Recombinant factor VIIa affects anastomotic patency of vascular grafts in a rabbit model

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Objective: Recombinant factor VIIa can decrease postoperative bleeding after cardiac surgery. However, the potential for recombinant factor VIIa to cause early vascular graft occlusion at the site of new vascular anastomoses has not been fully explored. We hypothesized that recombinant factor VIIa would cause a dose-dependent reduction in vascular graft patency in rabbits.

Methods: Reversed end-to-end interpositional vein grafts were sutured into the carotid artery of heparinized rabbits, and then recombinant factor VIIa (300 μg/kg, 90 μg/kg, or 20 μg/kg intravenously) or placebo was administered (n = 16/group). Graft patency was assessed at 24 hours using a vascular ultrasound probe. Factor VII activity levels were measured using a prothrombin time-based assay. In different rabbits, the patency of venous end-to-side anastomoses and simple carotid arterial repairs was assessed (recombinant factor VIIa, 300 μg/kg vs placebo, n = 8/group). Data were analyzed using Fisher's exact test, *t* tests, or analysis of variance.

Results: Physiologic variables (activated clotting time, hemoglobin, pH, Pao_2) and vessel diameter were not different between groups. Vein graft patency was reduced (93.8%, 81.2%, 13.8%, and 6.3%) as factor VII activity levels increased (1.8 ± 0.4, 4.4 ± 2.1, 11.8 ± 4.7, and 23.6 ± 16.9 U/mL, respectively) with increasing doses of recombinant factor VIIa administered (0, 20, 90, and 300 μg/kg, respectively, $P < .05$). Patency in the arterial repairs and end-to-side venous grafts was also reduced in recombinant factor VIIa-treated rabbits ($P < .05$ for both).

Conclusions: This study suggests that recombinant factor VIIa is associated with a dose-dependent increase in fresh vascular graft occlusion. Higher doses of recombinant factor VIIa may be associated with increased thrombotic outcomes. (J Thorac Cardiovasc Surg 2011;142:418-23)

Coronary artery bypass graft surgery is commonly performed to provide myocardial revascularization through arterial and venous bypass grafts. Postoperative hemorrhage occurs in up to 20% of these patients and has been associated with increased morbidity and mortality. Clinical management of postoperative bleeding includes multimodal therapeutic strategies with antifibrinolytics, cell salvage, and transfusion of allogeneic red blood cells, platelets, and coagulation factors. However, each of these therapies has been associated with significant risks and complications. A treatment that promotes hemostasis without compromising patency would be a valuable addition to current clinical practice. Recombinant factor VIIa (rFVIIa) is an approved therapy for treatment for bleeding episodes in patients with hemophilia, which has recently been used off-label to treat refractory bleeding in patients undergoing cardiac surgery.[1-5] The profound hemostatic effect of rFVIIa is thought to be a function of site-specific thrombin generation by tissue factor-mediated activation of coagulation or by tissue factor-independent thrombin generation on activated platelets at sites of vascular injury.[6] Although an increasing number of reports have described the successful use of rFVIIa to treat bleeding after cardiac surgery, the safety and efficacy of this treatment have not been well studied. This is of particular importance in patients with cardiovascular disease with fresh vascular anastomoses. The potential hemostatic benefits of rFVIIa could be negated if tissue factor expression and thrombin generation at the site of new anastomoses resulted in graft narrowing or

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Abbreviations and Acronyms
ACT = activated clotting time
rFVIIa = recombinant factor VIIa

thrombosis. Recent concerns of thrombotic complications with aprotinin and erythropoietin emphasize the importance of properly evaluating hemostatic drugs before widespread clinical use.[7-9] This study was designed to test the hypothesis that the administration of rFVIIa reduces graft patency at the site of new vascular anastomoses in a dose-dependent fashion, using a rabbit model.

MATERIALS AND METHODS

Animal Model and Surgical Preparation

All animal protocols were approved by the Animal Care and Use Committee at St Michael's Hospital in accordance with the requirements of Canadian Animal Care Committee. New Zealand White rabbits (3–4 kg) were premedicated with ketamine (10 mg/kg) and xylazine (2 mg/kg), and anesthetized with 1% to 3% isoflurane in oxygen delivered via face mask and spontaneous ventilation. Body temperature was maintained using radiant warming lamps. The ear artery was cannulated with a 25 G catheter for direct blood pressure measurement and blood sampling for blood gas analysis and measurement of hemoglobin concentration using co-oximetry (Radiometer ALB 500 and OSM 3, London Scientific, London, Canada). An ear vein was cannulated with a 22-g catheter for administration of study drug or saline vehicle. Intravenous isotonic saline was administered continuously at approximately 2 mL/kg/h throughout surgery to replace insensible fluid losses.

Through a midline neck incision, the right carotid artery was isolated and dissected free. A 2- to 3-cm portion of the ipsilateral jugular vein was then isolated, excised, and flushed with heparinized saline. The animals were then systemically anticoagulated with heparin (250–300 IU/kg intravenously) to achieve and maintain a target activated clotting time (ACT) of greater than 250 seconds during carotid occlusion and graft suturing. One of 2 experimental protocols was then performed, as outlined below. All anastomoses were performed with interrupted 9-0 Prolene sutures (AROSurgical, Newport Beach, Calif). Surgical hemostasis and graft patency were always ensured by inspection before wound closure. The isoflurane was then discontinued, and the animals were allowed to recover.

Experimental Protocol 1: Recombinant Factor VIIa Dose Response

In protocol 1, an interpositional graft of reversed jugular vein was fashioned in the carotid artery using 2 artery-to-vein anastomoses. The animals then received rFVIIa (Niastase; NovoNordisk, Mississauga, Canada) or placebo (n = 16 per group) in a blinded fashion, so that neither the person performing the anastomoses nor the person assessing the primary outcome was aware of treatment assignment. The study drug was prepared in accordance with the manufacturer's recommendations immediately before administration and injected intravenously via the ear vein. Three dosing regimens of rFVIIa were studied: 300, 90, and 20 μg/kg. These regimens were chosen because they span the range of doses given to humans. The currently approved dose for hemophiliac patients is 90 μg/kg. Before and approximately 20 minutes after drug (or placebo) administration, blood was drawn and centrifuged (3400 rpm $\times$ 12 minutes), and citrated plasma was frozen at–80 degrees for subsequent measurement of factor VII activity levels (see below).

Experimental Protocol 2: Effect of High-Dose Recombinant Factor VIIa on Arterial and End-to-Side Venous Anastomoses

In protocol 2, 2 different anastomotic techniques were evaluated in the same animal. The right carotid artery was ligated at its midpoint, and the vein segment was used as a bypass graft rather than an interposition graft by creating 2 end-to-side anastomoses. In addition, the contralateral carotid artery was transected and then repaired with a single end-to-end arterial repair. The end-to-side vein graft anastomoses were performed to model a saphenous vein to coronary artery graft. The simple arterial repair was performed to model vascular grafts using arterial conduits. In this protocol, the highest dose of rFVIIa was used (300 μg/kg) to maximize the ability to detect potential differences in graft occlusion with different graft techniques.

Outcomes/Measurements

Primary outcome. The primary outcome of this study was graft occlusion at 1 day as assessed by ultrasound or direct visual inspection in rabbits anesthetized as described. The investigators performing the anastomoses or assessing the primary outcome were blinded to study treatment. An HDI 5000cv ultrasound system (Philips Ultrasound, Mississauga, Canada), equipped with a high-frequency (15 MHz) linear array vascular probe (CL15-7), was used to visualize the body of the graft, including all anastomotic sites, using standard B-mode ultrasound in longitudinal and transverse axes. The grafts were defined as patent if there was demonstrable flow through them or there was no evidence of occlusion at autopsy. Filling defects representing intraluminal thrombi within the graft were identified, and the dimensions were measured in 2 orthogonal planes. Blood flow through patent grafts was assessed using standard color and spectral Doppler techniques. Quantitative blood flow measurements were performed in the proximal and distal carotid arteries using standard quantitative volume flow techniques (HDI5000cv). This technique uses mean velocities and vessel diameters to calculate volumetric flow through vessels and has been validated.[10]

Factor VII activity levels in the citrated plasma samples were measured in the dose-response experiments using standard 1-stage coagulation endpoint techniques, with rabbit brain thromboplastin (IL Test PT-Fibrinogen HS; Instrumentation Laboratory, Bedford, Mass) and immunoadsorbed factor VII deficient plasma (Hemoliance Deficient Plasma; Beckman-Coulter Canada, Mississauga, Ontario, Canada). The calibration curve used reference plasma calibrated against the World Health Organization standard.

Statistical Analyses

Statistical analyses were performed using the SAS System for Windows, version 8.02 (SAS Institute Inc, Cary, NC). The sample size was estimated assuming a 95% patency rate in the placebo group based on previous studies, a 40% reduction with rFVIIa, an alpha error of 0.05, and a beta of 0.20 (power 0.80).The primary outcome was assessed using Fisher's exact test. Other data were analyzed with *t* tests or 2-way analysis of variance for continuous variables as appropriate. When a significant F-ratio was present, multiple comparisons were made using Dunnett's and Tukey's tests. All data are reported as mean $\pm$ standard deviation or frequency $\pm$ 95% confidence intervals.

RESULTS

Fifteen of 95 rabbits entered into the protocol were excluded (surgical difficulties with anastomoses [n = 10], perioperative death [n = 2], problems with anticoagulation [n = 2], and problems with data acquisition [n = 1]), leaving a total of 80 animals. Sixty-four rabbits were used in the