Pharmacokinetics and Pharmacodynamic Effects of a New Antibody Glycoprotein IIb/IIIa Inhibitor (YM337) in Healthy Subjects

Sebastian Harder, MD; Carl M. Kirchmaier, MD; Hans Jürgen Krzywanek, MD; Dagmar Westrup, MSc; Jin-Woo Bae, MD; Hans Klaus Breddin, MD

Background—This study describes the first administration of YM337, the Fab fragment of a humanized monoclonal antibody against the fibrinogen GP IIb/IIIa receptor, in healthy male humans.

Methods and Results—Platelet aggregation (20 μmol/L ADP), platelet adhesion, fibrinogen binding, bleeding time, and YM337 concentrations in plasma were studied in substudy 1 after single boluses of 0.025, 0.05, 0.1, 0.2, and 0.4 mg/kg YM337 and in substudy 2 after a bolus (0.35 mg/kg) plus 6 hours of infusion at different dose levels of YM337 (0.5, 0.75, 1.0, 1.5 μg · kg⁻¹ · min⁻¹), with abciximab as reference drug (n=5 or 6 subjects per group). After the 0.2-mg/kg and 0.4-mg/kg boluses, fibrinogen binding was reduced by >80% and bleeding time was prolonged to ≈60 minutes. Bolus followed by infusion of 1.0 and 1.5 μg · kg⁻¹ · min⁻¹ YM337 maintained inhibition of platelet aggregation >80%. Aggregation and bleeding time returned to normal within 24 hours. A bolus of 0.25 mg/kg of abciximab followed by an infusion of 0.125 μg · kg⁻¹ · min⁻¹ showed effects similar to those observed with the 0.5- and 0.75-μg · kg⁻¹ · min⁻¹ infusion of YM337. In 53 subjects exposed to YM337, 1 case of transient thrombocytopenia and 3 minor bleeding events occurred. No human anti-chimeric antibodies were detected 2 weeks and 2 months after administration.

Conclusions—YM337 effectively inhibits IIb/IIIa-mediated platelet aggregation and adhesion in humans. The results of this phase 1 study will give rise to further clinical evaluation of YM337.

Key Words: platelets ■ antibodies ■ glycoproteins ■ YM337 ■ abciximab

Glycoprotein (GP) IIb/IIIa receptor antagonists prevent thrombotic occlusions in patients with acute coronary syndromes and reduce complications associated with high-risk percutaneous transluminal coronary angioplasty (PTCA). The first GP IIb/IIIa receptor antagonist that has been shown to be effective in larger clinical trials is the modified human-mouse chimeric monoclonal antibody (mAb) abciximab. YM337 is the Fab fragment of a humanized antibody against the fibrinogen receptor with only minor affinity to the αβ₃ receptor. For minimizing immunological responses, the murine antibody C4G1 was humanized by grafting only the mouse hypervariable regions onto a human IgG1 framework. Preclinical in vitro assessments of inhibitory effects on platelet function parameters showed a 2- to 4-fold stronger potency of YM337 compared with abciximab. Binding of fibrinogen to purified IIb/IIIa was inhibited by both agents with similar potency. Platelet aggregation investigated in rhesus monkeys was rapidly restored to 50% within 6 hours after a 0.5-mg/kg bolus of YM337, whereas after similar doses of abciximab, aggregation remained inhibited over 12 to 18 hours. Furthermore, bleeding time was found to be less prolonged than seen with abciximab. The time course of receptor occupancy with abciximab was more sustained than with YM337. From these preclinical data, a higher potency of YM337 in regard to platelet inhibition but a faster offset of effects after commencement of treatment would be expected. The aims of the phase 1 study presented here were (1) to evaluate the safety and tolerability of YM337 in healthy male volunteers as a single intravenous injection (substudy 1) or when given as a single bolus followed by a 6-hour infusion (substudy 2) and (2) to characterize the pharmacodynamic profile and the dose-response relationship of YM337 in humans.

Methods

Study Design

In substudy 1, each of 6 subjects received a single bolus of either 0.025, 0.05, 0.1, 0.2, or 0.4 mg/kg YM337 IV; 3 volunteers received placebo. The study started with the lowest dose level. It was intended to establish the dose that inhibited 20 μmol/L ADP–induced platelet aggregation by 80%, comparable to inhibition obtained after abciximab standard doses.

Received March 26, 1999; revision received June 7, 1999; accepted June 14, 1999.

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Circulation is available at http://www.circulationaha.org
In substudy 2, a bolus of YM337 (0.35 mg/kg IV) was followed by a 6-hour infusion at 4 different dose levels. Five or 6 subjects received either 0.5, 0.75, 1.0, or 1.5 mg/kg IV followed by an infusion of 0.125 mg/kg IV. Six other subjects received abciximab as a bolus of 0.25 mg/kg IV followed by an infusion of 0.125 mg · kg⁻¹ · min⁻¹, and 6 subjects received placebo.

Subject Selection and Study Protocol
Both substudies were approved by the local Institutional Review Board, and study subjects gave their written informed consent. Subjects in both substudies (n = 68) had a mean age of 28 years, a mean weight of 76 kg, and a mean height of 175 cm. All volunteers had normal findings in the physical and laboratory examinations and normal platelet counts (150 × 10⁹ to 400 × 10⁹/μL).

Investigations

Ex Vivo Platelet Aggregation
Samples were collected for platelet aggregation in substudy 1 at 0, 0.25, 1, 6, and 24 hours and in substudy 2 at 0, 0.25, 1, 2, 6, 8, 10, 24, and 48 hours. Platelet aggregation induced by 20 μmol/L ADP was studied in platelet-rich plasma (PRP) with a turbidimetric light-transmittance device (APACT, Labor). In substudy 2, a bolus of YM337 (0.35 mg/kg IV) was followed by a 6-hour infusion at 4 different dose levels. Five or 6 subjects received either 0.5, 0.75, 1.0, or 1.5 μg · kg⁻¹ · min⁻¹ of YM337. Six other subjects received abciximab as a bolus of 0.25 mg/kg IV followed by an infusion of 0.125 μg · kg⁻¹ · min⁻¹, and 6 subjects received placebo.

Fibrinogen Binding
Samples were collected for fibrinogen binding in substudy 1 at 0, 0.25, 1, 6, and 24 hours and in substudy 2 at 0, 0.25, 1, 2, 6, 8, 10, 24, and 48 hours. Samples were drawn into a tube containing 3.8% sodium citrate, and PRP was prepared. Platelets were counted by a cell counter, and PRP was diluted with platelet-poor plasma (PPP) to 250 000 platelets/μL. ADP was added at a final concentration of 100 μmol/L, and the samples were incubated for 10 minutes. Platelets were fixed with formaldehyde at a final concentration of 0.5%. After washing and resuspension, samples were incubated with fluorescein (FITC)-conjugated anti-human fibrinogen chicken antibodies (Biopool AB) for 30 minutes. Labeled platelets in PRP were analyzed by FACScan cytometer (Becton Dickinson). Acquisition and processing of data from 10 000 platelets were carried out with Lysis-II software. The mean fluorescence intensity values of the predose probes were set to 100%.

Table 1: Platelet Function and Fibrinogen Binding After a Single Bolus Dose of YM337 at Baseline, Maximum after 15 Minutes, and After 24 Hours

<table>
<thead>
<tr>
<th>YM337 0.5 μg · kg⁻¹ · min⁻¹ (n=6)</th>
<th>YM337 0.75 μg · kg⁻¹ · min⁻¹ (n=6)</th>
<th>YM337 1.0 μg · kg⁻¹ · min⁻¹ (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>6 h</td>
<td>24 h</td>
</tr>
<tr>
<td>ADP, %</td>
<td>0</td>
<td>54 (15)†</td>
</tr>
<tr>
<td>AI</td>
<td>1.47 (0.51)</td>
<td>0.22 (0.22)†</td>
</tr>
<tr>
<td>PITT, min</td>
<td>12.4 (6.5)</td>
<td>16.8 (3.7)†</td>
</tr>
<tr>
<td>BT, min</td>
<td>6.6 (1.3)</td>
<td>18.0 (6.1)†</td>
</tr>
<tr>
<td>FB, %</td>
<td>100</td>
<td>26 (8)†</td>
</tr>
<tr>
<td>PPP, ng/mL</td>
<td>0</td>
<td>181 (60)</td>
</tr>
<tr>
<td>PRP, ng/mL</td>
<td>0</td>
<td>2188 (375)</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1. Values are mean (SD).
* Significant difference from abciximab (P<0.01); † significant difference from placebo (P<0.01).
A cuff was placed around the arm and inflated to maintain a pressure of 40 mm Hg. Incision was made by a Simplate R device (Organon Inc). Blood was removed by a swab every 15 seconds, and the time until bleeding stopped was recorded up to 90 minutes.

Drug-Specific Antibodies
Plasma samples obtained before and 2 weeks and 2 months after exposure to the study drugs were tested on human anti-chimeric antibodies (HACAs). Determination of HACAs based on an enzyme immunoassay in which sera from YM337-, abciximab-, and placebo-treated subjects were incubated against YM337, abciximab, and normal human Fab molecules bound to microtiter plates. Blood samples were initially tested at dilutions of 1:10 down to 1:270. Depending on the optical density, samples were diluted further. Postdose samples were potentially scored seropositive for YM337 or abciximab when the optical density exceeded 0.4 and revealed an optical density twice that of the corresponding predose sample.

Pharmacokinetics
Concentrations of YM337 were determined in PRP and in PPP by an optical density twice that of the corresponding predose sample.

Statistical Evaluation
Results are presented as mean±SD. In substudy 1, the 0.25- and 24-hour target parameters were compared with their baseline value by use of Wilcoxon’s matched-pairs signed-rank test. In substudy 2, the 6- and 24-hour values were compared with the results of the placebo group and the group receiving abciximab by the Mann-Whitney U test.

Tolerability
A total of 53 subjects were exposed to YM337. Three minor bleeding events occurred (renewed bleeding from incision of the arm). Blood was removed by a swab every 15 seconds, and the time until bleeding stopped was recorded up to 90 minutes. Administration exceeding ±15% of the pretreatment values were observed.

Human Anti-chimeric Antibodies
Of the predose samples, 35% responded to human Fab fragments as well as to YM337 and abciximab, indicating the presence of nonspecific antibodies against Fab fragments. This anti-Fab response was stable over time and did not vary on administration of placebo, YM337, or abciximab, as was demonstrated by the optical density values obtained with the postdose samples 2 weeks and 2 months after the drug administration. None of the treated subjects revealed a specific HACA response against either YM337 or abciximab.

Pharmacokinetics
After the dose of 0.1 mg/kg, YM337 was detectable only in small amounts in PPP over 20 minutes after the bolus was given (Figure 1, top). If one considers the difference PRP–PPP as the drug concentration at the platelets, Figure 1 might be read as follows: After the 0.1-mg/kg bolus, YM337 is rapidly bound to platelets, and no drug appears as free drug in PPP. After the 0.2-mg/kg and the 0.4-mg/kg boluses, “excess” amounts of YM337 not bound at the platelets appear in PPP but are rapidly cleared after ≈4 hours (Table 2). The bottom of Figure 1 shows the PRP and PPP concentrations after the different infusion regimens. The initial drug concentrations were determined by the bolus dose, but PPP concentrations rose dose-dependently and fell immediately when the infusion was stopped. The PRP concentrations showed a less dose-dependent behavior, and at the end of the infusion, PRP concentrations varied between 2188 ng/mL (0.5 μg · kg⁻¹ · min⁻¹) and 3148 ng/mL (1.5 μg · kg⁻¹ · min⁻¹). After 24 hours, no drug was detectable in PPP, and PRP concentrations after the different infusion regimens were of a similar magnitude of ≈1300 ng/mL (Table 2).

Pharmacodynamics

Substudy 1
In general, no effect was seen after 0.025-μg/kg and 0.05-μg/kg IV bolus doses of YM337 (data not shown). Maximal inhibition of 20 μmol/L ADP–induced platelet aggregation averaged 94% after the 0.4-μg/kg bolus dose (Table 1, Figure 2). Pretreatment values for the platelet adhesion index varied
between 1.1 and 1.48; after 0.25 hours, the adhesion index was reduced to 0.001 after the 0.4-mg/kg bolus. Fibrinogen binding was maximally reduced to 53% after the 0.1-mg/kg bolus, 33% after the 0.2-mg/kg bolus, and 25% after the 0.4-mg/kg bolus. Bleeding time was prolonged to 62 minutes after the 0.4-mg/kg bolus. After 24 hours, fibrinogen binding returned to 65% to 100% (Table 1, Figure 2).

Substudy 2
Maximal inhibition of ADP-induced platelet aggregation was obtained at 0.25 hours and averaged between 90% and 95% after the various infusion regimens (Figure 3). At the end of the 6-hour infusion period, inhibition was between 54% (0.5 μg · kg⁻¹ · min⁻¹) and 93% (1.5 μg · kg⁻¹ · min⁻¹). Maximal inhibition immediately after the bolus abciximab was 77% and 37% after 6 hours of infusion. ADP-induced aggregation returned to normal 24 hours after start of the infusion (Table 2, Figure 4).

After the initial bolus, bleeding time was maximally prolonged to >60 minutes with YM337 as well as after abciximab. At 6 hours, YM337-induced prolongation of bleeding time increased dose-dependently and averaged 18 minutes with 0.5 μg · kg⁻¹ · min⁻¹ up to 65 minutes with 1.5 μg · kg⁻¹ · min⁻¹. Eighteen hours after the end of the infusion, bleeding times were normal or remained only slightly prolonged (Table 2, Figure 4).

PTT values determined 6 hours after start of the infusion were significantly prolonged with YM337 and abciximab, averages ranging between 13 minutes (1.0 μg · kg⁻¹ · min⁻¹ YM337) and 21 minutes (0.75 μg · kg⁻¹ · min⁻¹ YM337) (Table 2).

Fibrinogen binding was maximally reduced with YM337 to (average) values between 20% and 30% 0.25 hour after the bolus was given (Figure 3). At 6 hours, fibrinogen
With abciximab, fibrinogen binding was maximally reduced to 43% after 0.25 hour and averaged 50% after 6 hours of infusion. Eighteen hours after the end of the 6-hour infusion, binding remained significantly reduced to 45% with all doses of YM337 as well as abciximab (Figure 3).

Relationships between the fibrinogen binding and corresponding values of the 20 μmol/L ADP–induced platelet aggregation and bleeding times could be characterized by curvilinear or asymptotic monoeXponential functions, in which fibrinogen binding must be reduced to ∼30% to 40% to reach a 50% reduction in platelet aggregation as well as a 2-fold increase in bleeding time (Figure 5).

**Discussion**

The principal findings from these phase 1 studies were that (1) both IIb/IIIa antagonists lead to a complete reduction of platelet aggregation and adhesion, markedly prolonged bleeding times, and inhibition of platelet-induced thrombin generation; (2) all these parameters returned to baseline 18 hours after termination of the infusion; and (3) thrombocytopenia is also possible with the new, humanized mAb YM337. It must be emphasized that these results are limited to the typical phase 1 setting: altogether, 53 healthy male subjects were exposed, and possible interactions with drugs administered in clinical situations (aspirin and heparin) could not be taken into consideration.

The IIb/IIIa receptor antagonist abciximab and the peptide or peptidomimetic IIb/IIIa receptor antagonists eptifibatide and tirofiban are used in PTCA and in acute coronary syndromes. Other drugs, eg, humanized antibodies and orally available RGD peptidomimetics, are in different stages of clinical development. The preclinical pharmacology of the humanized mAb YM337 shows similar IIb/IIIa receptor binding but a faster decline of effects compared with abciximab in animal experiments. In these first human studies, however, the pharmacodynamic profile of YM337 resembles that of abciximab with regard to magnitude and rate of recovery of platelet inhibition, and in contrast to animal data, bleeding time was prolonged to a similar magnitude. Thrombocytopenia, possibly related to immune response mediated by anti-chimeric antibodies, is reported to occur in 2% to 4% of all patients treated with abciximab and other GP IIb/IIIa inhibitors. In our study,
Effects on platelet adhesion and thrombin generation have been only inconsistently reported, and the inhibitory effect on bleeding time were rapidly declining despite relatively high concentrations of YM337 in PRP after 24 hours. One explanation is that after short-term exposure with a IIb/IIIa antagonist (eg, after a bolus), receptors show up at the platelet surface (perhaps as a consequence of outside-in signaling), therefore perhaps capable of interfering with platelets during the activation process. This also applies to the bleeding time, in which the in vivo activation of platelets by the dermal incision is countered by free YM337. One further interesting observation in both studies was that antiplatelet effects and platelet aggregation shortly after the bolus by 80%, the standard bolus of abciximab decreased fibrinogen binding by only 55% (in contrast to 80% with YM337), whereas published data on abciximab show that this dose should block IIb/IIIa receptors by 80%. The discrepancy between our results on fibrinogen binding and reports on receptor occupancy of abciximab could not yet be explained. It might be noteworthy that the receptor binding assay used in the above-cited studies determines the amount of free abciximab binding sites by competition of receptor-bound abciximab with 125I-labeled abciximab, whereas the fibrinogen binding assay determines the amount of free IIb/IIIa binding sites by competition of mAb bound to IIb/IIIa receptors with the natural ligand fibrinogen, pointing to differences in the tightness of IIb/IIIa binding of the 2 mAbs in the presence of fibrinogen.

Despite the observation that fibrinogen binding 6 hours after start of the infusion was not different in the 4 dosing groups, a dose-dependent increase in the inhibition of ADP-induced aggregation and in bleeding time was seen (Figures 3 and 4). If the PPP and PRP concentrations are taken into consideration, this finding might be explained by the following model: PPP concentrations showed a dose-dependent increase, but the amount bound to platelets (the difference between PRP and PPP from Table 2) was quite similar with all infusion regimens, suggesting a saturation of IIb/IIIa binding sites in the unactivated state. Aggregation is induced in PRP, where free YM337 (the PPP level) is also present and therefore perhaps capable of interfering with platelets during the activation process. This also applies to the bleeding time, in which the in vivo activation of platelets by the dermal incision is countered by free YM337. One further interesting observation in both studies was that antplatelet effects and bleeding time were rapidly declining despite relatively high concentrations of YM337 in PRP after 24 hours. One explanation is that after short-term exposure with a IIb/IIIa antagonist (eg, after a bolus), receptors show up at the platelet surface (perhaps as a consequence of outside-in signaling), but in the absence of free drug levels, these new receptors are not inhibited. Supposing an “early” rearrangement of IIb/IIIa receptors after the bolus, a subsequent infusion might therefore be necessary to interfere with the new receptors,
leading to the preservation of $\geq 50\%$ reduction in fibrinogen binding even 18 hours after termination of the infusion, as seen in our study and by others. However, a steep and curvilinear relationship between fibrinogen binding and aggregation exists, as well as between fibrinogen binding and bleeding time, and fibrinogen binding must be decreased by $\geq 50\%$ to obtain a significant effect on these parameters (Figure 5). Nevertheless, because the curve describing the relationship between receptor binding and inhibition of functional parameters is very steep, even a small increase in unblocked receptors (also augmented by generation of new platelets within 24 hours) in the range $>50\%$ fibrinogen binding is followed by a marked restoration of aggregation properties and bleeding time, which is the final cause for the rapid decline of inhibitory effects on platelet function after cessation of the infusion.

In conclusion, the new humanized mAb YM337 effectively inhibits Ib/IIa-mediated platelet aggregation in humans. A bolus of 0.25 mg/kg abciximab IV followed by an infusion of 0.125 $\mu$g $\cdot$ kg$^{-1}$ $\cdot$ min$^{-1}$ showed effects similar to those observed with the 0.5- and 0.75-$\mu$g $\cdot$ kg$^{-1}$ $\cdot$ min$^{-1}$ infusions of YM337. In a total of 53 subjects exposed to YM337, 1 case of transient thrombocytopenia occurred, and no HACAs were detected 2 weeks and 2 months after administration. The results of this phase 1 study will give rise to further evaluation of YM337 in which the comparative clinical profile of abciximab has to be established.

Acknowledgment

This work was sponsored by Yamanouchi Europe BV. The valuable contributions of Dr T. Tan (Yamanouchi Europe BV) are appreciated.

References

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*Circulation*. 1999;100:1175-1181
doi: 10.1161/01.CIR.100.11.1175
*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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