Eptifibatide and Abciximab exhibit equivalent antiplatelet efficacy in an experimental model of stenting in both healthy volunteers and patients with coronary artery disease

Giovanni Amoroso¹
Ad J. van Boven¹
Dirk J. van Veldhuisen¹
René A. Tio¹
Corine P. Baljé-Volkers²
Anna S. Petronio³
Wim van Oeveren⁴

¹Department of Cardiology, Thoraxcenter, University Hospital of Groningen, The Netherlands

²Trial Co-ordination Center, Thoraxcenter, University Hospital of Groningen, The Netherlands

³Cardiothoracic Department, University of Pisa, Italy

⁴Department of Biochemical Engineering, University of Groningen, The Netherlands

J Cardiovasc Pharm 2001 (in press)
ABSTRACT

Platelet deposition and aggregation are the major determinants of acute thrombosis in coronary stents. We aimed to compare the antiplatelet efficacy of different treatments (GpIIbIIIa inhibitors and conventional antiaggregants) in an experimental model for stenting.

Methods and results
Blood samples were obtained from patients with coronary artery disease (n=15) and healthy volunteers (n=8) and incubated either with Eptifibatide (2.0 µg/ml), Abciximab (3.0 µg/ml), Indomethacin (15 µg/ml), or saline. Platelet ADP-induced aggregation in whole blood was assessed for all groups. Blood was also tested in an experimental circulation model containing metallic probes, on which platelet deposition in shear flow conditions was assessed by means of fluorescent-labelled platelet-specific (anti-GpIIIa and anti-GpIb) antibodies. Eptifibatide and Abciximab, in comparison to Indomethacin and no treatment, significantly reduced platelet aggregation (0, 0, 4, and 3 Arbitrary Units (AU), respectively; p<0.001), anti-GpIIIa (2.25, 1.83, 11.24, and 13.42 counts per second (cps)/mg, respectively; p<0.001), and anti-GpIb binding (0.61, 0.61, 1.00, and 1.83 cps/mg, respectively; p<0.001). Anti-GpIIIa and anti-GpIb binding were significantly correlated (R:0.36; p<0.01). Patients showed a higher anti-GpIIIa, but not anti-GpIb binding, than controls (8.43 vs. 3.33 cps/mg; p<0.01), irrespective of any treatment.

Conclusions
In conclusion, Eptifibatide and Abciximab show equivalent in-vitro antiplatelet efficacy, superior to that of Indomethacin. Given the occurrence of GpIIbIIIa platelet overexpression in the course of coronary artery disease, an extended use of GpIIbIIIa inhibitors may be proposed to prevent acute thrombosis during routine coronary stenting.

INTRODUCTION

Coronary stenting has progressively become a fundamental tool in the treatment of occlusive coronary artery disease (CAD) 1, 2. However, thrombosis and occlusion of the coronary artery can occur at an early stage after stenting 3. Prevention of stent thrombosis is mandatory, in order to decrease patients’ morbidity and mortality and the cost of health care needed for re-intervention 4, 5.

Conventional antiaggregant therapies (aspirin, ADP-receptor antagonists) have proven to be effective in reducing thrombotic complications after coronary stenting 6, 7. Arterial thrombus formation mainly depends on deposition and subsequent aggregation of circulating platelets on the damaged arterial wall and the implanted stent 8, 9. Although a targeted antagonism of the platelet GpIIbIIIa receptor clearly improves the clinical outcomes after coronary stenting 10, 11, the routine use of GpIIbIIIa inhibitors is still under debate 12. Moreover, currently available GpIIbIIIa inhibitors express a wide variability in pharmacokinetics and pharmacodynamics 13, 14 and, to date, only few studies explored the equivalency of these compounds during coronary interventions 15, 16.
The objective of the present study was to compare the efficacy of two different GpIIbIIIa inhibitors, also against conventional antiaggregant therapy, in inhibiting platelet deposition and aggregation in an experimental model for stenting.

METHODS

Study drugs
The study represents an in-vitro comparison of the antiplatelet effects of Eptifibatide (Integrilin®, Schering Plough BV, Maarssen, The Netherlands), Abciximab (ReoPro®, Eli Lilly BV, Nieuwegein, The Netherlands) and Indomethacin (Fluka Chemie AG, Buchs, Switzerland), an intravenous aspirin-like drug.

Blood sampling and preparation
Blood samples were collected immediately before the procedure at the University Hospital of Groningen (NL), from patients with CAD undergoing percutaneous coronary intervention. Exclusion criteria were unstable angina or impending myocardial infarction, abnormal platelet count, use of anticoagulation therapy and/or International Normalised Ratio (INR) > 2.0. Anticipating the potential implantation of a coronary stent, all patients had received within 12 hours prior to the procedure a 300mg loading dose of Clopidogrel Bisulphate (Plavix®, Sanofi-Synthelabo, Paris, France), a novel thienopyridinic platelet ADP-receptor antagonist. All patients were already receiving low-dose acetylsalicylic acid (100 mg/daily). Blood samples were also collected from healthy volunteers. Written informed consent for blood withdrawal was obtained from all participants. Blood from each individual was collected with PPACK (Calbiochem, La Jolla, CA, US) as anticoagulant, at a concentration of 0.5 μg/ml, 9.5 μM. Despite the hazard of excessive sample anticoagulation, PPACK was preferred to citrate, which enhances the activity of Eptifibatide via Ca²⁺ depletion 17. The final volume of the collected samples was 30±5 ml.

Table 1. Semiquantitative scale of ADP-induced platelet aggregation

<table>
<thead>
<tr>
<th>Resistance (%)</th>
<th>Category (AU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&gt;0 – 20</td>
<td>1</td>
</tr>
<tr>
<td>&gt;20 – 40</td>
<td>2</td>
</tr>
<tr>
<td>&gt; 40 – 60</td>
<td>3</td>
</tr>
<tr>
<td>&gt; 60 – 80</td>
<td>4</td>
</tr>
<tr>
<td>&gt; 80</td>
<td>5</td>
</tr>
</tbody>
</table>

AU: Arbitrary Units
Blood from each individual was separated into four equal aliquots, to which medication or saline (no treatment) was added. Selection of the final concentrations for GpIIbIIIa inhibitors was based on the known therapeutical active dose: Eptifibatide \(^{18}\) 2.0 mg/ml; Abciximab \(^{10}\) 3.0 mg/ml. For Indomethacin a concentration was chosen (15 mg/ml) in order to mimic the effect of 325 mg of oral acetylsalicylic acid \(^{19}\). Medications were incubated with blood for 10 minutes at room temperature. Subsequent analyses were performed by investigators blinded to treatment.

### Platelet aggregation

**In-vitro** platelet aggregation was induced by adding to whole blood ADP (5 µM), which provokes platelet interaction via fibrinogen binding to GpIIbIIIa receptors. Blood was equilibrated at 37°C for 2-5 minutes under constant stirring. Platelet aggregation was measured by decrease of impedance on a dual-channel ChronoLog aggregometer. Curve heights of the tracings generated by the apparatus (% of resistance in ohms) for each experiment were classified into 6 discrete categories of platelet aggregation, according to a semiquantitative scale (range: 0-5 Arbitrary Units: AU)(Table 1).

### Platelet deposition

A standardised experimental model was used to reproduce platelet-stent interaction in shear flow conditions. The system has been extensively described elsewhere \(^{20}\). Briefly, it is composed by a closed-loop circuit made of PVC tubing (3.0 mm inner diameter), kept at 37°C, and a semi-occlusive roller pump. A stainless steel probe was inserted in the system, as a model for coronary stent. Probes (20 mm long, 3-mm wide and 0.2 mm thick) were cut from high-grade quality stainless steel flat sheets (AISI 316L; Goodfellow Cambridge Ltd, London, UK), electropolished, cleansed by ultrasonication and rinsed in demineralised water and 95% methanol. Blood was recirculated through the tubing for 15 minutes, at a flow rate of 40 ml/min, then the steel probe was removed, gently washed in PBS and stored at –80°C.

Human platelet-specific, GpIIa (CD61) and GpIb (CD42b), antigen deposition on probes after circulation was assessed by specific fluorescent-labelled antibodies (Dako A/S, Glostrup, Denmark). Antibody labelling and binding was carried out as described elsewhere \(^{21}\). Results were corrected for controls, obtained by incubating antibodies with non-recirculated probes, and are expressed as counts of fluorescence per second, corrected for mg of metal (cps/mg).

### Statistical analysis

Since variables were predominantly not-normally distributed, they are presented as Median and Range (Min-Max). Differences for blood donors (patients or controls) were analysed using Wilcoxon 2-sample test/Wilcoxon rank-sum test, both irrespective of and according to treatment. Comparison between treatment groups (Eptifibatide, Abciximab, Indomethacin, no treatment) was performed using Friedman’s test for multiple comparisons for non-parametric randomised block design. Post-hoc analysis was performed by Bonferroni test. Spearman correlation analysis was performed for variables showing statistically significant changes. All statistical analyses were performed using SAS, Windows version 6.12 (SAS, Cary, NC).
RESULTS

Blood was withdrawn from 15 patients with CAD (11 males, mean age: 61 yrs) and 8 healthy volunteers (7 males, mean age: 42 yrs). However, blood from 2 patients coagulated before proper aggregation tests, thus 23 adhesion and 21 aggregation experiments, respectively, were carried out for each experimental group.

Figure 1.
Effect of no treatment (upper left), Indomethacin (upper right), Eptifibatide (lower left), and Abciximab (lower right) on ADP-induced in-vitro platelet aggregation. Platelet aggregation (x axis) has been quantified according to a semiquantitative scale in arbitrary units (AU; range 0-5). The percentage of experiments for each value is reported (y axis) for patients (black columns; n=13) and controls (grey columns; n=8). Eptifibatide and Abciximab inhibit almost completely platelet aggregation in comparison both to Indomethacin and no treatment (p<0.001).

Figure 2.
Linear correlation (full line) in the whole study group between anti-GpIb (x axis) and anti-GpIIa binding (y axis). A significant correlation was found (R:0.36, p<0.01).
Both Eptifibatide and Abciximab equally inhibited ADP-induced platelet aggregation in almost all samples (0 [0 – 1] and 0 [0 – 3] AU, respectively; \( p=0.57 \)), in comparison to Indomethacin (4 [0 - 5] AU; \( p<0.001 \) vs. Eptifibatide and Abciximab), which had almost no effect, and no treatment (3 [0 - 5] AU; \( p<0.001 \) vs. Eptifibatide and Abciximab). No differences between patients and controls were demonstrated (1 [0 - 5] and 2 [0 - 5] AU; \( p=0.20 \))(Figure 1).

Anti-GpIIa and anti-GpIb binding onto steel probes were significantly correlated (\( R:0.36, p<0.01 \))(Figure 2), as well as they were with ADP-induced platelet aggregation (for anti-GpIIa binding, \( R:0.61; p<0.001 \))(Figure 3).

When compared to Indomethacin or no treatment, Eptifibatide and Abciximab induced an equal and significant reduction both of anti-GpIIa binding (11.24 [4.16 – 21.08], 13.42 [1.71 - 22.35], 2.25 [-2.27 - 15.22], and 1.83 [-0.27 - 11.28] cps/mg, respectively; \( p=0.35 \) for Eptifibatide vs. Abciximab; \( p<0.001 \) for Indomethacin and no treatment vs. Eptifibatide and Abciximab)(Figure 4), and of anti-GpIb binding (1.00 [-0.75 - 4.61], 1.83 [0.25 - 4.05], 0.61 [-1.07 - 2.48] and 0.61 [-1.29 - 3.18] cps/mg, respectively; \( p=0.61 \) for Eptifibatide vs. Abciximab; \( p<0.001 \) for Indomethacin and no treatment vs. Eptifibatide and Abciximab) (Figure 5). However, among samples incubated either with Eptifibatide or Abciximab, 5 out of 26 from patients, but none from controls, exceeded median values, either for platelet aggregation or anti-GpIIa binding (Figure 3).

**Figure 3.**
Linear correlation (full line) in the whole study group for anti-GpIIa binding (x axis) and ADP-induced platelet aggregation (y axis). A significant correlation was found (\( R:0.61, p<0.001 \)). Experiments are classified as patients (triangles) and controls (dots), and as non-GpIIbIIIa-inhibitor incubated (white) and GpIIbIIIa-inhibitor incubated (black). In respect to median values (dashed lines), non-GpIIbIIIa-inhibitor incubated experiments are dispersed in all four quadrants. GpIIbIIIa-inhibitor incubated experiments are mostly grouped in the leftunderside quadrant, except for 5 out of 26 patients (pointed by tiny arrows).
Figure 4. Anti-GpIIa binding onto stainless steel probes after in-vitro recirculation with blood, withdrawn from patients (black dots; n=15) or controls (grey dots; n=8) and previously incubated with Eptifibatide, Abciximab, Indomethacin, or no treatment. Median values for treatment subgroups (white boxes), total of patients (black box) and controls (grey box) are also reported. Units are counts of fluorescence per second/mg of metal (cps/mg). Eptifibatide and Abciximab provoked a significant reduction in comparison both to Indomethacin and no treatment (p<0.001). Experiments with blood from patients showed a higher anti-GpIIa binding than controls (p<0.01).

Figure 5. Anti-GpIb binding onto stainless steel probes after in-vitro recirculation with blood, withdrawn from patients (black dots; n=15) or controls (grey dots; n=8) and previously incubated with Eptifibatide, Abciximab, Indomethacin, or no treatment. Median values for treatment subgroups (white boxes), total of patients (black box) and controls (grey box) are also reported. Units are counts of fluorescence per second/mg of metal (cps/mg). Eptifibatide and Abciximab provoked a significant reduction in comparison both to Indomethacin and no treatment (p<0.001). No difference was found for blood donors (patients or controls).
Irrespectively of any treatment, patients’ samples showed a significantly higher anti-GpIIIa binding than controls (8.43 [–0.51 – 22.35] vs.3.33 [–2.27 - 17.82] cps/mg, respectively; p<0.01)(Figure 4), while no significant difference was found for anti-GpIb binding (1.05 [–1.29 - 4.11] and 1.17 [–0.76 - 4.61] cps/mg, respectively; p=0.70)(Figure 5).

DISCUSSION

As expected, Eptifibatide and Abciximab exhibited an equivalent in-vitro efficacy in preventing ADP-induced platelet aggregation. In addition, both GpIIbIIIa inhibitors reduced platelet deposition onto stent-like surfaces exposed to blood shear flow, at a higher extent than an aspirin-like treatment (Indomethacin). Cumulatively, these data suggest a potential role for GpIIbIIIa inhibitors in the prevention of platelet deposition, aggregation and subsequent thrombosis after coronary stenting.

Previous studies have already demonstrated the involvement of activated platelet GpIIbIIIa receptors, other than in promoting aggregation 22, also in mediating platelet adhesion to fibrinogen-covered structures, as an alternative to the well-established von Willebrand factor-GpIb pathway 23, 24, 25. Our results follow previous findings, which demonstrated that, under low shear flow, anti-GpIIbIIIa antibodies (C7E3 Fabs) inhibit fibrinogen-mediated platelet adhesion 26. This capability is acknowledged by the present study also to currently-available drugs, such Eptifibatide and Abciximab.

Extending the role of GpIIbIIIa inhibitors beyond simply blocking platelet-to-platelet cohesion has important clinical implications. In fact, this treatment comes forward as a definitive solution for the problem of stent thrombosis. Indeed, while Abciximab has already given convincing evidences for significantly reducing 30 days-adverse events after stent placement (from 10.8 to 5.3%) 27, Eptifibatide has been acknowledged only in a recent trial for the same results (from 10.5 to 6.8%) 18. The clinical success of Abciximab has been also attributed to the additional blockade of non-GpIIbIIIa receptors, namely vitronectin and Mac-1. This capability is not shown by Eptifibatide, but it seems not to influence its inhibitory effect on platelet adhesion to the fibrinogen-covered metallic surface, probably the major determinant of thrombus formation in the immediate phases after coronary stenting. Our data then suggest that, for preventing acute stent thrombosis, Eptifibatide and Abciximab could be administered with equivalent results.

In our experimental model, an aspirin-like treatment (Indomethacin) was not as effective as GpIIbIIIa inhibitors in achieving platelet inhibition. This finding appears particularly relevant in patients, which, differently from controls, were also receiving Clopidogrel. In fact, a front-load dose of this compound, followed by a 28-days administration, has been recently proposed as the antithrombotic treatment of choice after stenting 28, 29. Instead, also considering that the adverse-event rate after stenting seems not to increase after the first 48 hours 10, 18, GpIIbIIIa inhibitors, administered at the time of intervention, could be proposed as a self-sufficient therapy. Patients in our study, however, could have profited by an additional platelet inhibition, provided by Clopidogrel. Moreover experimental studies have demonstrated that ADP-inhibitors enhance the platelet blockade of GpIIbIIIa
inhibitors. Then a combined strategy (parenteral GpIIbIIIa inhibitors during, and oral antiaggregants before and after stenting) could be advisable.

In the present study, tests with blood from patients, in comparison to controls and regardless of any antiplatelet treatment, resulted in an increased anti-GpIIIa, but not anti-GpIb, binding onto metallic probes. These data confirm an altered expression of surface platelet antigens in the course of CAD. A reduced expression of GpIb receptors (e.g. by cleavage or internalisation), partly concealing an increased platelet deposition, cannot be a priori excluded. However, CAD poorly influences GpIb expression, and only in parallel with that of GpIIbIIIa receptor. Instead, atherosclerosis and stable CAD can induce the overexpression of surface receptors, particularly those (like GpIIbIIIa receptor) involved in the process of platelet activation. Then, while anti-GpIb binding could be considered as a relatively constant indicator of platelet deposition, instead, to explain the increased anti-GpIIa binding found in the present study, we hypothesise an increased expression of activated GpIIbIIIa receptor (e.g. by externalisation and/or structural changes) on platelets from patients with CAD. Whether this phenomenon was already present, or it developed only after interaction with stent-like surfaces, could not be determined. Nevertheless, Gawaz et al. found that the relative risk of stent thrombosis is 18.5-fold for patients with enhanced GP IIbIIIa surface expression. On top of all that, GpIIbIIIa receptor expression manifests a wide inter-individual variability (e.g. by genetic polymorphisms). This is probably exacerbated, and can assume clinical relevance, in the presence of CAD, as, in the present study, a variability in the response to GpIIbIIIa inhibitors was demonstrated only among patients but not healthy controls (Figure 3). Extended GpIIbIIIa-receptor blockade in candidates to routine coronary stenting could then offer a broad protection against unexpected acute thrombotic complications.

**Study limitations**

In the present study a single concentration of both Eptifibatide and Abciximab was used, thus a dose-response curve could not be drawn. Potential differences in drug availability and distribution between *in-vivo* and *in-vitro* conditions should be also taken into account. Finally, gender and age bias, risk factors and/or concomitant medications could have induced differences in platelet receptors expression between patients and controls.

**Conclusions**

Eptifibatide and Abciximab at therapeutical concentrations exhibit an equivalent *in-vitro* efficacy in inhibiting platelet aggregation and deposition on stent-like surfaces.

Considering the occurrence of GpIIbIIIa-receptor overexpression in the course of CAD, and the incomplete blockade achieved by conventional antiplatelet therapy (aspirin-like and ADP-inhibitors), an extended use of these compounds may be justified, in order to prevent acute thrombosis in candidates to routine coronary stenting.
However, the enforcement in the clinical practice of this policy will require further investigations, taking into account also safety and economical profiles of different GpIIbIIIa inhibitors.

ACKNOWLEDGEMENTS

René A. Tio received financial support from The Netherlands Heart Foundation, grant D95-019. The study was supported by a grant from Schering Plough BV (Maarssen, NL). We are also indebted to Hemoprobe BV (Groningen, NL) for the laboratory investigations and to Paula J. Buentello for kindly reviewing the manuscript.
References


15. Lincoff AM. Trials of platelet glycoprotein IIb/IIIa receptor antagonists during percutaneous coronary revascularization. Am J Cardiol 1998;82:36P-42P.


34. Plow EF, D’Souza SE, Gimberg MH. Consequences of the interaction of platelet membrane glycoprotein GPllb-IIIa (alpha IIb beta 3) and its ligands. J Lab Clin Med 1992;120:198-204.
