The Role of Repeat Administration of Adventitial Delivery of Lentivirus-shRNA-*Vegf-A* in Arteriovenous Fistula to Prevent Venous Stenosis Formation

Rajiv Janardhanan, PhD, Binxia Yang, MD, PhD, Sreenivasulu Kilari, PhD, Edward B. Leof, PhD, Debabrata Mukhopadhyay, PhD, and Sanjay Misra, MD

ABSTRACT

Purpose: To determine if a second dose of a lentivirus mediated small hairpin RNA that inhibits *Vegf-A* gene expression (LV-shRNA-*Vegf-A*) can improve lumen vessel area (LVA) of the outflow vein of an arteriovenous fistula (AVF) and decrease venous neointimal hyperplasia.

Materials and Methods: Chronic kidney disease was created in C57BL/6 mice; 28 days later, an AVF was created by connecting the right carotid artery to the ipsilateral jugular vein. Immediately after AVF creation, 5×10^6 plaque-forming units of LV-shRNA-*Vegf-A* or control shRNA was administered to the adventitia of the outflow vein, and a second dose of the same treatment was administered 14 days later. Animals were sacrificed at 21 days, 28 days, and 42 days after AVF creation for reverse transcription polymerase chain reaction and histomorphometric analyses.

Results: By day 21, there was a 125% increase in the average LVA (day 21, $P = .11$), with a decrease in cell proliferation (day 21, $P = .0079$; day 28, $P = .28$; day 42, $P = .5$), decrease in α-smooth muscle cell actin staining (day 21, $P < .0001$; day 28, $P < .05$; day 42, $P = .59$), and decrease in hypoxic stress (day 21, $P < .001$; day 28, $P = .28$; day 42, $P = .46$) in LV versus control shRNA vessels.

Conclusions: A second dose of LV-shRNA-*Vegf-A* administration results in a moderate improvement in LVA at day 21.

ABBREVIATIONS

αSMA = α-smooth muscle actin, AVF = arteriovenous fistula, LV = lentivirus, LVA = lumen vessel area, RT-PCR = reverse transcription polymerase chain reaction, shRNA = small hairpin RNA, TUNEL = terminal deoxynucleotidyl transferase dUTP nick end labeling, VNH = venous neointimal hyperplasia

An arteriovenous fistula (AVF) is the preferred vascular access for hemodialysis, and maintaining its function is required for adequate hemodialysis. At 1 year, approximately 38% of AVFs fail because of venous stenosis formation, which is caused by venous neointimal hyperplasia (VNH) (1). Histologic analysis of VNH shows angiogenesis localized to the neointima and adventitia (2). This angiogenesis is accompanied by increased proliferation of cells staining positive for α-smooth muscle actin (α-SMA) in the neointima (2). A recent study demonstrated that transduction of a lentivirus mediated small hairpin RNA that inhibits *Vegf-A* gene expression (LV-shRNA-*Vegf-A*) to the adventitia of the outflow vein immediately after AVF creation led to an increase in lumen vessel area (LVA) compared with the

From the Amity Institute of Public Health (R.J.), Amity University Uttar Pradesh, Noida, Uttar Pradesh, India; Vascular and Interventional Radiology Translational Laboratory (B.Y., S.K., S.M.), Department of Radiology, and Department of Biochemistry and Molecular Biology (E.B.L., D.M., S.M.), Mayo Clinic, 200 First Street SW, Rochester, MN 55905. Received June 30, 2015; final revision received December 21, 2015; accepted December 22, 2015. Address correspondence to S.M.; E-mail: misra.sanjay@mayo.edu

S.M. has a patent for reducing venous stenosis formation of an arteriovenous fistula or graft pending. None of the other authors have identified a conflict of interest.

J Vasc Interv Radiol 2016; 27:576–583

http://dx.doi.org/10.1016/j.jvir.2015.12.751

outflow veins transduced with control shRNA. Moreover, fibroblast to myofibroblast differentiation along with a reduction in constrictive vascular remodeling was observed in outflow veins of AVFs transduced with *Vegf-A* shRNA (3). However, VNH started to recur 2 weeks after *Vegf-A* shRNA administration to the outflow veins of AVFs (3).

The aim of the present study is to determine whether two doses of LV-shRNA-*Vegf-A* could improve LVA and decrease VNH. Experiments were performed in a murine model of chronic kidney disease with AVF. The readouts were gene expression for *Vegf-A*, matrix metalloproteinase-2 (*Mmp-2*) and matrix metalloproteinase-9 (*Mmp-9*), proliferation, apoptosis, smooth muscle, and Hypoxyprobe (Hypoxyprobe, Inc, Burlington, Massachusetts) staining with histomorphometric analyses.

MATERIALS AND METHODS

Experimental Animals

Approval of the Institutional Animal Care and Use Committee was obtained before conducting any experiments. The animals were housed and handled in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals (4) (revised in 2000). C57BL/6 male mice (Jackson Laboratories, Bar Harbor, Maine) weighing 25–30 g were used for the study. The mice were maintained at room temperature of 22°C with 41% relative humidity and 12-hour/12-hour light/dark cycles. The animals were allowed access to water and food ad libitum.

Anesthesia was obtained using a combination of ketamine hydrochloride (0.1–0.2 mg/g) and xylazine (0.02 mg/g), which was administered using intraperitoneal delivery, and further anesthesia was maintained using a combination of ketamine hydrochloride (0.02 mg/g) and xylazine (0.002 mg/g), intraperitoneal delivery. All surgical procedures were performed by B.Y. Chronic renal insufficiency was created by performing a nephrectomy of the right kidney combined with ligation of the upper polar branch of the renal artery to the left kidney (5). In this model, after this procedure, the average blood urea nitrogen and creatinine are significantly elevated 28 days later (5). A carotid artery–to–jugular vein AVF was created 4 weeks later (**Fig 1a–c**) (5,6). The shRNA for *Vegf-A* was purchased from Open Biosystems, Inc (Huntsville, Alabama) and was prepared according to the manufacturer's protocol (7). At the time of AVF creation, 5×10^6 particle-forming units of either LV-shRNA-*Vegf-A* or scrambled-shRNA (control shRNA, nontargeting empty vector) in 5 μL of phosphate-buffered saline was administered to the adventitia of the proximal outflow vein using a 30-gauge needle. The same therapy was repeated 14 days later using a separate surgical procedure (8). Previously, experiments conducted using this technique showed that venous stenosis forms in this

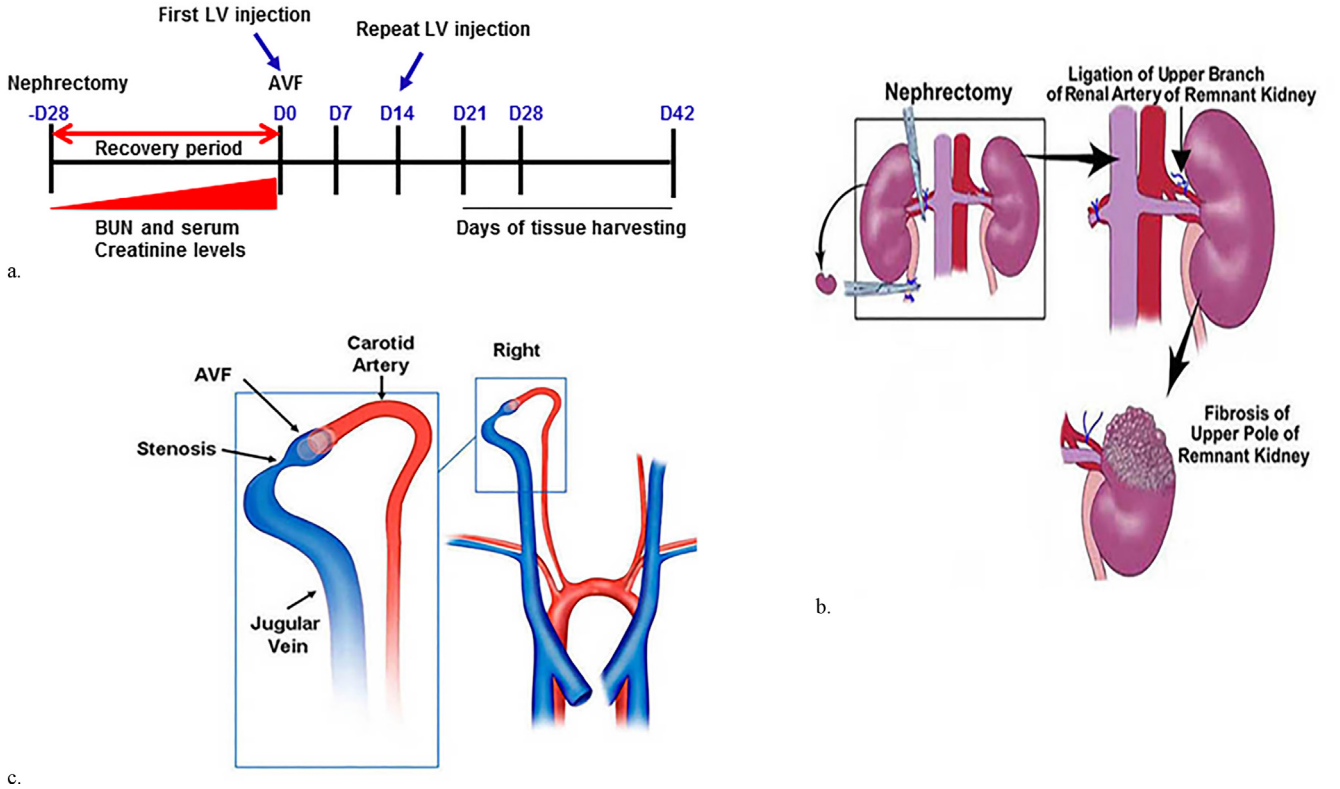

Figure 1. **(a–c)** Schematic of the study. BUN = blood urea nitrogen. (Adapted from Janardhanan R, Yang B, Vohra P, et al. Simvastatin reduces venous stenosis formation in a murine hemodialysis vascular access model. Kidney Int 2013; 84:338–352 [10].)