Insights into the mechanism(s) of von Willebrand factor degradation during mechanical circulatory support

Carlo R. Bartoli, MD, PhD,[a,b] Sujith Dassanayaka, MS,[c,e] Kenneth R. Brittian, BS,[c] Andrew Luckett, MD,[d] Srinivas Sithu, PhD,[e] Thorsten Siess, PhD,[f] Daniel H. Raess, MD,[f] Paul A. Spence, MD,[g] Steven C. Koenig, PhD,[h,i] Robert D. Dowling, MD,[j] and Stanley E. D'Souza, PhD[e]

Objective: Left ventricular assist device support produces a bleeding diathesis. Evidence suggests a major role for von Willebrand factor (vWF). We examined vWF metabolism in a preclinical model of short-term mechanical circulatory support.

Methods: In 25 calves (weight, 80-110 kg), the inflow/outflow graft of the Symphony Heart Assist System was sewn end-to-side to the carotid artery. Support was initiated (acute, n = 4; 1 week, n = 16; 2 weeks, n = 5). Acutely, carotid artery pressure and flow were measured to evaluate the hemodynamic changes near the anastomosis. At baseline and after ≤2 weeks of support, platelet aggregometry with adenosine 5′-diphosphate, collagen, and ristocetin was performed. Gel electrophoresis and wet immunoblotting qualitatively evaluated vWF multimers and quantified plasma ADAMTS-13, the vWF-cleaving protease. Carotid arterial rings near the anastomosis were studied with immunohistochemical staining for ADAMTS-13 and were cultured to quantify endothelial ADAMTS-13 production. Fluorescent resonance energy transfer was used to evaluate the enzymatic activity of ADAMTS-13 in the plasma and in supernatant from cultured carotid arterial rings. Plasma interleukin-6, which inhibits ADAMTS-13 activity, was measured using an enzyme-linked immunosorbent assay.

Results: During support, statistically significant ($P < .05$) changes in the carotid endothelium arterial hemodynamics were observed. The highest molecular weight vWF multimers were absent, and the vWF-ristocetin platelet aggregation pathway was significantly impaired. A modest but significant increase in plasma ADAMTS-13 protein and activity was observed. ADAMTS-13 decreased significantly in the carotid near the anastomosis but increased significantly in supernatant from cultured carotid arterial rings. The plasma interleukin-6 levels did not change significantly.

Conclusions: Hemodynamic activation of vWF and increased plasma ADAMTS-13 activity may have reduced high-molecular-weight vWF multimers and thereby impaired the vWF-platelet aggregation pathway. Additional delineation of these pathways may improve management of left ventricular assist device-associated bleeding. (J Thorac Cardiovasc Surg 2014;147:1634-43)

ET/BS

From the Division of Cardiovascular Surgery,[a] University of Pennsylvania School of Medicine, Philadelphia, Pa; MD/PhD Program,[b] University of Louisville School of Medicine, Louisville, Ky; Institute of Molecular Cardiology,[c] University of Louisville School of Medicine, Louisville, Ky; University of Louisville School of Medicine,[d] Louisville, Ky; Department of Physiology and Biophysics,[e] University of Louisville School of Medicine, Louisville, Ky; Abiomed Inc,[f] Danvers, Mass; SCR Inc,[g] Louisville, Ky; Cardiovascular Innovation Institute,[h] University of Louisville School of Medicine, Louisville, Ky; Department of Bioengineering,[i] University of Louisville, Louisville, Ky; and Dowling Consulting, PSC,[j] Louisville, Ky.

Funding for this project was provided by the National Institutes of Health grant HL43721 National Institutes of Health Small Business Innovation Research grants 2R44HL083586-02A1, 2R44HL088760-02, and R43HL102981, and Kentucky Science and Technology Corporation grants KSTC-184-512-08-054 and KSTC-184-512-08-054.

Disclosures: Drs Siess and Raess are paid employees of Abiomed, Inc. Dr Spence receives royalties for the Symphony pump. Dr Dowling is a paid consultant of Abiomed, Inc. All other authors have nothing to disclose with regard to commercial support.

Received for publication May 21, 2013; revisions received July 19, 2013; accepted for publication Aug 16, 2013; available ahead of print Oct 17, 2013.

Address for reprints: Stanley E. D'Souza, PhD, Department of Physiology and Biophysics, University of Louisville School of Medicine, 500 S Preston St, HSC A 1115, Louisville, KY 40292 (E-mail: sedsou01@louisville.edu).

0022-5223/$36.00

http://dx.doi.org/10.1016/j.jtcvs.2013.08.043

Recent reports have documented impairment of the von Willebrand factor (vWF) pathway[1-11] and nonsurgical bleeding[12] during mechanical circulatory support. In these studies, acquired von Willebrand syndrome developed in patients with continuous-flow[1-11] and pulsatile-flow[1,4,5,9] left ventricular assist devices (LVADs). The absence of high-molecular-weight vWF multimers contributed to a bleeding diathesis in these patients.

Limited data exist to explain the mechanisms of acquired von Willebrand syndrome in patients with an LVAD. During in vitro LVAD support in a mock circulatory loop, degradation of vWF occurred rapidly as a result of multiple mechanisms that occurred simultaneously.[13] Shear-induced mechanical demolition, in which vWF monomer subunits were broken off from vWF multimers, may have played a significant role. In parallel, ADAMTS-13, the vWF protease, constitutively cleaved vWF into fragments in the plasma.

To investigate the potential pathophysiologic mechanisms of acquired von Willebrand syndrome during mechanical circulatory support in vivo, we implanted the Symphony Heart

Abbreviations and Acronyms

ADP = adenosine 5′-diphosphate
FRET = fluorescent resonance energy transfer
IL = interleukin
LVAD = left ventricular assist device
PRP = platelet-rich plasma
SDS = sodium dodecyl sulfate
vWF = von Willebrand factor

Assist System[14] (Abiomed, Inc, Danvers, Mass), a novel partial-support counterpulsatile LVAD, in calves. Platelet aggregation, vWF multimers, ADAMTS-13, and interleukin (IL)-6, which inhibits ADAMTS-13, were examined.

METHODS

The present study was conducted in accordance with the National Institutes of Health guidelines for the care and use of animals in research. The Institutional Animal Care and Use Committee of the University of Louisville (Louisville, Ky) approved all experimental procedures.

Study Overview

The Symphony Heart Assist System (Abiomed, Inc) is a 30-mL displacement pump designed to deliver prolonged partial support in ambulatory patients with heart failure (Figure 1, *B* and *C*). The pump is implanted posterior to the pectoralis major muscle without entering the chest. A modified Gore-Tex graft (W. L. Gore & Associates, Flagstaff, Ariz) is sewn end-to-side to the subclavian artery. The patient's electrocardiogram is continuously monitored, and an R-wave–sensing algorithm controls filling and emptying of the pump. The Symphony fills during native cardiac systole to reduce left ventricular afterload and ejects during native cardiac diastole to augment the diastolic blood pressure. Improved coronary and systemic blood flow occurs. By these mechanisms, the myocardial oxygen supply/demand relationship improves,[14] and end-organ perfusion increases.

Aspects of the design, implantation strategy, support mechanism, and control algorithm of the Symphony pump are unique. The Symphony is designed with a valveless inflow/outflow cannula. A constant but shifting vortex continuously "washes" the interior of the pump to prevent thrombosis. Implantation (and explantation) with a simple subcutaneous procedure does not require extracorporeal circulation, sternotomy, or thoracotomy. The Symphony functions as a peripheral capacitance chamber that does not influence the internal impedance of the aorta. Adjustment of the timing of filling and ejection permit modest tradeoffs between improved coronary flow and reduced left ventricular workload. Consequently, subtle variations in the delivery of support may have important utility for incremental patient management on an individualized basis, especially during weaning of the device if explantation is indicated. Importantly, the low manufacturing cost and cost of implantation of the Symphony pump may expand the use of mechanical circulatory support in underserved foreign markets. A clinical trial with the Symphony pump is currently underway in Canada and France.[15]

We used 25 male calves (weight, 80-110 kg) to evaluate the Symphony pump. The study was performed according to the Good Laboratory Practices (GLP) guidelines to determine the preclinical safety of the pump. The calves were supported acutely (n = 4) or were provided uninterrupted support for 1 week (n = 16) or 2 weeks (n = 5) with the Symphony pump.

The Symphony pump was implanted subcutaneously in the neck. An anastomosis was performed between the pump graft and the carotid artery as described below. This approach was chosen because of the similar size (approximately 8-10 mm in diameter) of the bovine carotid artery and the human subclavian artery and the similar distance (approximately 8-10 cm in distance) from the anastomosis to the aortic valve in humans and cows.[16]

Experimental Design

Blood samples were collected at baseline before implantation of the Symphony pump and after 1 or 2 weeks of support prior to euthanasia. As such, each calf was its own control. Likewise, analysis of the carotid artery harvested near the anastomosis of the pump and the contralateral carotid artery enabled each calf to also be used as its own control.

Platelet aggregometry was performed on platelet-rich plasma (PRP, n = 14) to evaluate platelet aggregation pathways. Gel electrophoresis and wet immunoblotting were performed to evaluate plasma vWF multimers (n = 14) and to quantify plasma ADAMTS-13 (n = 14). Immunohistochemical staining for ADAMTS-13 was performed on carotid arterial rings near the anastomosis (n = 8) to evaluate endothelial ADAMTS-13. Later, carotid arterial rings were cultured in vitro to quantify endothelial ADAMTS-13 production near the anastomosis (n = 5). Fluorescent resonance energy transfer (FRET) was used to evaluate plasma ADAMTS-13 activity in the plasma (n = 14) and in the supernatant from cultured carotid arterial rings (n = 5). An enzyme-linked immunosorbent assay was performed to measure plasma IL-6 (n = 14). In addition to the histologic and molecular analyses, carotid artery hemodynamics were measured acutely (n = 4) to quantify the hemodynamic changes associated with partial support using an end-to-side anastomosis to a peripheral artery.

Surgical Preparation

The calves were anesthetized with 3% to 5% isoflurane and prepared for sterile surgery. Permanent fluid-filled catheters were placed in the right jugular vein for intravenous access and in the proximal right carotid artery for arterial blood pressure monitoring. A left fifth intercostal space minithoracotomy was performed to place screw-in epicardial electrocardiographic leads (Medtronic, Inc, Minneapolis, Minn).

The calf was repositioned, and a left neck incision was made. The Symphony pump (30-mL stroke volume) was implanted in a subcutaneous pocket (7 cm × 7 cm × 5 cm) in the anterior neck. The percutaneous driveline was tunneled to the nape of the neck, externalized, and attached to a pneumatic driver (iPulse, Abiomed, Inc). A modified 8-mm Gore-Tex vascular graft (W. L. Gore & Associates) was sewn end-to-side to the carotid artery and connected to the pump. Care was taken to completely de-air the pump. Pump filling and emptying were triggered by the electrocardiogram using R-wave recognition software. Device emptying and filling were timed to produce diastolic augmentation and afterload reduction, respectively.

In a subset of calves, carotid artery pressure and flow were measured acutely. Transit-time ultrasonic flow probes (Transonic, Inc, Ithaca, NY) were placed around the carotid artery proximally and distally to the anastomosis. High-fidelity single-tip micromanometer catheters (Millar Instruments, Inc, Houston, Tex) were placed directly into the carotid artery proximal and distal to the anastomosis. The Symphony was operated in an uninterrupted 1:1 support mode in which each ventricular systole resulted in pump filling and each ventricular diastole-triggered pump ejection. Hemodynamics were recorded for 30-s epochs at baseline and for 30-s epochs during 1:1 Symphony support.

Each calf was maintained on a daily dose of 75 mg of clopidogrel, which was started 1 day before surgery. Intraoperatively, the calves received 10,000 U of heparin before clamping the carotid artery to perform the end-to-side graft anastomosis. After anastomosis completion and initiation of device support, heparin was reversed with 100 mg of protamine. At 6 hours after surgery, a continuous heparin infusion was initiated and titrated to maintain an activated clotting time of >200 s. Warfarin was initiated on postoperative day 3 and titrated to maintain an international normalized