In vitro effects of the glycoprotein IIb/IIIa receptor antagonists abciximab and eptifibatide on platelet aggregation in healthy cats

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Objective—To determine effects of the glycoprotein IIb/IIIa receptor antagonists abciximab and eptifibatide on in vitro inhibition of cat platelets.

Sample—Venous blood samples from 10 healthy cats.

Procedures—Blood samples were anticoagulated with hirudin. Aliquots of whole blood from each cat were allocated to 5 treatments (baseline, 50 µg of abciximab/mL, abciximab volumetric control treatment, 4µM eptifibatide, and eptifibatide volumetric control treatment). Impedance platelet aggregometry was performed with 6.5µM ADP or 32µM thrombin receptor activator peptide (TRAP). Magnitude of platelet aggregation was determined by measuring the area under the curve 15 minutes after addition of ADP or TRAP.

Results—Eptifibatide caused a significant reduction in platelet aggregation, compared with baseline values, for aggregometry with both ADP (median, 50.0; range, 8 to 122 [baseline median, 306.0; baseline range, 130 to 664]) and TRAP (median, 75.5; range, 3 to 148 [baseline median, 219.0; baseline range, 97 to 578]). There was no significant difference in platelet aggregation with abciximab, the abciximab volumetric control treatment, or the eptifibatide volumetric control treatment for aggregometry with ADP or TRAP.

Conclusions and Clinical Relevance—Eptifibatide caused a significant reduction in platelet aggregation in vitro, but there was no identifiable antiplatelet effect for abciximab. Eptifibatide and abciximab have different binding and inhibitory actions; therefore, it can be hypothesized that abciximab would be ineffective in cats because of a lack of receptor binding, reduced binding kinetics, or lack of downstream signaling. Eptifibatide may be useful in identifying hyperreactive platelets in cats in an in vitro platelet inhibitory assay.

Cardiogenic embolism occurs in approximately 6% to 17% of cats with underlying cardiac disease and is associated with a mortality rate of approximately 66%.1–3 It is assumed that these cats have some type of hypercoagulable state; however, no hypercoagulable states have been definitively identified in these cats, although it is suspected that hyperactive platelets are involved.4 A variety of antithrombotic drugs are used in the prevention of thrombus formation and the treatment of cats with cardiogenic embolism.5,6 Antiplatelet drugs such as aspirin and clopidogrel have received wide interest and are tolerated well in cats.6,7 Aspirin irreversibly inhibits platelet cyclooxygenase, which decreases the conversion of arachidonic acid to thromboxane A₂, whereas clopidogrel irreversibly inhibits the ADP₂Y12 Platelet membrane receptor.7,8

Multiple intracellular pathways are involved in platelet aggregation; these pathways are specific to the physiologic stimulus (agonist) that initiates platelet activation.9 The GP IIb/IIIa receptor complex is the most abundant protein on the surface of both inactivated and activated platelets, with some 40,000 to 80,000 copies/platelet.8,10,11 This receptor plays a principal role in adhesion and aggregation of platelets. After platelet activation, the GP IIb/IIIa receptor complex undergoes a conformational change and develops high-affinity binding for fibrinogen, von Willebrand factor, and other adhesive proteins.11 Regardless of the agonist that activates the platelets, the cross-linking of adjacent GP IIb/IIIa receptor complexes is the final common pathway of platelet aggregation.4,11 Therefore, antagonism of the GP IIb/IIIa receptor complex would result in platelet inhibition regardless of the agonist that initiated platelet activation.
Abciximab and eptifibatide are GP IIb/IIIa receptor antagonists that can significantly reduce platelet aggregation and thrombotic complications associated with percutaneous coronary intervention in humans.13–17 Abciximab improves arterial flow in vivo in cats with experimentally induced arterial thrombosis, presumably through an antplatelet effect.12 Eptifibatide inhibits platelet aggregation on feline platelets in vitro; however, to the authors’ knowledge, there are no published reports of the objective in vitro effects of GP IIb/IIIa receptor antagonists on aggregation of feline platelets.

The purpose of the study reported here was to determine whether abciximab and eptifibatide could inhibit in vitro platelet aggregation in samples obtained from healthy cats. These results could then be used to develop an inhibitory assay to identify hyperreactive platelets as part of the risk assessment for cardioembolic embolism.

**Materials and Methods**

**Animals**—Ten healthy adult cats owned by veterinarians, veterinary technicians, and veterinary students at the Purdue University Veterinary Teaching Hospital were used in the study. Median age of the cats was 8.50 years (range, 5 to 14 years), and all 10 were neutered male domestic shorthair cats. Health status was determined on the basis of the medical history (ie, cats did not have clinical signs of disease) and results of physical examination, with emphasis placed on evaluation of the cardiovascular system. None of the cats received medications with reported platelet effects for a minimum of 1 month prior to the study. Informed owner consent was obtained for all cats, and the study was approved by the Purdue Animal Care and Use Committee.

**GP IIb/IIIa receptor antagonists**—Commercially available vials of abciximab and eptifibatide were used. Each vial of abciximab contained 2 mg of abciximab/mL in a buffered solution (0.01M sodium phosphate, 0.15M sodium chloride, and 0.001% polysorbate 80 in water [pH, 7.2]) for injection, with no preservatives. Abciximab was not diluted for use in the study. Each vial of eptifibatide contained 2 mg of eptifibatide/mL, 5.25 mg of citric acid/mL, and sodium hydroxide (pH, 5.35). Eptifibatide was diluted 1:10 with 0.9% NaCl solution prior to use in platelet aggregation assays.

**Sample collection**—Cats were manually restrained during blood collection. Blood samples were collected in a nontraumatic manner via jugular venipuncture by use of a 23-gauge butterfly catheter with a Luer adapter fitted with a needle at the distal end.4 Once blood flow was observed, the needle on the distal end of the catheter was inserted into blood collection tubes. The first 1 to 2 mL of blood was collected into a tube with no additives; these samples were discarded. The next 6 mL of blood was collected into 2 double-walled vacuum plastic tubes (3 mL of blood/tube) containing hirudin (final concentration of hirudin in fully filled tubes, 25 µg/mL). Including the initial volume of discarded blood, ≤8 mL of blood was collected from each cat at any point in the study. Tubes of whole blood containing hirudin were placed in a vertical (upright) position and allowed to sit at room temperature (approx 22°C) for 30 minutes.

**Platelet aggregation**—Aliquots (350 µL) of whole blood from each cat were pipetted from the hirudin-containing collection tubes into each of 10 plastic tubes. Nothing was added to tubes 1 and 2; they contained only the 350 µL of blood (baseline). Additions to the other tubes were as follows: tubes 3 and 4, 8.8 µL of 2 mg of abciximab/mL (final concentration, 50 µg of abciximab); tubes 5 and 6, 8.8 µL of 0.9% NaCl solution (volumetric control for abciximab); tubes 7 and 8, 5.8 µL of diluted eptifibatide (final concentration, 4µM eptifibatide); and tubes 9 and 10, 5.8 µL of 0.9% NaCl solution (volumetric control for eptifibatide). After addition of the abciximab, eptifibatide, and 0.9% NaCl solution, aliquots were incubated for 10 minutes at room temperature prior to performing platelet aggregation.

Whole blood impedance aggregometry was performed as previously described.18 Briefly, platelet aggregation was induced by adding ADP6 (6.5µM) or TRAP8 (32µM) to each of the aliquots. Maximal platelet aggregation was measured 15 minutes after the addition of ADP or TRAP and reported as the AUC.

**Statistical analysis**—Statistical analyses were performed by use of a commercial statistical software program.9 Normality of data distribution was assessed with the Shapiro-Wilk test. Nonparametric data were logarithmically transformed to achieve a normal distribution. A repeated-measures ANOVA was performed on transformed data, and pairwise comparisons were performed with paired t tests. Significance of all analyses was set at a value of P < 0.05.

**Results**

The median and range values for ADP- and TRAP-induced platelet aggregation were summarized in Table 1.
1). Eptifibatide resulted in a significant ($P < 0.001$) reduction in both ADP- and TRAP-induced platelet aggregation, compared with baseline values, whereas there were no significant differences in ADP- or TRAP-induced platelet aggregation with abciximab, compared with baseline values (Figure 1). There were no significant differences in ADP- or TRAP-induced platelet aggregation with the volumetric control treatments for abciximab or eptifibatide.

**Discussion**

In the study reported here, the addition of eptifibatide resulted in significant in vitro inhibition of platelet aggregation induced by ADP and TRAP. In a previous report, it was suggested that eptifibatide inhibits feline platelets, although the methods differ between the present study and the study of that report. Also, that report did not contain objective values. In contrast, we did not detect in vitro platelet inhibition with abciximab, even at concentrations 20-fold as high as those that will almost totally abolish platelet aggregation in other species, including humans, nonhuman primates, and dogs. Results of the present study are discordant with data reported for a study of an in vivo evaluation of cats with experimentally induced arterial thrombosis in which abciximab was associated with improved arterial flow in the face of intimal injury, presumably because of an antiplatelet effect, although platelet aggregation was not evaluated.

The GP IIb/IIIa receptor complex is a member of the receptor family known as integrins, which are calcium-dependent heterodimeric cell-surface proteins that play important roles in cell adhesion. Most integrins are widely distributed; however, the GP IIb/IIIa receptor complex is found only in platelets and cells of the megakaryocytic lineage. The GP IIb/IIIa receptor complex is composed of 2 subunits: GP IIb (α) and GP IIIa (β3). The GP IIb subunit has only been found in receptor complexes with GP IIIa. However, the GP IIIa subunit also forms complexes with another α subunit, αv, to form the vitronectin receptor (αvβ3). The αvβ3 integrin is widely distributed and is found on many cell types, including endothelial cells, osteoclasts, and smooth muscle cells.

Eptifibatide is a synthetic cyclic heptapeptide analog for the ligand recognition site of the β3 subunit of the GP IIb/IIIa receptor complex and a highly specific inhibitor of GP IIb/IIIa with no cross-reactivity on non-platelet cell types. Eptifibatide is similar in structure to the peptide barbourin, which belongs to a family of peptides known as disintegrins. Disintegrins bind to integrins, inhibiting the binding of the physiologic integrin ligands. Abciximab is the antigen-binding fragment of the chimeric human-murine MAb 7E3 and causes a high affinity steric hindrance antagonism of the GP IIb/IIIa receptor complex. The differences in structure and binding characteristics of eptifibatide and abciximab may explain their discordant effects on aggregation of feline platelets. Differential binding of these molecules to the GP IIb/IIIa receptor complex is highlighted in a study that involved the use of 2 MAbs. In that study, MAb1, which binds near the ligand recognition site, displaced abciximab binding but had no effect on eptifibatide binding, whereas MAb2, which binds at a location on the β3 subunit, displaced eptifibatide binding but had no effect on abciximab binding. Eptifibatide recognizes the RGD (Arg-Gly-Asp) sequence found on many molecules that are present in all mammals and therefore would reasonably be thought to be highly conserved among mammalian species. Abciximab is an MAb to the human GP IIb/IIIa receptor complex and may recognize an epitope on the receptor complex that is specific to humans or, at least, an epitope that is not highly conserved among species.

In addition to blockade of the GP IIb/IIIa receptor complex, abciximab has equivalent affinity for and functional blockade of the α3β1 integrin and interacts with an antigen present on the leukocyte integrin Mac-1 (αMβ2). The interaction of abciximab with α3β1 and αvβ3 may cause an antithrombotic action distinct from the interaction with the GP IIb/IIIa receptor complex, which was suggested in a clinical trial conducted to evaluate ischemic complications associated with coronary interventions in humans. It is not known whether abciximab has this effect in cats.

It should be emphasized that eptifibatide has promise only for use in an in vitro inhibitory assay to identify hyperreactive platelets in cats. Eptifibatide reportedly causes circulatory failure and sudden death when administered IV to some cats. This adverse effect of eptifibatide appears to be unpredictable and idiosyncratic. Those authors concluded that this adverse effect was unique to the feline species. Although the cause of the adverse reactions in cats remains unknown, eptifibatide is structurally similar to barbourin, a constituent of the venom of the dusky pigmy rattlesnake, *Sistrurus mili-
OCUS M. Barbouri, and may induce a unique antibody response in cats.22
The present study had limitations, including a relatively small sample size of 10 cats. Additionally, variation among cats may have obscured actual differences in platelet inhibition. However, the objective of this study was to determine whether either drug could be used in an in vitro inhibitory assay for clinical patients in which variability is inherent.
Analysis of results of the present study suggested that eptifibatide has promise for use in an in vitro inhibitory assay to identify hyperreactive platelets in cats. Further evaluation would include determination of the optimal in vitro eptifibatide concentration to distinguish functionally normal platelets from hyperreactive platelets in cats at risk for development of cardiogenic embolism. However, analysis of our data suggested that abciximab does not appear to have an inhibitory effect on feline platelets, most likely because of ineffective binding to the GP IIb/IIIa receptor complex.