C., Edgington, T.S., 1988. A monoclonal antibody reacting with distinct adhesion molecules defines a transition in the functional state of the receptor CD11b/CD18 (Mac-1). J. Immunol. 141, 2656–2660.

Antiplatelet Trialist' Collaboration, 1994. Collaborative overview of randomized trials of antiplatelet therapy I: Prevention of death, myocardial infarction and stroke by prolonged antiplatelet therapy in various categories of patients. Br. Med. J. 308, 81–106.

Aoki, T., Cox, D., Senzaki, K., Seki, J., Tanaka, A., Takasugi, H., Motoyama, Y., 1996. The anti-platelet and anti-thrombotic effects of FK633, a peptide-mimetic GPIIb/IIIa antagonist. Thromb. Res. 81, 439–450.

Bernardi, M.M., Califf, R.M., Kleiman, R.M., Ellis, S.G., Topol, E.J., 1993. Lack of usefulness of prolonged bleeding time in predicting hemorrhagic events in patients receiving the 7E3 glycoprotein IIb/IIIa platelet antibody. Am. J. Cardiol. 72, 1121–1125.

Bostwick, J.S., Kasiewski, C.J., Chu, V., Klein, S.I., Sabatino, R.D., Perrone, M.H., Dunwiddie, C.T., Cook, J.J., Leadley, R.J. Jr., 1996. Anti-thrombotic activity of RG13965, a novel platelet fibrinogen receptor antagonist. Thromb. Res. 82, 495–507.

Charo, I.S., Bekeart, L.S., Phillips, D.R., 1987. Platelet glycoprotein IIb-IIIa-like proteins mediate endothelial cell attachment to adhesive proteins and the extracellular matrix. J. Biol. Chem. 262, 9935–9938.

Co, M.S., Yano, S., Hsu, R.K., Landolfi, N.F., Vasquez, M., Cole, M., Tso, J.T., Bringman, T., Laird, W., Hudson, D., Kawamura, K., Suzuki, K., Furuichi, K., Queen, C., Masuho, Y., 1994. A humanized antibody specific for platelet integrin gpIIb/IIIa. J. Immunol. 152, 2968–2976.

Collen, D., Lu, H.R., Stassen, J.M., Vreys, I., Yasuda, T., Bunting, S., Gold, H.K., 1994. Antithrombotic effects and bleeding time prolongation with synthetic GPIIb/IIIa inhibitors in animal models of platelet-mediated thrombosis. Thromb. Haemost. 71, 95–102.

Coller, B.S., 1992. Antiplatelet agents in the prevention and therapy of thrombosis. Annu. Rev. Med. 43, 171–180.

Coller, B.S., Scudder, L.E., Beer, J., Gold, H., Folts, J.D., Cavagnaro, J., Jordan, R., Wagner, C., Iuliucci, J., Knight, D., Ghrayeb, J., Smith, C., Weisman, H.F., Berger, H., 1991. Monoclonal antibodies to platelet glycoprotein IIb/IIIa as antithrombotic agents. Ann. N.Y. Acad. Sci. 614, 193–213.

Cook, N.S., Kottirisch, G., Zerwes, H.G., 1994. Platelet glycoprotein GPIIb/IIIa antagonists. Drugs Future 19, 135–159.

Cox, D., Aoki, T., Seki, J., Motoyama, Y., Yoshida, K., 1994. The pharmacology of integrins. Med. Res. Rev. 14, 195–228.

Frishman, W.H., Burns, B., Atac, B., Alturk, N., Altajar, B., Lerrick, K., 1995. Novel antiplatelet therapies for treatment of patients with ischemic heart disease: Inhibitors of the platelet glycoprotein IIb/IIIa integrin receptor. Am. Heart J. 130, 877–892.

Harlow, E., Lane, D., 1988. Antibodies – A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 630–631.

Hynes, R.O., 1992. Integrins: Versatility, modulation and signaling in cell adhesion. Cell 69, 11–25.

Kaku, S., Kawasaki, T., Hisamichi, N., Sakai, Y., Taniuchi, Y., Inagaki, O., Yano, S., Suzuki, K., Terazaki, C., Masuo, Y., Satoh, N., Takenaka, T., Yanagi, K., Ohshima, N., 1996. Antiplatelet and antithrombotic effects of YM337, the Fab fragment of a humanized anti-GPIIb/IIIa monoclonal antibody in monkeys. Thromb. Haemost. 75, 679–684.

Kawamura, M., Imura, Y., Moriya, N., Kita, S., Fukushi, H., Sugihara, H., Nishikawa, K., Terashita, Z., 1996. Antithrombotic effects of TAK-029, a novel GPIIb/IIIa antagonist, in guinea pigs: Comparative studies with ticlopidine, clopidogrel, aspirin, prostaglandin E1 and argatroban. J. Pharmacol. Exp. Ther. 277, 502–510.

Lind, S.E., 1991. The bleeding time does not predict surgical bleeding. Blood 77, 2547–2552.

Mazur, C., Tschopp, J.F., Faliakou, E.C., Gould, K.E., Diehl, J.T., Pierschbacher, M.D., Connolly, R.J., 1994. Selective $\alpha_{IIb}\beta_3$ receptor blockage with peptide TP9201 prevents platelet uptake on Dacron vascular grafts without significant effect on bleeding time. J. Lab. Clin. Med. 124, 589–599.

Phillips, D.R., Charo, I.F., Parise, L.V., Fitzgerald, L.A., 1988. The platelet membrane glycoprotein IIb–IIIa complex. Blood 71, 831–843.

Pytela, R., Pierschbacher, M.D., Ginsberg, M.H., Plow, E.F., Ruoslaht, E., 1986. Platelet membrane glycoprotein IIb/IIIa: Member of a family of Arg–Gly–Asp-specific adhesion receptors. Science 231, 1559–1562.

Schrör, K., 1995. Antiplatelet drugs: A comparative review. Drugs 50, 7–28.

Tcheng, J.E., Ellis, S.G., George, B.S., Kereiakes, D.J., Kleiman, N.S., Talley, J.D., Wange, A.L., Weisman, L.R., Califf, R.M., Topol, E.J., 1994. Pharmacodynamics of chimeric glycoprotein IIb/IIIa integrin antiplatelet antibody Fab 7E3 in high-risk coronary angioplasty. Circulation 90, 1757–1764.

The EPIC Investigators, 1994. Use of a monoclonal antibody directed against the platelet glycoprotein IIb/IIIa receptor in high-risk coronary angioplasty. New Engl. J. Med. 330, 956–961.

The EPIC Investigators, Topol, E.J., Califf, R., Weisman, H.F., Elis, S.G., Tcheng, J.E., Worley, S., Ivanhoe, R., George, B.S., Fintel, D., Weston, M., Sigmon, K., Anderson, K.M., Lee, K.L., Willerson, J.T., 1994. Randomized trial of coronary intervention with antibody against platelet IIb/IIIa integrin for reduction of clinical restenosis: Results at six months. Lancet 343, 881–886.

Tschopp, J.F., Driscoll, E.M., Mu, D.X., Black, S.C., Pierschbacher, M.D., Lucchesi, B.R., 1993. Inhibition of coronary artery reocclusion after thrombolysis with an RGD-containing peptide with no significant effect on bleeding time. Coron. Artery Dis. 4, 809–817.

Tschopp, J.F., Mazur, C., Gould, K., Connolly, R., Pierschbacher, M.D., 1994. Inhibition of thrombosis by a selective fibrinogen receptor antagonist without effect on bleeding time. Thromb. Haemost. 72, 119–124.

Yano, S., Suzuki, K., Katoh, M., Sugita, Y., Kaku, S., Kawamura, K., Masuho, Y., 1994. Epitopes and biological activities of two monoclonal antibodies to platelet integrin $\alpha_{IIb}\beta_3$. J. Biochem. 116, 778–786.

Yano, S., Suzuki, K., Sato, K., Terazaki, C., Hisamichi, N., Kaku, S., Sakai, Y., Taniuchi, Y., Kawasaki, T., Inagaki, O., Sugita, Y., Satoh, N., Takenaka, T., Masuho, Y., 1995. Antiplatelet agent YM337 inhibits platelet function with little effect on bleeding time in monkeys. Blood 86, 912a, Abstract.

Zablocki, J.A., Nicholson, N.S., Feigen, L.P., 1994. Fibrinogen receptor antagonists. Exp. Opin. Invest. Drugs 3, 437–448.

Zhou, L., Lee, D.H.S., Plescia, J., Lau, C.Y., Altieri, D.C., 1994. Differential ligand binding specificities of recombinant CD11b/CD18 integrin I-domain. J. Biol. Chem. 269, 17075–17075.