Glycoprotein IIb/IIIa Antagonist FK633 Could Not Prevent Neointimal Thickening in Stent Implantation Model of Canine Coronary Artery

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Abstract—The platelet glycoprotein (GP) IIb/IIIa receptor antagonist appears to reduce the need for revascularization after coronary angioplasty. However, since the effect of GP IIb/IIIa receptor antagonist on the in-stent neointimal thickening has not been clarified, we examined it in the canine model. The beagle dogs were assigned to the control (n = 7) or the GP IIb/IIIa receptor antagonist FK633 group (n = 7). FK633 was administered by subcutaneous osmotic pumps (0.2 mg/h) and an intravenous bolus injection (1 mg/kg) before stenting. A coil stent was implanted in the left circumflex coronary arteries. The platelet aggregation capability was significantly (<5%) and consistently reduced by FK633 except for the mild elevation (10% to 30%) on the next day of stenting. Hearts were excised 3 months after stent implantation. The area of intima and media and the area stenosis were not different between the groups. We conclude that, although GP IIb/IIIa antagonist FK633 prevented the platelet aggregation significantly and consistently, it could not prevent the neointimal thickening after stent implantation in canine coronary artery. (Arterioscler Thromb Vasc Biol. 1999;19:343-347.)

Key Words: restenosis | platelet | glycoprotein IIb/IIIa | stent

Although restenosis after coronary intervention has been a major issue for a long time, a viable or effective means of prevention has not been found. Several prospective randomized trials1,2 revealed that coronary stents, which prevent elastic recoil and vascular remodeling, reduce the incidence of restenosis. However, in-stent restenosis is still observed in 20% to 30% of patients and is mainly attributable to neointimal thickening.3–6 Although various drugs have proven effective in reducing neointimal thickening in animal experiments, no drug is clinically available at present to prevent restenosis. Although the platelet glycoprotein (GP) IIb/IIIa antagonists have been evaluated recently in clinical trials7–8 and are reported to reduce the ischemic events after coronary angioplasty and need for revascularization, their effectiveness in preventing restenosis has been refuted by the increasing evidence from clinical trials so far.

The current study was designed to evaluate the effect of GP IIb/IIIa antagonist FK6339–10 (Fujisawa Pharmaceutical Co) on neointimal thickening after stent implantation in canine coronary arteries.

Study Protocol
The animal study was approved by the Animal Care and Use Committee of the Animal Research Institute of Osaka University. Fourteen specific-pathogen-free, one-year-old beagle dogs weighing 8 to 12 kg (Oriental Yeast Co, Tokyo, Japan) were randomly assigned to the FK633 group (n = 7) or the control group (n = 7). A day before the stent implantation, the platelet aggregation induced by 20 μmol/L ADP (Sigma) was measured by standard aggregometer (Nikobioscience) in all animals using the blood samples obtained with 0.38% sodium citrate. Because the measurement of platelet aggregation is known to be unchanged by the platelet counts when they are >5×10^4/μL, the platelet counts were semiquantitatively measured and confirmed to be >5×10^4/μL. Three hours before stent implantation, osmotic pumps (alzet model 2 ML4, Alza Co) were subcutaneously implanted to administer FK633 at 0.2 mg/h kg^(-1) h^(-1) in 7 dogs (the FK633 group). The pumps were replaced with new ones 1 and 2 months after the initial implantation for the continuous administration of FK633 for 3 months, until the completion of the study. The platelet aggregation capability was also measured 1 to 2 days; 2 weeks; and 1, 2, and 3 months after stent implantation in the FK633 group and 1 month after the implantation in the control group. The platelet and white blood cell counts, hematocrit, hemoglobin concentration, prothrombin time (PT), and activated partial thromboplastin time (APTT) were measured at...
baseline and at 2 and 3 months after stent implantation. PT and APTT were measured with the Chromoquick kit and CTS Neotomin (Dade Behring Inc), respectively.

Dogs were anesthetized with intravenous pentobarbital (30 mg/kg) administration. Under sterile conditions, the right femoral artery was exposed and an 8F sheath was inserted. After systemic heparinization (200 U/kg) and the administration of FK633 (1 mg/kg IV in the FK633 group), a coil stent made of tantalum (~15 mm long) was placed in the proximal left circumflex coronary artery by a trained interventionist using the standard PTCA technique with a 7F guiding catheter JSL20 (Toray Medical), a 0.014-in guidewire (Boston Scientific), a 2.5-mm balloon catheter (Boston Scientific), and the nonionic contrast agent Iopamiron 370 (Schering). The stent was manually crimped onto the balloon and deployed by its inflation at 10 atm for 30 seconds. After the stent implantation and the administration of antibiotics (2 g cefazolin sodium, Fujisawa), all equipment was removed and the femoral artery was ligated.

For histological analysis, the heart was excised 3 months after stent implantation and perfusion-fixed with 95% ethanol for 24 hours by a pathologist. The left circumflex coronary artery, including implanted stent, was densely dissected from the heart. The dissected whole artery was embedded in glycol methacrylate (HistoResin Plus, Leica), leaving the stent wires intact to minimize potential artifacts from removal or cutting of stent wires. The artery embedded in glycol methacrylate was cut at 3-mm intervals with an electric cutting machine that had a circular saw blade and subdivided into 6 to 8 blocks. The blocks were cut at 4-μm thickness with tungsten carbide blades using AS500 semi-thin microtome (Anglia Scientific). The sections were stained with hematoxylin-eosin and Weigert-van Gieson stains.

**Evaluation of Neointimal Thickening**

On the photographs acquired from the stained histology slides, the area within the external elastic lamina (AEE) and the area of intima and media (AIM) were measured with an NIH image on a Macintosh computer (Apple Computer) by an analyst blinded to the background data. The area stenosis was calculated as AIM/AEE. These data were acquired on all cross-sections and were averaged for each stented artery. Since (1) the media usually thinned at the portion of stent wire implantation and (2) the clinically evaluated neointima by intravascular ultrasound imaging included intima and media, we used AIM and the area stenosis to evaluate the degree of neointimal thickening.

**Statistical Analysis**

All data were presented as mean±SD. The differences in the repeatedly measured data were analyzed by ANOVA with Scheffe’s comparison test. The histologically measured data were analyzed by Student’s t test. The value of $P<0.05$ was considered significant.

**Results**

**Stent Implantation and Postprocedural Events**

The implantation of stent was successful (without delivery failure) in all dogs. Neither coronary dissection or abrupt closure was detected angiographically after stent implantation. No dog died during the study, and no dog pathologically presented stent occlusion at 3 months. Although no severe hemorrhagic complication was detectable through general observations of the dogs, a hematoma at the femoral wound was observed in all dogs in the FK633 group but in none of the dogs in the control group on the day after stent implantation. The hematoma did not increase in size thereafter and was absorbed by 1 month. The hematocrit (53.7±5.4% at baseline) and hemoglobin concentration (17.9±1.4 g/dL at baseline) decreased at 2 months (40.0±11.1%, 13.0±3.9 g/dL) and recovered at 3 months (55.3±5.5%, 17.0±1.9 g/dL) in the FK633 group but were unchanged throughout the study in the control group.

**Effect of GP IIb/IIIa Antagonist on Platelet Aggregation**

The platelet aggregation (Table 1) induced by 20 μmol/L ADP was almost completely (~5%) and consistently prevented by the administration of FK633 (Figure 1) throughout the study, except for mild elevation (10% to 30%) on the day after stent implantation. Because FK633 (1 mg/kg) was administered intravenously before stenting, in addition to the administration by implanted osmotic pumps, platelet aggregation was supposed to be prevented almost completely before the stent was implanted. There was no difference in platelet aggregation before and 1 month after stent implantation in the control group. The platelet count (26.5±8.8×10^4/μL at baseline) decreased in the FK633 group at 2 months (11.2±6.1×10^4/μL) but recovered at 3 months (25.3±9.7×10^4/μL). The white blood cell counts (10 917±4380/μL at baseline) also decreased in the FK633 group at 2 months (8471±2841/μL) but recovered at 3 months (13 214±2936/μL). The platelet and white blood cell counts did not change throughout the study in the control group. The plasma fibrinogen, PT, and APTT did not change throughout the study in both groups.

<table>
<thead>
<tr>
<th>Maximum Platelet Aggregation Induced by 20 μM ADP</th>
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<tbody>
<tr>
<td><strong>Time After Stenting</strong></td>
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<td>----------------------------</td>
</tr>
<tr>
<td>Number</td>
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<td>1 day before</td>
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ND indicates not done. *$P<0.0001$ vs 1 day before; †$P<0.0001$ vs control group.

![Figure 1](image-url)
Effect of GP IIb/IIIa Antagonist on Neointimal Thickening

The histology of the stented coronary arteries at 3 months (Figure 2) showed significant thickening of the intima. Stent wires were placed adjacent to the external elastic lamina compressing the media with or without slight disruption of the internal elastic lamina in all sections. However, no disruption of the external elastic lamina was observed. Therefore, the severity of vascular injury caused by the stent wires was similar in both groups. No thrombus was observed within the neointima at 3 months. The neointimal area (AIM, $1.3\pm0.2$ and $1.3\pm0.2$ mm$^2$ in the FK633 and the control groups, respectively; Figure 3) and the area stenosis ($41.8\pm7.5\%$ and $33.9\pm6.7\%$ in the FK633 and the control groups, respectively; Figure 4) were not different between the groups.

Discussion

Mechanisms of Restenosis and the Model of Coronary Stenting

The mechanisms involved in restenosis after coronary angioplasty are (1) vascular recoil, (2) coronary dissection, (3) thrombosis, (4) intimal hyperplasia, (5) matrix production, and (6) vascular remodeling. All clinical trials performed so far used drugs (eg, anticoagulant or antiproliferative drugs) against only one of these mechanisms. Although these drugs were ineffective for preventing restenosis in the clinical trials, it is unknown whether they actually are ineffective in reducing intimal thickening or vascular remodeling in particular. We should evaluate the same parameters in clinical trials as in the animal experiments to determine whether the experimental results are also true in humans. The mechanism of in-stent restenosis, on the other hand, is mainly neointimal thickening promoted by thrombosis, smooth muscle cell proliferation, and matrix production. We can measure the in-stent neointimal area both in clinical trials and in animal experiments. Clinically evaluated neointimal area by intravascular ultrasound imaging corresponds to the AIM in our animal model. Therefore, we might be able to predict the clinical outcome from our experimental results without being disturbed by the effects of vascular recoil or remodeling. Since the use of stents has become common recently, the experimental results in the model of coronary stenting would be clinically informative.

Effects of GP IIb/IIIa Antagonist on Platelet Aggregation and In-Stent Neointimal Thickening

The platelet aggregation induced by 20 \(\mu\text{mol/L}\) ADP was $<3\%$, $10\%$, and $20\%$ at 60, 120, and 180 minutes, respectively, after intravenous bolus administration of 1 mg/kg FK633 in the preliminary study. Therefore, the platelet aggregation would be $<3\%$ in the FK633 group when the stent was implanted. However, mild elevation of platelet aggregation (10% to 30%) was observed (Figure 1) in some dogs on the day of after stent implantation, possibly because of the platelets activated by coronary and/or femoral injuries. The platelet aggregation was almost completely ($<3\%$) and continuously prevented after 2 weeks until the end of the study. FK633 had no effect on the coagulation system so far as it was detected by plasma fibrinogen, PT, or APTT. The transient decrease in the number of platelets, white blood cells, and red blood cells may be the effect of FK633, which seems beneficial for reducing neointimal thickening by reducing thrombosis. Despite the significant suppression of platelet aggregation, the in-stent neointimal thickening was not at all reduced by the drug. This result was not changed even by...
excluding the dog that presented rather high (29%) platelet aggregation on the day after stent implantation.

**GP IIb/IIIa Antagonists and Restenosis in Previous Reports**

Recently, the Evaluation of c7E3 for Prevention of Ischemic Complications (EPIC) trial\(^8\)\(^{-11}\) demonstrated that the monoclonal antibody Fab fragment (c7E3) directed against GP IIb/IIIa reduces the incidence of adverse events and restenosis after angioplasty. However, some GP IIb/IIIa antagonists\(^12\)\(^{-14}\) do not reduce intimal hyperplasia in animal experiments. These investigators used the animal models of vascular endothelial denudation, which did not mimic the vascular injury caused by balloon angioplasty in the clinical interventions. The current report evaluated, for the first time, the effect of GP IIb/IIIa antagonist on the neointimal hyperplasia in the model of coronary stenting, which closely mimicked the clinical interventions, and revealed that the drug was ineffective in reducing neointimal hyperplasia. The discrepancy in the results of the studies reported so far may be explained by the differences in (1) animal species, (2) type of injury, and (3) drug characteristics. Some anti-GP IIb/IIIa (integrin \(\alpha_{\text{IIb}}\beta_3\)) drugs are known to antagonize vitronectin receptors (integrin \(\alpha_{\text{IIb}}\beta_3\)) and are reported\(^15\) to reduce intimal hyperplasia and the incidence of restenosis, whereas selective GP IIb/IIIa (integrin \(\alpha_{\text{IIb}}\beta_3\)) antagonists do not. Because integrin \(\alpha_{\text{IIb}}\beta_3\) is responsible for binding platelets to endothelial cells, this may be the cause of the differences in the results. Because FK633 does not antagonize vitronectin receptors,\(^10\) our result is consistent with those of previous reports. Although the prevention of thrombus formation by anticoagulant drugs\(^16\)\(^{-19}\) reduces the intimal thickening, we have shown that the continuous prevention of platelet aggregation by a GP IIb/IIIa antagonist does not reduce intimal thickening. We evaluated the inhibition of platelet aggregation by the ex vivo examination commonly used in clinical trials. A possible explanation is that the selective inhibition of GP IIb/IIIa to the level we achieved in the current study would not prevent platelet adhesion or local thrombus formation at the stented coronary segment. Platelet-derived growth factor or thrombin generated locally would work as a strong promoter of neointimal hyperplasia. Therefore, our results would not necessarily deny the importance of platelets or thrombus in the mechanisms of restenosis. Due to lack of knowledge of the molecular and cellular mechanisms of restenosis and especially of the involvement of platelets in it, we do not know whether managing the function of platelets can lead to the prevention of restenosis.

**Conclusions**

We demonstrated that the prevention of platelet aggregation by intravenous bolus injection and continuous subcutaneous infusion of a selective GP IIb/IIIa antagonist FK633 does not reduce in-stent neointimal thickening, although the prevention of platelet aggregation reached a level similar to or more severe than the level achieved in clinical trials. Therefore, selective GP IIb/IIIa antagonists do not appear to prevent restenosis after stent implantation.

**Limitations of the Study**

Because mild elevation of platelet aggregation (10% to 30%) was observed in some dogs on the day after stent implantation, it may be possible that mild elevation of platelet aggregation early after stenting critically influenced the results. Because vascular responses to angioplasty-induced injury may differ between species or between normal and atherosclerotic arteries, our results with normal dog coronary arteries does not necessarily predict the clinical outcome. Because intimal hyperplasia induced by angioplasty is milder in dogs than in pigs and the severity of vascular injury in the current study was relatively mild, the sensitivity of the ability to detect the effect of drugs may not be high. However, FK633 did not have antiplatelet effect in pigs; therefore, we could not use a pig model. Because in the current study FK633 did not reveal any tendency to reduce neointimal thickening, its effect, if any, may be a limited one. To test our result in humans, the effect of GP IIb/IIIa antagonists on the in-stent restenosis should be investigated in clinical trials.

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