Antithrombotic Effect of Tissue Factor Inhibition by Inactivated Factor VIIa
An Ex Vivo Human Study

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Abstract—FFR-rFVIIa is an inactivated recombinant factor VIIa (rFVIIa) that inhibits the binding of factor VIIa to tissue factor (TF). It has been shown to prevent TF-induced thrombosis in animals. The present study is a substudy of the Active Site Inhibited Seven (ASIS) trial and examines the antithrombotic effect of 3 doses of FFR-rFVIIa in 24 patients undergoing percutaneous coronary intervention (PCI). Group 1 (n = 9) received 400 μg/kg FFR-rFVIIa and 40 to 50 U/kg heparin, group 2 (n = 7) received 200 μg/kg FFR-rFVIIa and 100 U/kg heparin, and group 3 (n = 8) received 50 μg/kg FFR-rFVIIa and 100 U/kg heparin. Blood thrombogenicity was assessed as total thrombus area and fibrin deposition on the perfusion chamber at shear rate conditions typical of mild-moderate coronary stenosis. Baseline blood thrombogenicity was evaluated a day before PCI, after heparin administration. A second perfusion chamber study was performed just before PCI, 15 minutes after the administration of heparin and FFR-rFVIIa. Thrombus formation at a high shear rate was markedly reduced in groups 1 and 2 after drug administration, by 79% to 84% and 76% to 87%, respectively (P < 0.004 [group 1], P < 0.04 [group 2]). In group 3, moderate thrombus reduction of 46% to 48% was achieved (P < 0.04). Fibrin deposition in all 3 groups was nearly eliminated after drug administration. Our data demonstrate that FFR-rFVIIa has a potent antithrombotic effect at different shear rates and severe arterial injury conditions. (Arterioscler Thromb Vasc Biol. 2002;22:III-39–III-44.)

Key Words: tissue factor; thrombus formation; factor VIIa; blood flow; fibrin; platelets

Percutaneous coronary interventions (PCIs) are increasingly performed for the treatment of severe coronary stenosis. Such interventions cause mechanical vascular injury, inducing a series of events that may give rise to acute thrombus formation as well as restenosis.1–3 Thrombin generation, platelet activation, and fibrin deposition are the main events leading to acute thrombosis.4,5 Inhibition of thrombin generation has been associated with a decrease in acute thrombosis as well as restenosis after PCI.6–9 and is therefore considered to be an important therapeutic target.

Tissue factor (TF) is a membrane-bound glycoprotein that initiates the coagulation cascade and is considered to be a major regulator of hemostasis and thrombosis. TF and factor VIIa form complexes that catalyze the activation of factors IX and X, which, in turn, lead to in vivo thrombin generation.10,11 Vessel wall injury caused by PCI is associated with rapid induction of TF mRNA and activity as well as increased exposure of circulating blood to vascular TF.10,12,13 Therefore, inhibition of TF activity during PCI may reduce thrombosis.

FFR-rFVIIa (NovoSeven) is a modified recombinant factor VIIa (rFVIIa) with the active site irreversibly blocked by a synthetic tripeptide, chloromethyl ketone. The resulting molecule retains its TF binding capacity but is enzymatically inactive. FFR-rFVIIa exerts its antithrombotic effect by competing with native factor VIIa (FVIIa) for TF binding and consequently impeding TF/FVIIa activity.14,15 FFR-rFVIIa has been shown to have a higher affinity to TF than native FVIIa.16 In rabbit and baboon models, FFR-rFVIIa efficiently prevented TF-induced arterial thrombosis.17–20 FFR-rFVIIa also significantly reduced restenosis after vascular injury in a rabbit model.7 Furthermore, FFR-rFVIIa has been shown to exert an antithrombotic effect in human blood in the parallel-plate perfusion device, with the drug being added ex vivo.14,21
The antithrombotic activity of the drug has been demonstrated in this model at venous and arterial shear rates. A phase I clinical study has reported that administration of single doses of FFR-rFVIIa up to 400 μg/kg to 64 healthy subjects did not affect the safety of the subjects nor the hemostatic function, except for the expected prolongation of the prothrombin time (PT). On the basis of these findings, the Active Site Inhibited Seven (ASIS) phase II study was performed. This was a multicenter, double-blind, dose-escalation, randomized trial evaluating the efficacy and safety of FFR-rFVIIa in patients undergoing elective or urgent PCI. In association with this trial, we performed a single-center substudy. The aim of the substudy was to evaluate the antithrombotic effect of FFR-rFVIIa in an ex vivo perfusion chamber connected directly to the patients’ blood streams.

**Methods**

**Patients**

Thirty patients eligible for the ASIS phase II trial at Mount Sinai Medical Center, New York, NY, were also enrolled in the substudy. The substudy and main trial were performed in a blinded manner, with data from each cohort being unblinded only after its completion. Three patients withdrew from the substudy after enrollment and randomization, thus leaving 27 patients who completed the substudy and the main ASIS trial. All patients had planned to undergo elective or urgent PCI. Main exclusion criteria were acute myocardial infarction or unstable angina within the prior 24 hours, contraindications to anticoagulant treatment, uncontrolled hypertension at presentation (>180/100 mm Hg), thrombocytopenia (<100 000 cells/μL), recent or planned use of glycoprotein IIb/IIIa inhibitors, or recent surgery. PCI within the previous 3 months. The present study was approved by the institutional review board of the Mount Sinai Medical Center, and informed consent was obtained from each patient. The study was performed from July 1999 through July 2000.

**Medications**

Three patients were randomized to receive placebo (plus heparin), and 24 patients received active drug. The substudy consisted of 3 groups, corresponding to the last 3 cohorts of the ASIS trial with different FFR-rFVIIa dosages. FFR-rFVIIa was administered as an intravenous injection over 5 minutes in the catheterization laboratory, followed by an intravenous bolus of heparin, just before crossing the lesion with a guidewire. Group 1 (n=9) received 400 μg/kg of FFR-rFVIIa and 40 to 50 U/kg of heparin. Group 2 (n=7) received 200 μg/kg of FFR-rFVIIa and 100 U/kg of heparin. Group 3 (n=8) received 50 μg/kg of FFR-rFVIIa and 100 U/kg of heparin. All patients received concomitant therapy with aspirin (325 mg orally daily) and clopidogrel (75 mg orally daily) after the PCI.

**Perfusion Chamber**

Each patient underwent 2 perfusion chamber studies: a baseline chamber study, performed a day before the PCI, and a second study performed in the catheterization laboratory, just before the PCI. This design allows each patient’s pretreatment chamber value to serve as his/her own control. The first chamber (baseline) was performed 10 minutes after a heparin bolus, which was given at the same dosage the patient would be receiving the following day. The second chamber study was performed just before PCI, 15 minutes after FFR-rFVIIa administration, and 10 minutes after the heparin bolus.

The perfusion chamber used in the present study for quantification of thrombus formation has been extensively described elsewhere. It consists of a cylindrical flow channel (1- or 2-mm diameter, 2-cm length) that allows the flowing of blood, pumped directly from the patient, over an exposed thrombogenic surface. Local flow conditions mimicking mild to moderately stenotic coronary arteries were kept constant in all experiments: blood flow rate of 10 mL/min and a shear rate of 1690 per second (high shear rate [HSR]) or 212 per second (low shear rate [LSR]). Our previous work demonstrated that these rheological conditions resulted in consistent levels of platelet deposition and thrombus formation. We have also previously shown that heparin administration does not significantly alter thrombus formation in the perfusion chamber.

**Thrombogenic Substrates**

To trigger thrombus formation, the chamber contained porcine aortic tunica media sections. The substrates (25×10-mm sections) were surgically prepared to simulate the degree of severe arterial injury induced by PCI, as previously described. Some of the tunica media segments were later coated with human TF (Thromborel) to enhance TF-induced thrombus formation. TF coating was based on studies with Thromborel-coated coverslips, which have shown that Thromborel offers a well-characterized TF surface, allowing reproducible TF-dependent thrombus formation. One vial of Thromborel was dispersed in 2 mL distilled water, incubated for 15 minutes at 37°C, and diluted 1:50 in coating buffer (0.1 mol/L sodium carbonate, pH 9.5). The tunica media segments were incubated at 4°C for ∼17 hours in the Thromborel dilution and then rinsed and immersed in PBS before use in the perfusion studies.

**Evaluation of Thrombus Formation**

After perfusion, specimens were removed from the chamber and immediately fixed in 4% phosphate-buffered paraformaldehyde. Specimens were then transversely cut into 2- to 4-mm-thick pieces and paraffin-embedded. Histological sections (5 μm) from each specimen were prepared and stained with 2 types of stain: combined Masson’s trichrome-elastin, which stains the total thrombus, and a murine monoclonal anti-human B2/15-42 antibody, which reacts with fibrin polymer. Morphometric analyses were conducted at 100-fold magnification, and images were digitalized with a Sony DKC-5000 camera and Adobe Photoshop 4.0 software. Thrombus area was measured on each section by computerized planimetry with the use of Image-Pro Plus software. The results of 6 sections were averaged to determine the total thrombus and fibrin area for each chamber.

**Platelet Function Evaluation: CPA**

Just before each perfusion chamber study, venous blood was drawn in Vacutainers containing 3.8% sodium citrate and evaluated for platelet function, PT, activated partial thromboplastin time, and complete blood count. Platelet function was assessed by the cone and platelet assay (CPA) test, which quantifies platelet deposition under flow conditions. Citrated whole blood was placed in polystyrene wells and circulated at an HSR of 1300 per second with a rotating cone. The wells were then thoroughly washed with PBS, stained with May-Grünwald stain, and analyzed with an inverted light microscope connected to an image analysis system (NIH Image Version 1.60). Results were expressed as the percentage of total surface area covered by platelets.
**TABLE 1. Clinical Characteristics**

<table>
<thead>
<tr>
<th>Age, y</th>
<th>Group 1, n=9</th>
<th>Group 2, n=7</th>
<th>Group 3, n=8</th>
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<tr>
<td>57.8±6.4</td>
<td>58.9±11.9</td>
<td>61.1±7.3</td>
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<tr>
<td>Men, n (%)</td>
<td>6 (66.7)</td>
<td>4 (57.1)</td>
<td>5 (62.5)</td>
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<td>Diabetes, n (%)</td>
<td>5 (55.5)</td>
<td>3 (42.9)</td>
<td>5 (62.5)</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>3 (33.3)</td>
<td>2 (28.6)</td>
<td>5 (62.5)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>7 (77.8)</td>
<td>4 (57.1)</td>
<td>6 (75)</td>
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<tr>
<td>Smoking, n (%)</td>
<td>5 (55.5)</td>
<td>3 (42.9)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Unstable angina, n (%)</td>
<td>5 (55.5)</td>
<td>5 (71.4)</td>
<td>6 (75)</td>
</tr>
<tr>
<td>Previous MI, n (%)</td>
<td>4 (44.4)</td>
<td>2 (28.6)</td>
<td>3 (37.5)</td>
</tr>
</tbody>
</table>

MI indicates myocardial infarction.

**Hematological Tests**

Plasma levels of P-selectin, β-thromboglobulin (β-TG), thrombin-antithrombin complex (TAT), and prothrombin fragment 1+2 (F1.2) were evaluated from frozen plasma samples collected before FFR-rFVIIa administration (baseline) and 1 hour after drug injection. Quantification of levels of the various parameters was performed according to the manufacturer’s instructions. Human soluble P-selectin and β-TG assays use a quantitative sandwich immunoassay technique with a monoclonal antibody specific for P-selectin (R & D Systems) and β-TG, respectively (Diagnostica Stago). TAT and F1.2 levels were measured by enzyme immunoassay kits (Enzygnost, Dade Behring).

**Statistical Analysis**

Results are expressed as mean±SEM. Paired continuous variables were compared by the Wilcoxon signed rank test. Categorical variables were compared with the Fisher exact test. Statistical significance was set at a value of *P*<0.05 (2-tailed).

**Results**

**Study Population**

The baseline characteristics of the patients are outlined in Table 1. There were no significant differences in any of the clinical parameters between the 3 groups. All patients underwent percutaneous coronary procedures.

**Thrombus Formation**

Total thrombus formation results are shown in Figure 1. In all 3 groups, the average thrombus area decreased significantly from baseline to the postdrug study, although the decrease noted in group 3 was less marked than in the other 2 groups. In the first flow chamber (tunica media without TF coating at HSR), thrombus formation declined by 84% in group 1, 87% in group 2, and 48% in group 3. In the second flow chamber (TF-coated tunica media at LSR), the thrombus area decreased by 94% in group 1, 96.5% in group 2, and 79% in group 3. In the third flow chamber (TF-coated tunica media at HSR), thrombus formation decreased by 79% in group 1, 76% in group 2, and 46% in group 3. As expected, the flow chamber coated by TF exhibited more thrombus formation (at baseline and also after drug administration) than the flow chamber at the same shear rate without TF coating (chamber type III versus type I), although the differences were not statistically significant. Photomicrographs of total thrombus in a representative patient from group 1 are shown in Figure 2.

It should be noted that compared with groups 1 and 2, group 3 had lower average thrombus area at baseline. This was due to lower thrombus areas in 2 patients (baseline areas ranging from 4000 to 5000 μm²). However, the proportional decrease in thrombus area from the baseline to the posttreatment value was consistent and to a similar degree among all patients in this group.

In the 3 patients who received only heparin (and placebo), the mean thrombus area did not change significantly from baseline to the postdrug samples (HSR without TF coating was 8412±1756 and 10 402±2164 μm², LSR with TF coating was 10 414±1211 and 8182±1042 μm², and HSR with TF coating was 11 452±1146 and 10 108±1665 μm² in the baseline and postdrug chambers, respectively).

**Fibrin Deposition**

Fibrin deposition was nearly abolished in groups 1 and 2 after FFR-rFVIIa administration. In group 1, the average fibrin area at baseline in the 3 types of flow chambers ranged from 4487 to 5492 μm² and declined to 22 to 30 μm² after the FFR-rFVIIa injection (*P*(<0.004). In group 2, the average baseline fibrin area ranged from 6266 to 6971 μm² and declined to 19 to 31 μm² after treatment (*P*(<0.01). The fibrin deposition in group 3 also decreased markedly after FFR-rFVIIa administration, although it was not abolished. The average baseline fibrin area in group 3 ranged from 3413 to 3906 μm² in the 3 types of flow chambers and declined to 178 to 679 μm² after treatment (*P*(<0.01). In the 3 patients who received heparin alone, the mean fibrin area did not change significantly after FFR-rFVIIa administration.

**Platelet Function and Coagulation Tests**

Results of complete blood count, platelet function, and coagulation tests are presented in Table 2. There were no significant differences between the 3 groups in the baseline values of the blood count and coagulation tests, except for baseline platelet levels, which were lower in group 2 than in group 1 (*P*(<0.02). In all 3 groups, hemoglobin levels decreased significantly (by an average of 0.9 g/dL) from baseline to the posttreatment sample taken the next day (*P*(<0.02). This decrease was probably partly due to blood drawing. As expected, the administration of FFR-rFVIIa resulted in a significant prolongation of the PT in all 3 groups (*P*(<0.01) but did not affect the activated partial thromboplastin time. The PT prolongation in group 3 (the lower dosage group) was less marked than in groups 1 and 2 (average posttreatment PT of 34.9±1.7 seconds in group 3 compared with 58.7±1.3 and 55.5±4.5 seconds in groups 1 and 2,
Figure 1. Total thrombus formation in the 3 treatment groups at baseline (black bars) and after drug administration (white bars). Group 1 (n=9) received 400 μg/kg FFR-rFVIIa, group 2 (n=7) received 200 μg/kg FFR-rFVIIa, and group 3 (n=8) received 50 μg/kg FFR-rFVIIa. Results are presented for the 3 types of flow chambers (tunica media without TF coverage, 0.1-mm diameter; tunica media with TF, 0.2-mm diameter; and tunica media with TF, 0.1-mm diameter). Total thrombus results are expressed as mean ± SEM (in micrometers squared). *P < 0.02, †P < 0.01, and ‡P < 0.03 for baseline vs after drug administration.

Figure 2. Representative photomicrographs of porcine media exposed to flowing blood from a group 1 patient. Micrograph A was taken at baseline (after heparin bolus); micrograph B was taken after FFR-rFVIIa + heparin administration. Sections were stained with combined Masson’s trichrome-elastic (CME) to demonstrate total thrombus deposition. Original magnification ×100.
Table 2. Results of Complete Blood Count, Platelet Function and Coagulation Tests

<table>
<thead>
<tr>
<th></th>
<th>Group 1, n=9</th>
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<th>Group 2, n=7</th>
<th></th>
<th>Group 3, n=8</th>
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</thead>
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<tr>
<td></td>
<td>Baseline</td>
<td>Postdrug</td>
<td>Baseline</td>
<td>Postdrug</td>
<td>Baseline</td>
<td>Postdrug</td>
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<tr>
<td>Hg, gm/dL</td>
<td>12.9±0.5*</td>
<td>12.1±0.5*</td>
<td>13.6±0.5*</td>
<td>12.9±0.5*</td>
<td>13.1±0.7*</td>
<td>11.9±0.6*</td>
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<tr>
<td>Plt, 1000/μL</td>
<td>251.9±14.9</td>
<td>234.3±11.6</td>
<td>187.3±18.1</td>
<td>175.1±17.5</td>
<td>226.9±26.9</td>
<td>208.4±25</td>
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<td>PT, s</td>
<td>14.2±0.6</td>
<td>58.7±1.3†</td>
<td>15.5±1</td>
<td>55.5±4.5†</td>
<td>14.3±0.6</td>
<td>34.9±1.7†</td>
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<td>APTT, s</td>
<td>90.3±5.3</td>
<td>98.1±6.8</td>
<td>90.4±6.8</td>
<td>97.9±2.2</td>
<td>93.6±4.9</td>
<td>94.4±5.6</td>
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<tr>
<td>Fibr, mg/dL</td>
<td>414±34.5</td>
<td>414.1±36.7</td>
<td>443.4±73.4</td>
<td>434.6±74.8</td>
<td>412.6±21.3</td>
<td>395.3±25.8</td>
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<td>CPA, %covered</td>
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<td>5.7±0.8</td>
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<td>TAT, μg/L</td>
<td>8.6±2.6</td>
<td>3.7±1.4</td>
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<td>F1.2, nmol/L</td>
<td>2.1±1.1</td>
<td>0.75±0.1†</td>
<td>1.4±0.3</td>
<td>0.9±0.2‡</td>
<td>7.8±2.0</td>
<td>4.4±1.3</td>
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<td>P-sel, ng/mL</td>
<td>45.7±3.3</td>
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<td>29.3±2.5§</td>
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<td>β-TG, IU/mL</td>
<td>156.9±17.2</td>
<td>104±17.8</td>
<td>135.7±23.6</td>
<td>122.3±18.8</td>
<td>143.2±19.4</td>
<td>79.2±12.8‡</td>
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Values are mean±SEM. *P<0.02, †P<0.01, ‡P<0.03, §P<0.05, all for baseline vs postdrug in the specific group.

Discussion

This is the first study to evaluate the antithrombotic efficacy of inactivated FVIIa administered systemically to coronary patients. The present study clearly demonstrated that FFR-rFVIIa has a potent antithrombotic effect, confirming previous reports in which the drug was locally added to human blood.14 The study was performed with the use of a well-characterized perfusion chamber system exposing different types of thrombogenic substrates to blood flowing at HSR and LSR conditions. Our results showed a significant inhibitory effect on both thrombus and fibrin deposition on severely injured arterial surfaces. Furthermore, there appears to be a dose-dependent effect, whereby the 2 higher doses of the drug (200 and 400 μg/kg) achieved marked thrombus inhibition (76% to 87% reduction at HSR), and the lower dosage (50 μg/kg) achieved only moderate inhibition (46% to 48% at HSR). This dose effect was also apparent in the PT prolongation achieved by the various drug doses.

TF has been shown to be an important determinant of the thrombogenicity of human atherosclerotic lesions after spontaneous or mechanical plaque disruption.32 It is also considered to play a pivotal role in the initiation of coagulation and thrombus formation in vivo.10,11,33 In animal models, inhibition of the TF pathway by anti-TF antibodies,34,35 recombinant TF pathway inhibitors,36,37 and inactivated FVIIa7,17–20 has been shown to prevent arterial thrombosis as well as intimal hyperplasia. More recently, several observations have suggested that high levels of plasma TF37 or microparticles present in the blood with TF activity38 are associated with acute coronary syndromes and an increased risk for adverse outcomes.37 We evaluated the potential antithrombotic effect of the inhibition of the TF pathway by FFR-rFVIIa in patients undergoing PCI.

In the present study, fibrin deposition was nearly abolished by administration of FFR-rFVIIa, especially at the 2 higher doses. Therefore, it can be assumed that most of the residual thrombus was composed of platelet aggregates. Whole platelet function as assessed by a flow-dependent assay (CPA) was not significantly affected by FFR-rFVIIa administration, nor was the platelet activation marker β-TG in most groups. Only P-selectin decreased modestly. These results are in accordance with animal studies17 and the phase I study, which showed that platelet aggregation (induced by ADP and thrombin receptor–activating peptide) and bleeding time were not significantly affected by FFR-rFVIIa administration at various doses.32 However, given the clear reduction in thrombin generation (F1.2) in the high-dose groups and, to a lesser extent, the decrease in thrombin activity (TAT), it would have been expected that platelet activation and deposition would also be significantly affected. Possibly, platelets adhered and were activated by other thrombus-promoting components in the vessel wall lesion, such as collagen fibrils.29 In addition, minute quantities of thrombin can be generated independent of the TF-FVIIa pathway33 and may contribute to platelet reactivity.

A limitation of the present study is the small size of the 3 groups; however, the design of the study allowed each patient’s pretreatment value to serve as his/her own control and, therefore, enabled statistically significant results. It should also be noted that the marked antithrombotic effect of FFR-rFVIIa was achieved 15 minutes after the drug bolus (at its peak effect). It remains to be established whether this peak antithrombotic effect is translated to clinical efficacy over time and whether a maintenance infusion of the drug is required.

In conclusion, our results demonstrate that inhibition of the TF pathway by FFR-rFVIIa markedly reduces thrombus...
formation and fibrin deposition in conditions of severe arterial injury. Such a novel approach may be beneficial in preventing thrombotic complications of PCI, although clinical refinement of the optimal dosage and method of administration of inactivated FVIIa may be required.

Acknowledgments

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References


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