Recombinant Activated Factor VII: The Controversial Conundrum Regarding Its Off-Label Use

Keyvan Karkouti, MD, MSc, FRCPC,* and Jerrold H. Levy, MD, FAHA†

Recombinant factor VIIa (rFVIIa; NovoSeven®, Novo Nordisk A/S, Bagsværd, Denmark) is a widely used recombinant protein currently licensed for treating bleeding episodes or preventing bleeding in surgical interventions or invasive procedures in patients with hemophilia A or B who have antibody inhibitors to factors VIII or IX or in patients with congenital factor VII deficiency. Since its original approval, rFVIIa has also been extensively reported and evaluated in a number of off-label uses. Over recent years, the off-label use of rFVIIa has turned out to be an increasingly controversial issue. Initially, rFVIIa was thought to potentially represent a "universal hemostatic agent" without serious safety issues.[1] Controversy arose after recent studies demonstrated that its efficacy varies depending on the clinical setting and that it may increase the risk of thromboembolic complications in some clinical settings.[2,3] Adding to the controversy was the observation that clinicians are increasingly using the drug off-label to treat life-threatening refractory bleeding after major surgery or trauma,[4] a practice that has not been studied by randomized controlled trials and has recently been criticized in an editorial as being unhelpful, dangerous, and costly.[5]

In this context, what then can we learn from the phase I study published in this issue of the *Journal*?[6] Surprisingly, a lot! Skolnick et al. reported a randomized, placebo-controlled study that assessed the effect of low-dose rFVIIa on blood loss (using a punch biopsy-induced bleeding model) in 40 healthy male volunteers who were treated with, and responded to, oral clopidogrel (300 mg loading, followed by 75 mg for 2 additional days). Importantly, to obtain 40 responders to clopidogrel (defined liberally as a >30% platelet inhibition as measured by VerifyNow® [Accumetrics, San Diego, CA] after 3 days of therapy), the investigators had to treat 104 patients, which means that the nonresponse rate was 56%. This illustrates the wide variability in responsiveness to clopidogrel that has often been reported,[7] and highlights the need for individualized transfusion management when patients who are on clopidogrel therapy present for urgent surgery. More specifically, it clearly illustrates that the decision to preemptively transfuse platelets to patients receiving clopidogrel therapy on the basis of the expectation of excessive blood loss is inappropriate, because many of them may not have excessive blood loss owing to variability of its effect.

In the 37 clopidogrel responders who were evaluated, 10 and 20 mcg/kg of rFVIIa (n = 6 and 12, respectively) significantly improved ex vivo measures of whole blood clot dynamics, as measured by thromboelastography performed about 3 hours after administration of rFVIIa. This, by itself, does not necessarily translate into clinical benefit, because the authors illustrated this in a previous study that used the same human bleeding model to assess the effects of rFVIIa in the setting of warfarin therapy.[8] In that study, despite causing robust improvements in ex vivo measures of clot dynamics, rFVIIa had no effect on blood loss or bleeding duration in a human model of reversing warfarin therapy,[8] which is not surprising because agents that contain factors II, VII, IX, and X are more appropriate for reversing the effects of warfarin.[9,10] In the current trial, however, subjects who received 10 and 20 mcg/kg of rFVIIa had an approximately 50% reduction in blood loss and 20% reduction in bleeding duration in comparison with those who received placebo (although the difference in bleeding duration did not reach the threshold of statistical significance). These clinical data support other data suggesting that rFVIIa may be effective for reversing thienopyridine-induced platelet dysfunction.[11]

The differing efficacy results of these 2 early-stage volunteer studies are emblematic of what numerous clinical and nonclinical trials have clearly illustrated in the last decade, which is that rFVIIa is not a universal hemostatic agent. Rather, it is a drug that seems to improve clot formation by enhancing the rate of thrombin generation on thrombin-activated platelet surfaces, thereby increasing the activation of platelets, thrombin-activatable fibrinolysis inhibitor, and factor XIII.[12] Thus, rather than being a universal hemostatic agent, rFVIIa is a potent but specific prohemostatic agent that requires the presence of adequate amounts of hemostatic substrates to be effective. In other words, it is a drug that could potentially be considered to be a "clot-booster."

Regarding rFVIIa as a clot booster rather than a universal hemostatic agent has important practical implications for the clinician. First, it highlights its potential safety risks. Just as we would not consider using a fibrinolytic agent or clot buster without considering its risk for bleeding complications, we should not consider using a potent prohemostatic clot booster without considering its risk for thromboembolic complications, which may outweigh its benefits in certain scenarios. Second, it highlights the fact that rFVIIa is not the correct choice for all bleeding problems. In some scenarios, such as the massively bleeding patient whose coagulation proteins have been depleted, it will likely be ineffective as a sole therapy, and in other scenarios, such as warfarin reversal, it will likely not be the best treatment option.

Considering rFVIIa as a prohemostatic clot booster may also help explain the discordance between the increasing

From the *Department of Anesthesiology and Health Policy, Management, and Evaluation, Toronto General Hospital, University Health Network, University of Toronto, Toronto, Ontario, Canada; and †Department of Anesthesiology, Emory University School of Medicine, Cardiothoracic Anesthesiology and Critical Care, Emory Healthcare, Atlanta, Georgia.

Accepted for publication June 7, 2011.

Funding: Funded by the Department of Anesthesiology, Toronto General Hospital, University of Toronto, and the Emory University School of Medicine.

Recuse Note: Jerrold H. Levy is section Editor of Hemostasis and Transfusion Medicine for the *Journal*. This manuscript was handled by Steve Shafer, Editor-in-Chief, and Dr. Levy was not involved in any way with the editorial process or decision.

Conflict of Interest: See Disclosures at the end of the article.

Address correspondence to Keyvan Karkouti, MD, Toronto General Hospital, 200 Elizabeth St., 3EN-402, Toronto, Ontario, Canada M5G 2C4. Address e-mail to keyvan.karkouti@uhn.on.ca.

DOI: 10.1213/ANE.0b013e318228c6a9

off-label use of rFVIIa to treat refractory bleeding after major surgery or trauma in view of randomized clinical trial data showing it to be ineffective and possibly harmful in various other clinical settings.[2,9] When presented with a patient who continues to bleed despite administration of all available therapies, clinicians have only 2 choices: they can keep administering the standard interventions that have failed to work in that patient, or they can administer a potent clot-booster like rFVIIa. We believe that in the setting of refractory blood loss, clinicians are justified in choosing the latter course of action for several reasons. First, it is clinically evident that patients with massive refractory bleeding will have dismal outcomes unless the blood loss is controlled in a timely manner.[13] Second, persisting with standard interventions will likely not achieve this goal and will unnecessarily expose patients to the risks of these interventions. Third, the efficacy and safety data from most randomized trials are not applicable to this scenario because they did not study patients with refractory blood loss. Fourth, even if the safety data from randomized trials do apply, which indicate that rFVIIa doubles the risk of thrombotic complications,[3] this risk is likely dwarfed by the risk of allowing blood loss to continue unabated. Fifth, there is an expanding body of observational data,[14] and some randomized trial data in bleeding patients,[15] that suggest that rFVIIa is an effective therapy for refractory blood loss. Sixth and perhaps most important, this is a scenario for which additional applicable data from placebo-controlled randomized trials will not be forthcoming in the foreseeable future, primarily because of feasibility issues (e.g., difficulty in obtaining informed consent in a timely manner, ethical concern of administering a placebo to patients with refractory blood loss, and lacking standardized alternative therapies), but also because further clinical development of rFVIIa or its analogs by Novo Nordisk will be for licensed indications.[6]

Does this mean that the controversy surrounding the off-label use of rFVIIa is unwarranted? Probably not, for it is an expensive drug with the potential for serious adverse responses that is increasingly being used off-label for indications for which its risk–benefit profile has not been fully elucidated. Nevertheless, we believe that blanket criticisms of the off-label use of this drug are at best unhelpful, and at worst harmful. At the same time, it would also be harmful to use this drug outside of approved indications without first fully considering its risk–benefit profile in the specific setting where its use is being considered. In this regard, clinicians need to carefully scrutinize data from randomized trials for applicability and data from observational studies for selection bias (for or against the drug). It would be most harmful if we resign ourselves to the current state of knowledge about this drug specifically, and refractory blood loss in general. New, potent, and irreversible antiplatelet agents (e.g., clopidogrel, prasugrel) and anticoagulant agents (e.g., dabigatran) are being introduced into clinical practice, which will likely increase the burden of refractory blood loss. We believe, therefore, that clinical studies (randomized where possible, well-designed observational where not) for patients who are at high risk for refractory blood loss and may benefit from novel prohemostatic therapies such as rFVIIa are urgently needed.

DISCLOSURES

Name: Keyvan Karkouti, MD, MSc, FRCPC.
Contribution: Wrote first draft of manuscript and added additional information and references.
Conflicts of Interest: Serves on a steering committee for Novo Nordisk regarding recombinant factor XIII; received funding from Novo Nordisk for a rFVIIa registry.
Name: Jerrold H. Levy, MD, FAHA.
Contribution: Reviewed and revised manuscript and oversaw ongoing revisions and corrections.
Conflict of Interest: Serves on a steering committee for Novo Nordisk regarding recombinant factor XIII.

REFERENCES

1. Hedner U. NovoSeven as a universal haemostatic agent. Blod Coag Fibrinol 2000;11[Suppl 1]:S107–11
2. Yank V, Tuohy CV, Logan AC, Bravata DM, Staudenmayer K, Eisenhut R, Sundaram V, McMahon D, Olkin I, McDonald KM, Owens DK, Stafford RS. Systematic review: benefits and harms of in-hospital use of recombinant factor VIIa for off-label indications. Ann Intern Med 2011;154:529–40
3. Levi M, Levy JH, Andersen HF, Truloff D. Safety of recombinant activated factor VII in randomized clinical trials. N Engl J Med 2010;363:1791–800
4. Logan AC, Yank V, Stafford RS. Off-label use of recombinant factor VIIa in U.S. hospitals: analysis of hospital records. Ann Intern Med 2011;154:516–22
5. Avorn J, Kesselheim A. A hemorrhage of off-label use. Ann Intern Med 2011;154:566–7
6. Skolnick BE, Shenouda M, Khutoryansky NM, Pusateri AE, Gabriel D, Carr ME. Reversal of clopidogrel-induced bleeding with rFVIIa in healthy subjects: a randomized, placebo-controlled, double-blind, exploratory study. Anesth Analg 2011;113:703–10
7. Ben-Dor I, Kleiman NS, Lev E. Assessment, mechanisms, and clinical implication of variability in platelet response to aspirin and clopidogrel therapy. Am J Cardiol 2009;104:227–33
8. Skolnick BE, Mathews DR, Khutoryansky NM, Pusateri AE, Carr ME. Exploratory study on the reversal of warfarin with rFVIIa in healthy subjects. Blood 2010;116:693–701
9. Levy JH, Tanaka KA, Dietrich W. Perioperative hemostatic management of patients treated with vitamin K antagonists. Anesthesiology 2008;109:918–26
10. Holland L, Warkentin TE, Refaai M, Crowther MA, Johnston MA, Sarode R. Suboptimal effect of a three-factor prothrombin complex concentrate (Profilnine-SD) in correcting supratherapeutic international normalized ratio due to warfarin overdose. Transfusion 2009;49:1171–7
11. Szlam F, Tanaka KA, Rumph B, Bolliger D, Levy JH. In vitro effects of recombinant activated factor VIIa (NovoSeven) on clopidogrel-induced platelet inhibition. Thromb Haemost 2010;103:863–5
12. Hedner U. Mechanism of action, development and clinical experience of recombinant FVIIa. J Biotechnol 2006;124:747–57
13. Karkouti K, Beattie WS, Wijeysundera DN, Yau TM, McCluskey SA, Ghannam M, Sutton D, van Rensburg A, Karski J. Recombinant factor VIIa (rF-VIIa) for intractable blood loss after cardiac surgery: a propensity-score matched case-control analysis. Transfusion 2005;45:26–34
14. Karkouti K, Beattie WS, Arellano R, Aye T, Bussieres JS, Callum JL, Cheng D, Heinrich L, Kent B, Lee TW, MacAdams C, Mazer CD, Muirhead B, Rochon AG, Rubens FD, Sawchuk C, Wang S, Waters T, Wong BI, Yau TM. Comprehensive Canadian review of the off-label use of recombinant activated factor VII in cardiac surgery. Circulation 2008;118:331–8
15. Gill R, Herbertson M, Vuylsteke A, Olsen PS, von HC, Mythen M, Sellke F, Booth F, Schmidt TA. Safety and efficacy of recombinant activated factor VII: a randomized placebo-controlled trial in the setting of bleeding after cardiac surgery. Circulation 2009;120:21–7

Reversal of Clopidogrel-Induced Bleeding with rFVIIa in Healthy Subjects: A Randomized, Placebo-Controlled, Double-Blind, Exploratory Study

Brett E. Skolnick, PhD,* Magdy Shenouda, MD,† Naum M. Khutoryansky, PhD,* Anthony E. Pusateri, PhD,‡ Don Gabriel, MD, PhD,§ and Marcus E. Carr, MD, PhD, FACP*

BACKGROUND: Clopidogrel (Plavix®) therapy, although effective for minimizing risk of thrombotic events, is also associated with potential bleeding risk. Recombinant activated FVII (rFVIIa, NovoSeven®) induces hemostasis in hemophilia patients with inhibitors (alloantibodies) and has been proposed as potential treatment for mitigating clopidogrel therapy–mediated bleeding.
METHODS: In this single-center, randomized, placebo-controlled, double-blind, dose-escalation, exploratory phase I trial, we assessed the safety and effects of rFVIIa in reversing clopidogrel-enhanced bleeding in an experimentally induced punch biopsy in healthy subjects. Efficacy assessments included the reversal of bleeding characteristics (bleed duration [BD], the primary end point and blood loss volume [BV] induced by punch biopsy, and thromboelastograph [TEG®] parameters) with rFVIIa or placebo after clopidogrel treatment.
RESULTS: A significant number of subjects (56%) had limited response to clopidogrel (defined as ≤30% platelet aggregation inhibition) and were discontinued from study. The remaining subjects continued and had 4 biopsies. Of 40 subjects randomized, 37 were evaluated for efficacy. Clopidogrel treatment increased BD and BV compared with the baseline biopsy. Recombinant FVIIa (10 and 20 μg/kg) significantly mitigated the clopidogrel-induced effects on BV ($P = 0.007$ and $P = 0.001$, respectively). Early trial termination limited the evaluation of effects of higher rFVIIa doses. Subgroup analyses of subjects biopsied by the same physician demonstrated significant reduction of clopidogrel-induced BD with 20 μg/kg rFVIIa ($P = 0.048$). Ex vivo analysis of rFVIIa demonstrated clotting dynamics presented by parameters time to clot onset (TEG®-R) and clot angle (TEG®-A) ($P < 0.005$).
CONCLUSIONS: In this clinical study, rFVIIa (10 and 20 μg/kg) reversed the effect of clopidogrel on blood loss. (Anesth Analg 2011;113:703–10)

Clopidogrel (Plavix®; Bristol-Myers Squibb Co., Princeton, NJ) is an antiplatelet drug indicated for acute coronary syndrome and peripheral artery disease. In recent years, clopidogrel has become widely used to minimize thrombosis risk after cardiovascular stenting. As such, many patients remain on long-term clopidogrel treatment. However, the use of clopidogrel carries the risk of bleeding as a potential side effect in patients with recent ischemic stroke, recent myocardial infarction, or peripheral arterial disease (CAPRIE Trial)[1] and in patients symptomatic of coronary artery disease with evidence of ischemia (CREDO Trial).[2]

Recombinant activated FVII (rFVIIa) (NovoSeven®; Novo Nordisk A/S, Bagsværd, Denmark) is indicated for the treatment of bleeding episodes in hemophilia A or B patients with inhibitors (i.e., alloantibodies) to FVIII or FIX and in patients with acquired hemophilia, the prevention of bleeding in surgical interventions or invasive procedures in hemophilia A or B patients with inhibitors to FVIII or FIX and in patients with acquired hemophilia, and the treatment of bleeding episodes in patients with congenital factor VII deficiency and prevention of bleeding in surgical interventions or invasive procedures in patients with congenital FVII deficiency.[3] A number of published ex vivo and retrospective studies have reported the use of rFVIIa to mitigate the bleeding associated with clopidogrel therapy.[4–9] Several features make rFVIIa a candidate for the mitigation of antiplatelet-induced bleeding. These include rapid action localized to the site of vascular injury,[10,11] low volume required for administration, allowing for rapid infusion, and demonstrations of effectiveness with good safety profiles.[4,7,9]

Data regarding bleed mitigation in clopidogrel-treated animals[12,13] and in patients undergoing cardiac surgery[14] are contradictory and provide little insight as to the use of rFVIIa in mitigating spontaneous or trauma-induced bleeds in patients receiving clopidogrel therapy. The current study was designed to assess the utility of rFVIIa, in vivo, to mitigate bleeding induced via a punch biopsy bleeding model in healthy volunteers treated with clopidogrel. The punch biopsy model has been shown to produce wounds of reproducible depths and widths to give measurable bleeding duration (BD) and blood volume (BV).[15] Previous clinical trials using the punch biopsy technique to evaluate

From *Novo Nordisk Inc., Princeton, New Jersey; †MDS Pharma Services, Neptune, New Jersey; ‡Novo Nordisk Research Facility US, New Brunswick, New Jersey; and §Department of Medicine, Division of Hematology and Oncology, University of North Carolina, Chapel Hill, North Carolina.

Accepted for publication June 8, 2011.

Supported by the sponsor, Novo Nordisk Inc., Princeton, NJ. The sponsor was responsible for providing clinical study supplies, preparing the study protocol, data management, statistical analysis, and preparing the clinical study report.

Conflict of Interest: See Disclosures at the end of the article.

Authors' current affiliations are listed at the end of the article.

Reprints will not be available from the authors.

Address correspondence to Brett E. Skolnick, PhD, Novo Nordisk, 100 College Rd. West, Princeton, NJ 08540. Address e-mail to BRES@novonordisk.com.

DOI: 10.1213/ANE.0b013e318228c690

rFVIIa to mitigate bleeding provided BD sufficiently prolonged to allow for the detection of treatment differences.[15] The results from the current study could provide additional information regarding the use of rFVIIa for clopidogrel-treated patients in instances where there is a need to mitigate spontaneous or trauma-induced bleeds.

METHODS

This study was conducted from May 2008 to January 2009 in accordance with the Declaration of Helsinki.[16] MDS Pharma Services IRB reviewed and approved the study conduct. Signed informed consent was obtained from each subject. This was a single-center, randomized, placebo-controlled, double-blind, dose-escalation, phase I study.

Study Population

Healthy male subjects answering advertisements were recruited locally and paid for their participation in the study. All subjects were between 18 and 45 years of age, had normal platelet counts (150–352 $\times$ 10^9 cells/L), normal coagulation screening assays (prothrombin time of 9.4–12 seconds, and activated partial thromboplastin time of 25.4–38.4 seconds), and met the strict cardiovascular criteria [HDL-cholesterol $\geq$40 mg/dL, LDL-cholesterol $<$189 mg/dL, or apolipoprotein B 100 or lipoprotein(a) below the 90th percentile]. Subjects were withdrawn if there was excessive bleeding at the initial biopsy (>25 minutes, n = 5). Subjects taking investigational drugs, oral anticoagulant therapy, aspirin, or nonsteroidal antiinflammatory drugs were also excluded.

Study Design

Novo Nordisk provided the randomization schedule. Subjects were randomized (6:2 or 6:6) to receive either the trial product (a single IV dose of rFVIIa) or placebo. The planned rFVIIa dose tiers were 5, 10, 20, 40, and 80 μg/kg. The punch biopsy was performed on the back of the thigh (after local anesthetic with 2% lidocaine without epinephrine) to a subcutaneous depth of 4 to 6 mm using a disposable punch biopsy instrument (Miltex Inc., York, PA) with a diameter of 5 mm. Subjects underwent 4 biopsies: before clopidogrel administration (biopsy Bx0), approximately 4 days after initiation of clopidogrel treatment (biopsy Bx1), 2 hours and 13 minutes after administration of the trial product (rFVIIa or placebo, biopsy Bx2), and then 5 hours after trial product (approximately 2 half-lives of rFVIIa) (biopsy Bx3).

Treatment

All subjects received clopidogrel treatment orally with an initial 300-mg loading dose on day 1, followed by daily 75-mg doses of clopidogrel for 2 additional days. Two hours after Bx1, trial product (rFVIIa or placebo) was administered IV. There were no delays in performing the biopsies. Study subjects and all clinical staff involved in assessing outcomes were blinded. To maintain blinding, subjects were dosed based on their weights and given an equal weight-based volume of rFVIIa or placebo. The unblinded pharmacist prepared the trial product. Clopidogrel (clopidogrel bisulfate, Plavix®) was provided as 75-mg tablets for oral administration. Recombinant FVIIa and placebo were provided as identical freeze-dried powder in single-use vials (4.8 mg) to be reconstituted with sterile water for injection (USP) to be administered as a slow IV bolus injection. The reconstituted vials have a pH of approximately 6.0 and consist of sodium chloride (2.3 mg/mL), calcium chloride dehydrate (1.5 mg/mL), glycylglycine (1.3 mg/mL), polysorbate 80 (0.1 mg/mL), and mannitol (25 mg/mL). The rFVIIa vials also have 0.6 mg/mL rFVIIa.

Assessment of Clopidogrel Effects on Platelet Inhibition

Platelet inhibition (PI) in subjects treated with clopidogrel was assessed using the VerifyNow® P2Y12 assay (Accumetrics®, San Diego, CA).[17] Based on previous studies, the PI cutoff adopted in this study was a 20% PI at approximately 16 hours after the 300-mg initial loading dose of clopidogrel (day 1), and was set at 30% after a minimum of 2 additional days of 75 mg clopidogrel (day 3).[17,18]

Assessment of Clopidogrel and rFVIIa Effects

BD and BV were evaluated after each biopsy as described previously.[15] In addition to in vivo bleeding evaluations, thromboelastography (TEG®) provided ex vivo sequential coagulation profiles of whole blood coagulation.[19,20] The primary end point was the punch biopsy–induced BD after trial product administration (biopsy Bx2). Secondary end points included BV and clot dynamics assessed by TEG®.

Assessment of Safety

Safety was continuously monitored during the study, and blinded safety data (i.e., adverse events/serious adverse events, electrocardiograms, and safety laboratory values) were evaluated before dose escalation. Safety variables were assessed in the phase I unit that conducted the trial. As part of the discharge from the phase I unit, an electrocardiogram, troponin I measurement, and assessment of adverse events were performed. Subjects returned to the phase I unit after 8 to 15 days for suture removal and adverse event and vital sign assessment.

During the course of the study, there was a change in the physician performing the biopsies. Therefore, post hoc analyses included using the physician as a covariate in analyses for BD and BV, as well as an analysis of subjects biopsied only by the first physician. Analyses of TEG® parameters included TEG® values at Bx1 as a covariate. No adjustment for changes in physician was made because blood sampling is not influenced by biopsy technique. Duration of rFVIIa effects (as reflected by biopsy 3) was to be evaluated only if the preplanned analyses were significant.

Statistical Analyses

The null hypothesis for the primary end point was that BD at Bx2 after rFVIIa treatment was more than or equal to placebo and the alternative hypothesis was that BD after rFVIIa treatment was less than that after placebo. All subjects who received clopidogrel and trial product or placebo were included in the safety analyses. All randomized subjects who underwent biopsies 1 and 2 were included in the intent-to-treat (ITT) analysis.

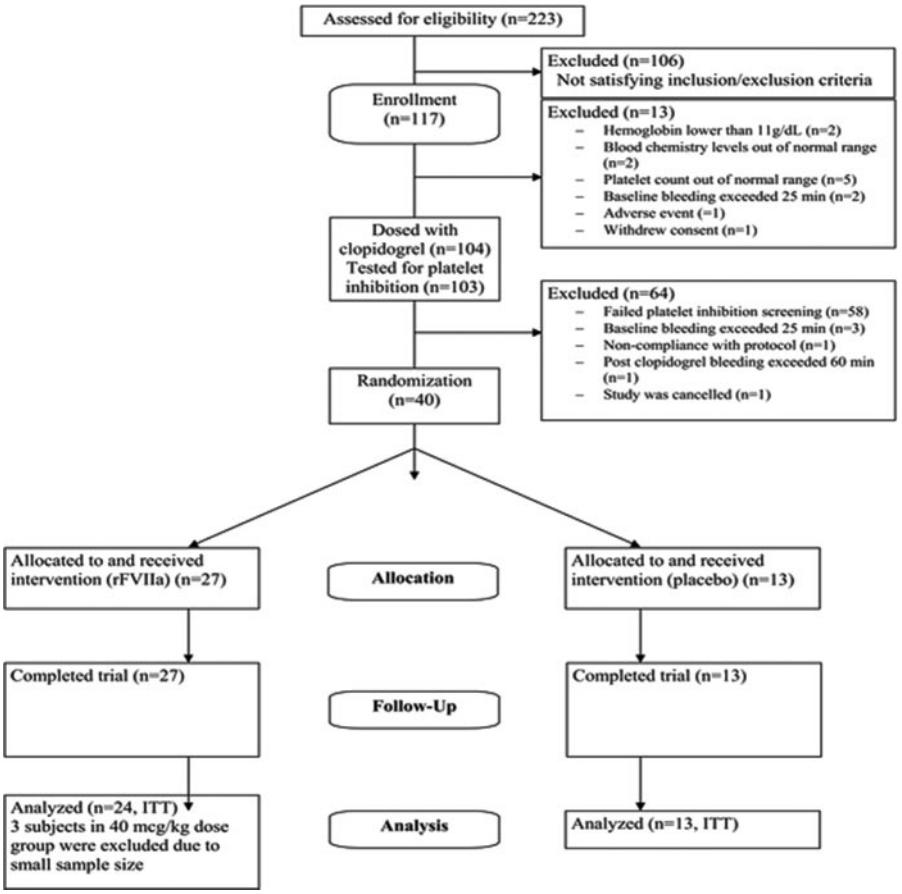

Figure 1. CONSORT (Consolidated Standards of Reporting Trials) flow diagram. rFVIIa = recombinant activated FVII. ITT = intent-to-treat.

The planned sample size allowed up to 108 subjects based on an adaptive study design that accommodated incremental progression of rFVIIa dose based on the results of the interim analyses and safety review. The design involved systematic evaluation at each rFVIIa dose tier and accommodated small initial sample sizes while allowing rapid determination of the optimally effective rFVIIa dose, compared with a more conventional study design. The study started with 8 subjects (6 rFVIIa and 2 placebo) in the 5 μg/kg dose tier and was planned to progress in ascending dose tiers to 80 μg/kg. It was planned that statistical comparisons should start at the first dose where the normalization of the clopidogrel-induced BD was assessed or at the highest dose (80 μg/kg) if there was no normalization at lower doses. Normalization (ratio of BD at Bx2 to BD at initial biopsy $\leq$1.1) was not achieved at any completed dose tier in this study. The statistical inferential comparisons of the 3 rFVIIa dose groups (5, 10, and 20 μg/kg) with placebo for the BD and blood loss were performed using a generalized linear model with the logarithmic link and the γ distribution for errors. This approach is preferable for data with constant coefficient of variation (CV).[21] The assumption of constant CV is approximately satisfied for BD and blood loss data in the different dose groups of the trial. The model for BD and blood loss at Bx2 included adjustments for logarithmically transformed baseline values (at Bx1) of the dependent variable as a covariate and investigator as a fixed factor. The statistical analysis for repeated TEG® data was based on a mixed-effect model with subject as a random effect. This model allows for the accounting of correlations between data for different time points.

RESULTS

This study was terminated to align with the sponsor's (Novo Nordisk A/S) decision to only pursue the development of rFVIIa within the licensed indications. At the time the study was stopped, only 3 subjects were enrolled in the 40 μg/kg dose tier, and no subjects in the 80 μg/kg dose tier. The results of the 40 μg/kg dose tier were not included in the analyses because of the small sample size, although no apparent differences were noted in safety variables. Efficacy end points for each rFVIIa dose tier (5, 10, and 20 μg/kg rFVIIa) were tested separately with no estimation of the α level inflation because of multiplicity of testing, which is often the case for exploratory analyses. Therefore, these results should be interpreted with caution.

Subject Disposition

Subject disposition is presented in Figure 1. One hundred seventeen subjects fulfilled the screening criteria and were enrolled in the study. Of the 104 subjects who received clopidogrel, 56% (58 of 103) did not meet the initial PI cutoff level of 30% on day 3, and were therefore removed from the study. The safety analysis set comprised 40 subjects who received trial product (rFVIIa or placebo).

Table 1. Demographics and Medical History of Clopidogrel Nonresponders and Responders

	Clopidogrel nonresponders	Clopidogrel responders[a]
Total no. of randomized subjects, *n*	58	37
Age, mean (SD), y	32.5 (7.7)	28.1 (6.8)*
Weight, mean (SD), kg	82.7 (14.4)	77.4 (9.1)
Body mass index, mean (SD), kg/m^2	26.8 (3.8)	25.1 (2.7)*
Ethnicity, *n* (%)		
Caucasian	54 (93.1)	36 (97.3)
Asian	4 (6.9)	1 (2.7)
Total no. of subjects with illness/surgery in medical history, *n* (%)[b]	19 (32.8)	12 (32.4)
Surgical and medical procedures[c]	16 (27.6)	9 (24.3)
Fractures	3 (5.2)	1 (2.7)
Deafness unilateral	—	1 (2.7)
Blindness unilateral	—	1 (2.7)
Seasonal allergy	2 (3.4)	2 (5.4)
Drug hypersensitivity	1 (1.7)	—
Infections	1 (1.7)	3 (11.1)
Radial tunnel syndrome	—	1 (2.7)
Coma (past)	1 (1.7)	—
Dysuria	—	1 (2.7)
Epistaxis	—	1 (2.7)
Ex-tobacco user	1 (1.7)	—

[a] Clopidogrel-treated subjects who were withdrawn for reasons other than clopidogrel nonresponse were excluded from this table.
[b] Subjects reporting >1 illness or surgery are recorded only once in the total within each category.
[c] Surgical and medical procedures included appendectomy, chondroplasty, ear tube insertion, hernia repair, peripheral nerve decompression, suture insertion, and tonsillectomy in clopidogrel responders; arthroscopic surgery, bone, limb, maxillofacial, bunion, tendon, or venous operations, hip surgery, wrist surgery, renal surgery, keratomileusis, liposuction, muscle reattachment, patellectomy, and rotator cuff repair in clopidogrel nonresponders; and endodontic procedures and wisdom teeth removal in both clopidogrel responders and nonresponders.
* Significantly different from the clopidogrel nonresponder group ($P < 0.05$).

Table 2. Percentage Platelet Inhibition on Day 3

	Day 1		Day 3	
	n	Mean (SD)	*n*	Mean (SD)
Total subjects	46	44.0 (18.1)	46	58.9 (20.3)
Nonresponders[a]			3	25.3 (3.1)
Randomized population	40	45.4 (17.8)	40[b]	60.1 (18.1)

[a] Percentage platelet inhibition cutoff level for clopidogrel nonresponders on day 3 was <30%.
[b] Of the 103 subjects treated with clopidogrel and tested for platelet inhibition, 63 subjects were withdrawn (58 clopidogrel nonresponders and 5 who met other withdrawal criteria).

Demographics

Demographics and baseline characteristics of the clopidogrel nonresponders and clopidogrel responders are presented in Table 1. The mean age of the clopidogrel nonresponders (32.5 years) was older than the mean age of the clopidogrel responders (28.1 years) ($P < 0.05$). The mean body mass index of the clopidogrel nonresponders (26.8 kg/m^2) was higher than the mean body mass index of the clopidogrel responders (25.1 kg/m^2) ($P < 0.05$). All other demographic and baseline characteristics are presented in Table 1.

Effect of Clopidogrel on PI

Of the 103 subjects who received the 300-mg clopidogrel initial loading dose on day 1, 55 did not reach the initial PI cutoff level of 20% and 2 subjects were withdrawn from the study because of excessive bleeding at Bx0. In the total population of subjects ($n = 46$), mean ± SD PI levels increased from 44.0% ± 18.1% after the 300-mg loading dose on day 1 to 58.9% ± 20.3% on day 3 after a minimum of 2 additional maintenance doses of 75 mg clopidogrel (Table 2). Three subjects from the total subject population did not meet the PI cutoff level of 30% on day 3 and 3 subjects were withdrawn from the study because of noncompliance with the investigator's instructions or excessive bleeding (i.e., BD [Bx1] >60 minutes) The mean ± SD PI level for the randomized population on day 3 ($n = 40$) was 60.1% ± 18.1%. Total clopidogrel exposure ranged from 450 to 525 mg in the randomized subjects, dependent on whether subjects received a third 75-mg dose.

Effect of rFVIIa on Bleeding Characteristics

After clopidogrel treatment, BD increased from 10.7 ± 4.8 minutes to 32.3 ± 15.8 minutes (the mean difference was statistically significant; $P < 0.001$) and BV increased from 5.0 ± 4.8 mL to 17.7 ± 18.8 mL ($P < 0.001$). Treatment with rFVIIa had no significant effect on BD compared with placebo (Table 3, Fig. 2). However, BV was significantly reduced with the 10 and 20 μg/kg rFVIIa doses compared with placebo (Table 3, Fig. 2).

The mean values and variation in the measurements for BD and BV were higher for the second physician performing the biopsies. Subjects in the 5 and 10 μg/kg rFVIIa treatment groups were biopsied only by the first physician.

Table 3. Effect of rFVIIa and Placebo Treatment on Bleeding Duration and Blood Loss After Platelet Inhibition with Clopidogrel

	n	Post-clopidogrel (Bx1) mean[a] (CV)	Post-rFVIIa or placebo treatment (Bx2) mean[b] (CV)	Ratio of means at Bx2 (rFVIIa vs placebo) (95% CI)	*P* value
Bleeding duration (min)					
Placebo	13	30.7 (0.4)	30.4 (0.3)		
5 μg/kg rFVIIa	6	40.3 (0.4)	36.9 (0.4)	1.22 (0.88, 1.70)	0.255
10 μg/kg rFVIIa	6	26.1 (0.4)	24.0 (0.3)	0.81 (0.58, 1.12)	0.145
20 μg/kg rFVIIa	12	29.2 (0.4)	25.1 (0.3)	0.83 (0.66, 1.06)	0.106
Blood loss (mL)					
Placebo	13	15.9 (0.7)	23.2 (0.5)		
5 μg/kg rFVIIa	6	28.1 (0.8)	18.9 (0.6)	0.81 (0.45, 1.48)	0.501
10 μg/kg rFVIIa	6	11.3 (0.8)	10.3 (0.6)	0.44 (0.25, 0.80)	0.007
20 μg/kg rFVIIa	12	15.6 (0.8)	11.6 (0.6)	0.50 (0.33, 0.76)	0.001

CI = confidence interval; CV = coefficient of variance; rFVIIa = recombinant activated FVII.
[a] Mean: geometric mean, adjusted for surgeon.
[b] Mean: geometric mean, adjusted for baseline (Bx1) value and surgeon, based on a generalized linear model with logarithmic link and γ distribution.

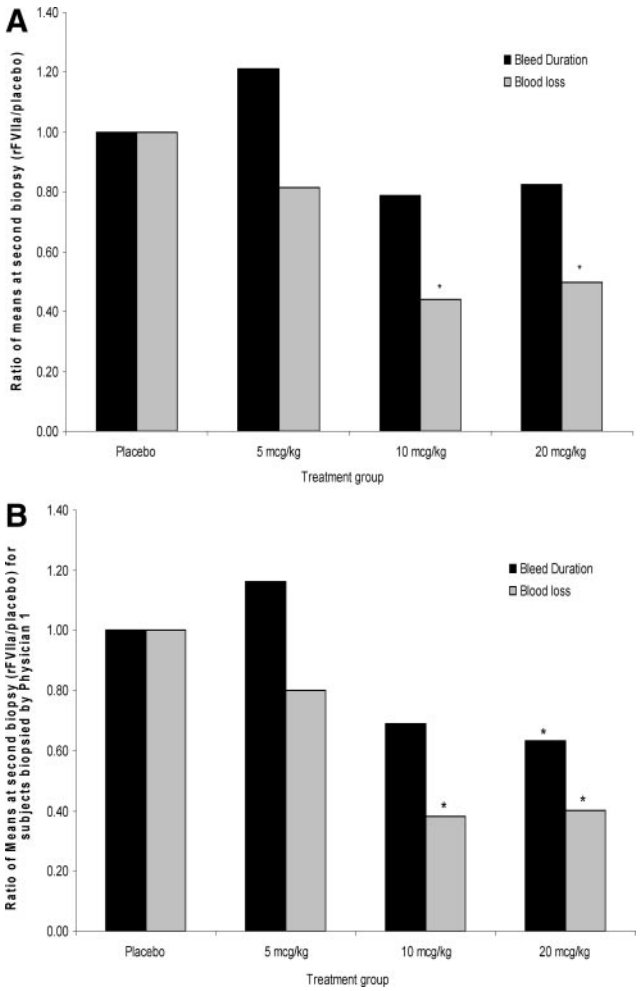

Figure 2. Ratio of means comparison (recombinant activated FVII versus placebo) of bleed duration and blood loss at the second biopsy for (A) all subjects and (B) subjects biopsied by physician 1. *Significant difference from placebo ($P < 0.05$).

The treatment groups had smaller standard deviations for BD (3.6 and 6.6 minutes) and BV (8.7 and 5.6 mL). The subjects in the placebo and 20 μg/kg rFVIIa groups were biopsied either by the first or second physician and had larger standard deviations for BD (16.8 and 19.7 minutes) and BV (12.0 and 30.4 mL).

In an attempt to account for the influence of the variability between physicians, analyses of rFVIIa effects on BD and BV incorporated adjustment for physician. In addition, the statistical analysis model used for BD and BV performs satisfactorily even for large changes in standard deviation if the CV is approximately constant across treatment groups. In these analyses, monotonicity was observed for the effect of rFVIIa dose on BD and BV, although this was only significantly different from placebo for blood loss in the 10 μg/kg ($P = 0.007$) and 20 μg/kg ($P = 0.001$) dose groups (Table 3). However, a separate analysis for physician 1, who was completely responsible for the 5 and 10 μg/kg dose groups, can be useful. The effects of rFVIIa on BV in subjects biopsied by physician 1 were significant for the 10 and 20 μg/kg dose groups for physician 1 ($P = 0.006$ and $P = 0.013$, respectively) (Table 4). The effect of rFVIIa on BD was significant only in the 20 μg/kg dose group for physician 1 ($P = 0.048$) (Table 4).

Effect of rFVIIa on Clot Dynamics

Clot dynamics were assessed using TEG®. A longitudinal approach was performed using a mixed-effect model. Treatment with rFVIIa significantly decreased time to clot onset (R) and increased the clot angle (A), compared with placebo ($P < 0.005$ for all dose groups) (Fig. 3). The effects of rFVIIa on any parameter of clot dynamics were not observed 3 hours after Bx2 or 1 hour after Bx3. No significant differences were observed in other TEG® parameters across treatment groups.

Safety

The adverse events reported in this study were burning sensation (placebo, $n = 3$), suture-related complications (placebo, $n = 2$), eczema (40 μg/kg rFVIIa, $n = 2$), catheter site hemorrhage (20 μg/kg rFVIIa, $n = 1$), vessel puncture at site of hematoma (20 μg/kg rFVIIa, $n = 1$), postprocedural complication (10 μg/kg rFVIIa, $n = 1$), dizziness (20 μg/kg rFVIIa, $n = 1$), headache (placebo, $n = 1$), agitation (20 μg/kg rFVIIa, $n = 1$), ecchymosis (placebo, $n = 1$), erythema (40 μg/kg rFVIIa, $n = 1$), and rash (placebo, $n = 1$). All adverse events were graded as mild or moderate in

Table 4. Effect of rFVIIa and Placebo Treatment on Bleeding Duration and Blood Loss After Platelet Inhibition with Clopidogrel in Analyses of Subjects Biopsied by Physician 1

	n	Post-clopidogrel (Bx1) mean[a] (CV)	Post-rFVIIa or placebo treatment (Bx2) mean[b] (CV)	Ratio of means at Bx2 (rFVIIa vs placebo) (95% CI)	*P* value
Bleeding duration (min)					
Placebo	5	27.2 (0.3)	20.0 (0.3)		
5 μg/kg rFVIIa	6	30.8 (0.3)	23.3 (0.4)	1.16 (0.78, 1.76)	0.477
10 μg/kg rFVIIa	6	19.9 (0.3)	13.8 (0.4)	0.69 (0.45, 1.04)	0.078
20 μg/kg rFVIIa	5	18.8 (0.3)	12.7 (0.4)	0.63 (0.40, 0.99)	0.048
Blood loss (mL)					
Placebo	5	15.0 (0.6)	15.8 (0.6)		
5 μg/kg rFVIIa	6	20.2 (0.6)	12.7 (0.6)	0.80 (0.42, 1.53)	0.501
10 μg/kg rFVIIa	6	8.1 (0.6)	6.0 (0.6)	0.38 (0.19, 0.76)	0.006
20 μg/kg rFVIIa	5	7.7 (0.6)	6.4 (0.6)	0.40 (0.20, 0.82)	0.013

CI = confidence interval; CV = coefficient of variance; rFVIIa = recombinant activated FVII.

[a] Mean: geometric mean.

[b] Mean: geometric mean, adjusted for baseline (Bx1) value based on a generalized linear model with logarithmic link and γ distribution.

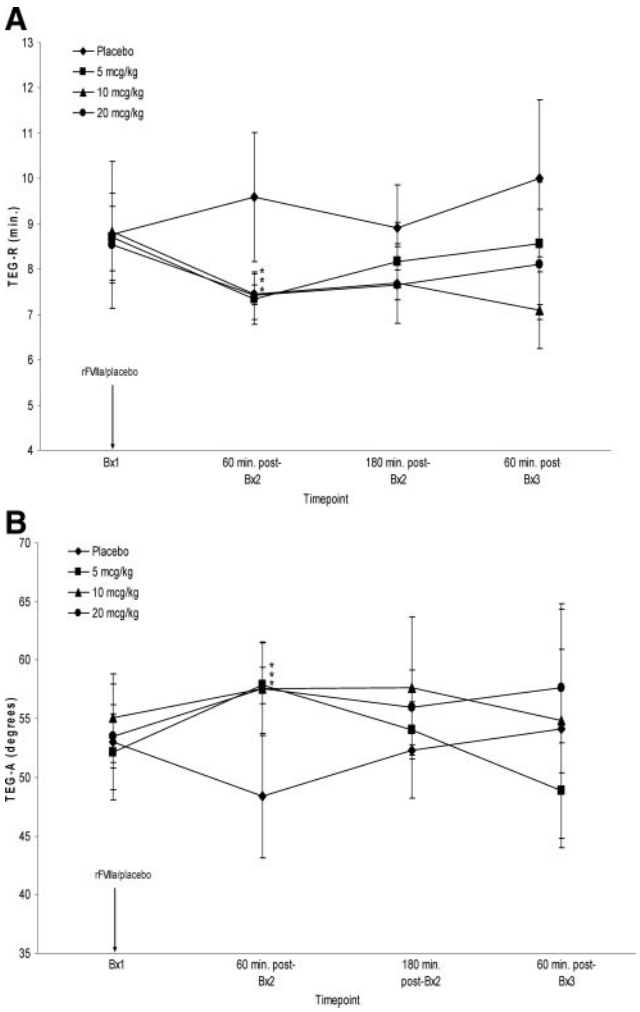

Figure 3. Thromboelastograph (TEG®) parameters in placebo or recombinant activated FVII (rFVIIa)-treated subjects over time. A, TEG®-R = time to clot onset B, TEG®-A = clot angle. Data points were missing for 3 randomized subjects (1 in placebo and 2 in 20 μg/kg rFVIIa treatment groups) because of issues pertaining to equipment/supplies, staff, or samples. *Significant difference from placebo ($P < 0.05$).

severity and were related to the biopsy procedure. No thromboembolic complications were reported.

DISCUSSION

Clopidogrel Response

Because platelet response to clopidogrel (i.e., inhibition of platelet aggregation by clopidogrel) is highly variable,[22–24] with poor response rates occurring in as many as 40% to 60% of patients,[25–27] it was critical to include reliable PI screening to exclude subjects nonresponsive to clopidogrel. In the current study, 56% of subjects failed to demonstrate an antiplatelet effect measured as PI. Previous studies report that up to 60% of clopidogrel-treated subjects exhibit a poor clopidogrel response, a poor response being arbitrarily defined as less than 30% to 40% inhibition of platelet aggregation.[25–27] Furthermore, the variability (20.3% SD) in PI in those subjects who met the cutoff levels was similar to the variability reported from secondary post hoc analyses of clopidogrel-treated subjects by Serebruany et al.[28] (20.8% SD). Factors such as age (older than 55 years),[27] increased body weight,[26,29] and conditions that increase platelet reactivity, including diabetes,[27,30] acute coronary syndrome, and acute stroke[31] may affect response to clopidogrel. It is more likely that other factors affected subject response to clopidogrel because most subjects in this study did not have any of these characteristics that affect response to clopidogrel. Factors that influence the absorption and biotransformation of clopidogrel and/or variability in the $P2Y_{12}$ adenosine diphosphate receptor affecting platelet activation and aggregation[32] may explain the clopidogrel response pattern. Furthermore, there are several other mechanisms and pathways for platelet aggregation (e.g., activation of platelet aggregation by thromboxane A2).[33,34] Therefore, targeting activation of the P2Y12 adenosine diphosphate–receptor pathway alone may not sufficiently attenuate the entire aggregation process.

Bleeding Characteristics

The punch biopsy model has advantages over the original Ivy and Simplate bleeding tests. The Ivy method has limited use in the clinic because of its poor correlation with actual patient bleeding.[35] When healthy volunteers were treated with acetylsalicylic acid, bleeding times did not significantly increase using the Simplate bleeding test ($P > 0.05$).[36] The punch biopsy model produced significantly longer BD and BV in healthy volunteers after anticoagulant therapy.[15] For these reasons, the punch biopsy model was deemed a suitable model to investigate the ability of rFVIIa to mitigate the effects of clopidogrel on BD and BV. The reasons for the limited ability of the punch biopsy model to demonstrate an effect of rFVIIa on mitigating drug-induced (warfarin[15] or clopidogrel) coagulopathies measured by BD remain unclear. Other than the inter-investigator variability that was specific to this study, the nature of the injury (capillary effects), the interaction of the local anesthetic with rFVIIa at the area of injury, or simply the limited effects of rFVIIa in such a small vessel bleeding model are possible explanations.

The effects of rFVIIa on BV, unlike BD, were consistent with the ex vivo time to clot onset (TEG®-R) and clot angle (TEG®-A) results (Fig. 3). These results are similar to the previously reported findings in which rFVIIa corrected the effect of warfarin on all ex vivo TEG® parameters,[15] and suggest that time to clot formation affects BV but not BD.

Limitations of the Study

Dual therapy with aspirin and clopidogrel is the common practice for inhibition of platelet aggregation for preventing cardiovascular events.[34] This study might have benefited from the use of dual antiplatelet drugs and/or increased loading/maintenance dose[26,37,38] to allow a larger proportion of subjects to achieve the PI cutoff levels and possibly a more uniform clopidogrel response. However, the percentage of subjects who did not respond to clopidogrel treatment (56%) was similar to that reported in a previous study that used dual therapy with aspirin.[18]

A signal on BV for the 10 and 20 μg/kg rFVIIa dose groups and for BD in the 20 μg/kg rFVIIa dose group, for the initial physician, clearly underscores the impact of

technical skill in this bleeding model. The premature termination of the study restricted enrollment of subjects in the 40 μg/kg dose group and did not allow for the investigation of the 80 μg/kg dose group, a dose that is within the trial product label. Even though a signal showed an rFVIIa effect for BV in the 10 and 20 μg/kg rFVIIa dose groups as compared with placebo, the results for BD at these doses were not convincing.

Despite these noted limitations of the punch biopsy model, rFVIIa mitigated the effects of clopidogrel-induced bleeding on BV. It is possible that the inhibition of platelet aggregation was compensated by rFVIIa-mediated enhancement of thrombin generation and thrombin-mediated activation of platelet aggregation,[33,39] which was reflected more in the BV measurements than in BD. As such, the results of this study cannot be extrapolated to clinical hemorrhages because the effectiveness of rFVIIa to mitigate clopidogrel-associated bleeding has not been tested.

CONCLUSIONS

This exploratory study was designed to investigate the effect of escalating doses of rFVIIa in clopidogrel-mediated bleeding in a punch biopsy model. Despite study limitations, rFVIIa (10 and 20 μg/kg) significantly mitigated clopidogrel-induced effects on BV. Furthermore, in subgroup analyses of subjects, 20 μg/kg rFVIIa showed a significant reduction of clopidogrel-induced BD as well as on BV. The clinical enhancement of coagulation by rFVIIa was also reflected in ex vivo clotting parameters (TEG®-R and TEG®-A).

CURRENT AFFILIATIONS

Brett E. Skolnick, PhD, is currently affiliated with Novo Nordisk Inc, Princeton, NJ; Magdy Shenouda, MD, is currently affiliated with Iberia Clinical Research, Eatontown, NJ; Anthony E. Pusateri, PhD, is currently affiliated with US Army Medical Research and Materiel Command, Fort Detrick, MD; and Marcus E. Carr, MD, PhD, FACP, is currently affiliated with Pfizer Inc., Collegeville, PA.

DISCLOSURES

Name: Brett E. Skolnick, PhD.
Contribution: This author helped design the study, conduct the study, analyze the data, and write the manuscript.
Attestation: Brett E. Skolnick designed the study, has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.
Conflicts of Interest: Brett E. Skolnick is an employee of Novo Nordisk.
Name: Magdy Shenouda, MD.
Contribution: This author helped conduct the study and write the manuscript.
Attestation: Magdy Shenouda has seen the original study data and approved the final manuscript.
Conflicts: Magdy Shenouda received financial compensation for his role as investigator of the study.
Name: Naum M. Khutoryansky, PhD.
Contribution: This author helped design the study, analyze the data, perform statistical analysis, and write the manuscript.
Attestation: Naum M. Khutoryansky has seen the original study data, supervised the analysis and review of the data, and approved the final manuscript.
Conflicts of Interest: Naum M. Khutoryansky is an employee of Novo Nordisk.
Name: Anthony E. Pusateri, PhD.
Contribution: This author helped conduct the study, write the manuscript, and contributed to acquisition of laboratory data.
Attestation: Anthony E. Pusateri has seen the original study data and approved the final manuscript.
Conflicts of Interest: Anthony E. Pusateri was an employee of Novo Nordisk at the time of study.
Name: Don Gabriel, MD, PhD.
Contribution: This author helped conduct the study, write the manuscript, and served as an external independent Safety Officer.
Attestation: Don Gabriel has seen the original study data and approved the final manuscript.
Conflicts of Interest: Don Gabriel received financial compensation for his role as Safety Officer of the study.
Name: Marcus E. Carr, MD, PhD, FACP.
Contribution: This author helped write the manuscript.
Attestation: Marcus E. Carr has seen the original study data and approved the final manuscript.
Conflicts of Interest: Marcus E. Carr was an employee of Novo Nordisk at the time of study.

ACKNOWLEDGMENTS

We thank all the study subjects for their participation. We also thank Dr. Sandra Connolly and other study staff at MDS Pharma Services, Neptune, NJ, and the facility staff at Novo Nordisk Research Facility US (NNRUS), New Brunswick, NJ, including Jun D. Guzman, and Petula Fraser-Davies at Novo Nordisk, Princeton, NJ, for acquisition of data, study supervision, and administrative, technical, and/or material support. Dr. Alvin Estilo, Novo Nordisk's Safety Officer, assembled a safety group (including internal Novo Nordisk members not involved in the study conduct and an external independent Safety Officer, Dr. Don Gabriel) to review safety findings at the conclusion of each dose tier. We thank Abha Chandra, PhD, Neal Okarter, PhD, and Charlotte Yap, MSc, of Novo Nordisk, for providing writing and editorial assistance with the manuscript. We also thank Hongli Wang, MSc, and Thomas Henschel, employees of Novo Nordisk, for performing the statistical analyses.

REFERENCES

1. The CAPRIE Steering Committee. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). CAPRIE Steering Committee. Lancet 1996;348:1329–39
2. Steinhubl SR, Berger PB, Mann JT III, Fry ET, DeLago A, Wilmer C, Topol EJ. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. JAMA 2002;288:2411–20
3. NovoSeven® RT Prescribing Information. Princeton, NJ: Novo Nordisk Inc., 2010
4. Altman R, Scazziota A, de Lourdes HM, Gonzalez C. Recombinant factor VIIa reverses the inhibitory effect of aspirin or aspirin plus clopidogrel on in vitro thrombin generation. J Thromb Haemost 2006;4:2022–7
5. Cherfan A, Arabi Y, Al Askar A, Al Shimemeri A. Recombinant activated factor VII treatment of retroperitoneal hematoma in a patient with renal failure receiving enoxaparin and clopidogrel. Pharmacotherapy 2007;27:755–9
6. Hyllner M, Houltz E, Jeppsson A. Recombinant activated factor VII in the management of life-threatening bleeding in cardiac surgery. Eur J Cardiothorac Surg 2005;28:254–8

7. Loertzer H, Soukup J, Fornara P. Recombinant factor VIIa reduces bleeding risk in patients on platelet aggregation inhibitors immediately prior to renal transplantation: a retrospective analysis. Urol Int 2007;78:135–9
8. Pfau G, Schilling T, Kozian A, Huth C, Schneemilch CE, Heim MU. A single dose of recombinant activated factor VII (NovoSeven®) did not impair the function of the coronary artery bypass grafts: successful treatment of critical bleeding after cardiac surgery in two cases. Transfus Med Hemother 2007;34:204–7
9. von Heymann C, Schoenfeld H, Sander M, Ziemer S, Grubitzsch H, Spies C. Clopidogrel-related refractory bleeding after coronary artery bypass graft surgery: a rationale for the use of coagulation factor concentrates? Heart Surg Forum 2005;8: E39–41
10. Diness V, Bregengaard C, Erhardtsen E, Hedner U. Recombinant human factor VIIa (rFVIIa) in a rabbit stasis model. Thromb Res 1992;67:233–41
11. Fattorutto M, Tourreau-Pham S, Mazoyer E, Bonnin P, Raphael M, Morin F, Cupa M, Samama CM. Recombinant activated factor VII decreases bleeding without increasing arterial thrombosis in rabbits. Can J Anaesth 2004;51:672–9
12. Hindy-Francois C, Bachelot-Loza C, Le Bonniec B, Grelac F, Dizier B, Godier A, Emmerich J, Gaussem P, Samama CM. Recombinant activated factor VII does not reduce bleeding in rabbits treated with aspirin and clopidogrel. Thromb Haemost 2010;104:823–30
13. Lauritzen B, Tranholm M, Ezban M. rFVIIa and a new enhanced rFVIIa-analogue, NN1731, reduce bleeding in clopidogrel-treated and in thrombocytopenic rats. J Thromb Haemost 2009;7:651–7
14. Hyllner M, Houltz E, Jeppsson A. Recombinant activated factor VII in the management of life-threatening bleeding in cardiac surgery. Eur J Cardiothorac Surg 2005;28:254–8
15. Skolnick BE, Mathews DR, Khutoryansky NM, Pusateri AE, Carr ME. Exploratory study on the reversal of warfarin with rFVIIa in healthy subjects. Blood 2010;116:693–701
16. World Medical Association. Declaration of Helsinki: ethical principles for medical research involving human subjects. J Int Bioethique 2011;15:124–9
17. Malinin A, Pokov A, Spergling M, Defranco A, Schwartz K, Schwartz D, Mahmud E, Atar D, Serebruany V. Monitoring platelet inhibition after clopidogrel with the VerifyNow-P2Y12(R) rapid analyzer: the VERIfy Thrombosis risk ASsessment (VERITAS) study. Thromb Res 2007;119:277–84
18. Price MJ, Coleman JL, Steinhubl SR, Wong GB, Cannon CP, Teirstein PS. Onset and offset of platelet inhibition after high-dose clopidogrel loading and standard daily therapy measured by a point-of-care assay in healthy volunteers. Am J Cardiol 2006;98:681–4
19. Takamiya O, Sakata M. Ex vivo coagulation test on tissue factor-expressing cells with a calibrated automated thrombogram. Lab Hematol 2008;14:39–44
20. Young G, Ebbesen LS, Viuff D, Di PJ, Konkle BA, Negrier C, Pasi J, Ingerslev J. Evaluation of thromboelastography for monitoring recombinant activated factor VII ex vivo in haemophilia A and B patients with inhibitors: a multicentre trial. Blood Coagul Fibrinolysis 2008;19:276–82
21. McCullagh P, Nelder J. Generalized Linear Models. London: Chapman and Hall, 1996
22. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramirez C, Sabate M, Banuelos C, Hernandez-Antolin R, Escaned J, Moreno R, Alfonso F, Macaya C. High clopidogrel loading dose during coronary stenting: effects on drug response and interindividual variability. Eur Heart J 2004;25:1903–10
23. Gurbel PA, Bliden KP, Samara W, Yoho JA, Hayes K, Fissha MZ, Tantry US. Clopidogrel effect on platelet reactivity in patients with stent thrombosis: results of the CREST Study. J Am Coll Cardiol 2005;46:1827–32
24. Hochholzer W, Trenk D, Frundi D, Blanke P, Fischer B, Andris K, Bestehorn HP, Buttner HJ, Neumann FJ. Time dependence of platelet inhibition after a 600-mg loading dose of clopidogrel in a large, unselected cohort of candidates for percutaneous coronary intervention. Circulation 2005;111:2560–4
25. Barsky AA, Arora RR. Clopidogrel resistance: myth or reality? J Cardiovasc Pharmacol Ther 2006;11:47–53
26. Lee DH, Arat A, Morsi H, Shaltoni H, Harris JR, Mawad ME. Dual antiplatelet therapy monitoring for neurointerventional procedures using a point-of-care platelet function test: a single-center experience. AJNR Am J Neuroradiol 2008;29:1389–94
27. Prabhakaran S, Wells KR, Lee VH, Flaherty CA, Lopes DK. Prevalence and risk factors for aspirin and clopidogrel resistance in cerebrovascular stenting. AJNR Am J Neuroradiol 2008;29:281–5
28. Serebruany VL, Steinhubl SR, Berger PB, Malinin AI, Bhatt DL, Topol EJ. Variability in platelet responsiveness to clopidogrel among 544 individuals. J Am Coll Cardiol 2005;45:246–51
29. Feher G, Koltai K, Alkonyi B, Papp E, Keszthelyi Z, Kesmarky G, Toth K. Clopidogrel resistance: role of body mass and concomitant medications. Int J Cardiol 2007;120:188–92
30. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramirez C, Sabate M, Jimenez-Quevedo P, Hernandez R, Moreno R, Escaned J, Alfonso F, Banuelos C, Costa MA, Bass TA, Macaya C. Clopidogrel withdrawal is associated with proinflammatory and prothrombotic effects in patients with diabetes and coronary artery disease. Diabetes 2006;55:780–4
31. Matetzky S, Shenkman B, Guetta V, Shechter M, Bienart R, Goldenberg I, Novikov I, Pres H, Savion N, Varon D, Hod H. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. Circulation 2004;109:3171–5
32. Wiviott SD. Clopidogrel response variability, resistance, or both? Am J Cardiol 2006;98:18N–24N
33. Savage B, Cattaneo M, Ruggeri ZM. Mechanisms of platelet aggregation. Curr Opin Hematol 2001;8:270–6
34. Varon D, Spectre G. Antiplatelet agents. Hematol Am Soc Hematol Educ Program 2009;2009:267–72
35. Willoughby ML, Allington MJ. The rate of blood loss from skin punctures during the Ivy bleeding time test. J Clin Pathol 1961;14:381–4
36. Kumar R, Ansell JE, Canoso RT, Deykin D. Clinical trial of a new bleeding-time device. Am J Clin Pathol 1978;70:642–5
37. von Beckerath N, Taubert D, Pogatsa-Murray G, Schomig E, Kastrati A, Schomig A. Absorption, metabolization, and antiplatelet effects of 300-, 600-, and 900-mg loading doses of clopidogrel: results of the ISAR-CHOICE (Intracoronary Stenting and Antithrombotic Regimen: Choose Between 3 High Oral Doses for Immediate Clopidogrel Effect) Trial. Circulation 2005;112:2946–50
38. Montalescot G, Sideris G, Meuleman C, Bal-dit-Sollier C, Lellouche N, Steg PG, Slama M, Milleron O, Collet JP, Henry P, Beygui F, Drouet L. A randomized comparison of high clopidogrel loading doses in patients with non-ST-segment elevation acute coronary syndromes: the ALBION (Assessment of the Best Loading Dose of Clopidogrel to Blunt Platelet Activation, Inflammation and Ongoing Necrosis) Trial. J Am Coll Cardiol 2006;48:931–8
39. Hedner U. Factor VIIa and its potential therapeutic use in bleeding-associated pathologies. Thromb Haemost 2008;100: 557–62