Video Article

Intraluminal Drug Delivery to the Mouse Arteriovenous Fistula Endothelium

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URL: https://www.jove.com/video/53905
DOI: doi:10.3791/53905

Keywords: Medicine, Issue 109, vascular biology, medicine, arteriovenous fistula, aorta, inferior vena cava, aorto-caval fistula, mouse, animal model, drug delivery, surgical techniques

Date Published: 3/4/2016

Citation: Hashimoto, T., Yamamoto, K., Foster, T., Bai, H., Shigematsu, K., Dardik, A. Intraluminal Drug Delivery to the Mouse Arteriovenous Fistula Endothelium. *J. Vis. Exp.* (109), e53905, doi:10.3791/53905 (2016).

Abstract

Delivery of therapeutic agents to enhance arteriovenous fistula (AVF) maturation can be administered either via intraluminal or external routes. The simple murine AVF model was combined with intraluminal administration of drug solution to the venous endothelium at the same time as fistula creation. Technical aspects of this model are discussed. Under general anesthesia, an abdominal incision is made and the aorta and inferior vena cava (IVC) are exposed. The infra-renal aorta and IVC are dissected for clamping. After proximal and distal clamping, the puncture site is exposed and a 25 G needle is used to puncture both walls of the aorta and into the IVC. Immediately after the puncture, a reporter gene-expressing viral vector was infused in the IVC via the same needle, followed by 15 min of incubation. The intraluminal administration method enabled more robust viral gene delivery to the venous endothelium compared to administration by the external route. This novel method of delivery will facilitate studies that explore the role of the endothelium in AVF maturation and enable intraluminal drug delivery at the time of surgical operation.

Video Link

The video component of this article can be found at https://www.jove.com/video/53905/

Introduction

The murine aortovenous fistula (AVF) puncture model between the aorta and the inferior vena cava (IVC) is now an established technique.[1] In this model, both walls of the infra-renal aorta are punctured with a 25 G needle, exiting into the adjoining infra-renal vena cava; the anterior aortic entrance hole is repaired with simple compression, and does not require suture repair. Serial follow-up examination by high-resolution Doppler ultrasound and histological analysis shows the AVF to have a maturation phase and then a failing phase, recapitulating the known pathophysiology of human AVF.[2]

To explore mechanisms that modulate AVF maturation, improved methods for delivery of therapeutic agents to the maturing AVF endothelium are needed. Delivery of therapeutic agents to vessels can be either via endovascular delivery to the lumen, or via external delivery to the adventitia. One example of external delivery is the commonly used adventitial application of Pluronic gel. This copolymer is thermo-reversible and transformed from liquid to solid gel when warmed to body temperature. Prior studies have shown sustained drug delivery is achieved when drug mixed in pluronic gel is applied topically *in vivo*.[3,4] Adventitial application of viral vectors or siRNA with Pluronic gel has been reported to be effective as a perivascular delivery system.[5,6] We have also reported that treatment of explanted human saphenous veins with adventitial stimulation by peptides resulted in phosphorylation of endothelial receptor proteins.[7]

On the other hand, investigators have also used intraluminal delivery of both viral and nonviral vectors in canine[8-10] and rabbit[11,12] models of vein grafts. In these reports, gene transfer was performed *ex vivo* after vein harvest. Eslami *et al.* reported endovascular viral gene delivery to carotid veins *in situ* without creating a bypass.[13] Gloverman et al. reported intraluminal and adventitial delivery of naked DNA in rat femoral artery-superficial epigastric vein fistulae.[14] The Mayo group reported adventitial drug delivery in mouse carotid artery-jugular vein fistulae.[15,16] However, these previously reported models required a sutured anastomosis to create the AVF. In this report, intraluminal drug delivery with simultaneous AVF creation in mice is described, using a suture-less model of AVF creation. By using this modified murine AVF model a simple method for intraluminal drug delivery to the venous limb of the fistula can be performed.

Protocol

Approval by the appropriate Institutional Animal Care and Use Committee is obtained.

1. Anesthesia and Pre-operative Procedures

1. Anesthetize male C57Bl/6 ice, aged 8 weeks, with vaporized 3% isoflurane and 0.8 L/min oxygen administered into an acrylic induction chamber.
2. Confirm adequate anesthesia by lack of reaction to toe pinch. Position the mouse supine on the operation table and position a silicone mask to deliver vaporized 2 - 3% isoflurane by continuous inhalation.
3. Remove ventral hair from the neck to lower abdomen using a chemical depilatory cream.
4. Perform Doppler ultrasound examination prior to AVF surgery to record baseline characteristics of arterial and venous flow and vessel diameter at the areas of interest.[1,2]
5. Attach a 1 ml syringe to a 25 G needle and load the syringe with the desired drug. Bend the needle to a 60 degree angle approximately 4 mm from the needle tip. Grasp the 25 G needle with a curved needle holder.

2. Operative Procedures

1. Prepare the incision site with a topical antiseptic and apply a surgical drape. Use sterile gloves and instruments to maintain aseptic technique throughout the surgery.
2. Make a midline abdominal incision with a scalpel extending from the level of the lower liver edge to just above the pubis.
3. Insert a retractor and eviscerate all bowels from the abdominal cavity toward the right side. Wrap the bowels in gauze soaked with saline. Dissect the membrane connecting the retro-peritoneum and lower colon to obtain full view of the aorta and IVC.
4. Dissect the infra-renal aorta and IVC from surrounding tissues, preparing for proximal and distal clamping.
5. Place a single microsurgery clip across both the proximal aorta and the proximal IVC at the level just below the left renal vein. Place a second microsurgery clip across both the distal aorta and the distal IVC.
6. Grasp the connective tissue surrounding the aorta and rotate medially so that the dorsal surface of the aorta is slightly exposed for the arterial puncture, as described previously.[1]
7. Quickly expose the puncture site. The puncture site will be at the caudal aspect of the vessels, approximately three quarters of the distance from the left renal vein to the aortic bifurcation. Keeping the aorta in a rotated position with the left hand, dissect the left lateral margin of the aorta so that there is ample exposure to allow puncture with the right hand. Be careful not to dissect between the aorta and the IVC.
8. Maintain the aorta in a rotated position and puncture the aorta through into the IVC using a 25 G needle with an attached 1 ml syringe containing drug solution. (**Figure 1A**)
9. Infuse the drug solution (100 - 200 µl) using the left hand. The needle can be seen through the dilated and thin IVC wall when the transparent drug solution displaces the venous blood out of the IVC (**Figure 1B,C**). Remain still and maintain the needle in position for 15 min.
10. Remove the distal microsurgery clip to de-clamp only the distal aorta and the distal IVC.
11. Remove the needle and then cover the puncture site of the aorta by pulling up adjacent retro-peritoneal tissue.
12. Remove the proximal microsurgery clip to de-clamp the proximal aorta and the proximal IVC. Upon de-clamping, arterial blood is observed flowing into the IVC instead of dark venous blood flow. Keep covering the puncture hole for 1 min.
13. After confirmation of hemostasis by observation for 30 sec without compression, return the bowels into their natural position and close the abdomen with a running suture according to your approved animal protocol.

3. Post-operative Procedures

1. After closure of the abdomen, discontinue anesthesia. Apply post-operative care including analgesia and wound care in accordance with instructions recommended by the Institutional Animal Care and Use Committee. For analgesia we use buprenorphine at 0.1 mg/kg intrasmuscularly every 12 hr for 24 hr following the surgical procedures.
2. On the first day after operation, perform Doppler ultrasound to confirm patency of the AVF. In addition, measure other vessel and flow characteristics serially and compare for changes from pre-operative baseline values.[1,2]

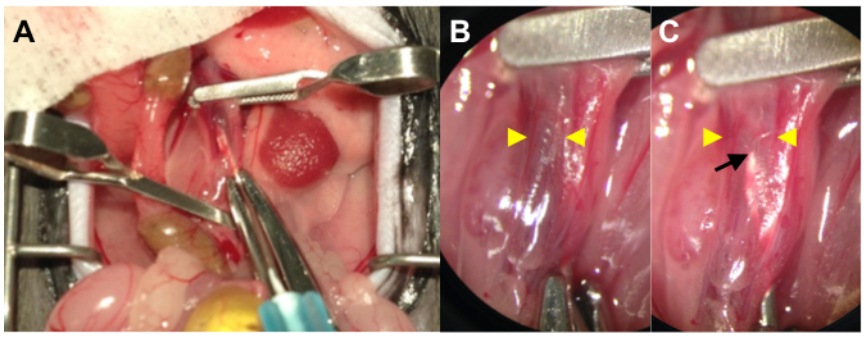

Figure 1. (A) Operative Photo Showing Intraluminal Delivery during AVF Surgery. Clamp the proximal and distal aorta, as well as the IVC by applying microsurgery clips. Puncture the aorta through into the IVC using a 25 G needle with attached syringe containing drug solution. **(B)** A Higher Power Picture (4X magnification) of the Punctured IVC before Infusion. The needle tip is obscured by dark colored venous blood. Yellow arrowheads denote the wall-to-wall diameter of the IVC. **(C)** A Higher Power Picture (4X magnification) of the Punctured IVC after Infusion. The needle tip (black arrow) can be seen through the gently distended and thin IVC wall (yellow arrowheads) as the transparent drug solution displaces the venous blood.

Representative Results

In a series of 33 mice, survival on the first post-operative day was 97.0%; AVF patency, as determined by ultrasound, was 84.9%.

Gene transduction efficiency of this endovascular delivery route with the traditional external route was compared. For intraluminal delivery (ILD), immediately after the puncture, 200 µl of Adenovirus-GFP (Ad-GFP) vector solution (1×10^9 PFU/ml) was infused into the IVC via the puncturing needle, followed by 15 min incubation time. Control mice received adventitial delivery (AD) of Ad-GFP (1×10^9 PFU/ml) to the IVC using Pluronic gel. Specimens were explanted at 24 hr after exposure to adenovirus vectors. Expression of GFP at the IVC wall and the fistula was assessed by en-face observation from the intima by fluorescence microscopy, as well as histologic examination in sections using an anti-GFP antibody.

En face observation showed stronger expression of GFP in the IVC wall in the mice treated with ILD compared with AD, with characteristic augmentation around the fistula at 24 hr (**Figure 2A**). GFP expression was persistent in both groups at 72 hr after transfection (data not shown). Histological analysis with anti-GFP antibody showed more robust and site-specific GFP expression in the intima of the IVC in the mice treated with ILD compared with AD. (**Figure 2B**). To confirm these results, infra-renal IVCs treated with adenovirus-GFP by AD or ILD were harvested for Western blot. GFP protein expression was sufficient for detection only after ILD, suggesting superior viral drug delivery to the IVC wall via the intraluminal route at 24 hr after transfection.

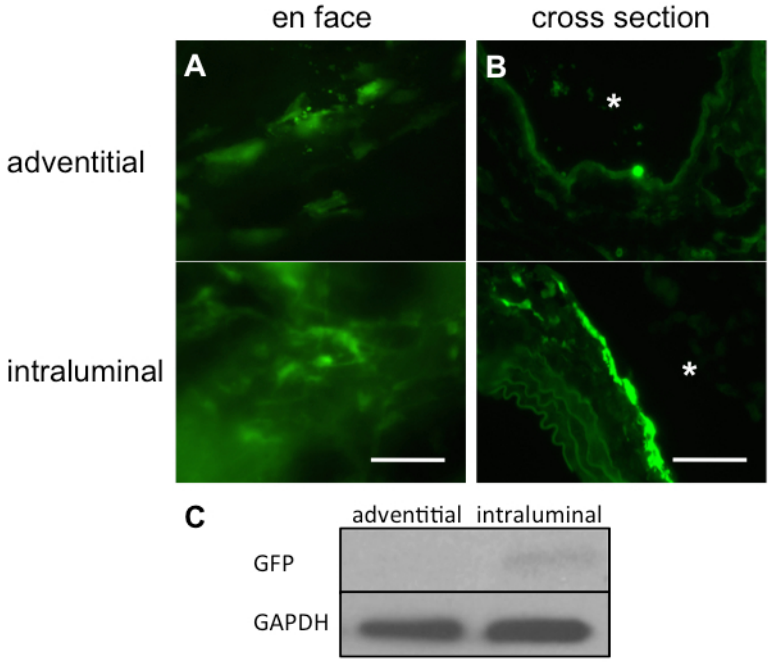

Figure 2. Delivery of Adenovirus-GFP to the AVF Lumen Enhances GFP Expression. (A) Representative immunofluorescence showing en face view of the AVF endothelium (24 hr) in mice receiving adenovirus-GFP applied by adventitial delivery (top) or by intraluminal delivery (bottom). Bar indicates 25 µm. **(B)** Representative immunofluorescence showing sections of the AVF endothelium (24 hr) in mice receiving adenovirus-GFP applied by adventitial delivery (top) or by intraluminal delivery (bottom). * denotes lumen of the IVC; bar indicates 25 µm. **(C)** Representative Western blot showing detection of GFP in AVF treated with adenovirus-GFP applied via the adventitia or intraluminal route.

Discussion

This modification of the murine AVF model incorporates intraluminal drug delivery to the venous endothelium at the time of AVF creation. An AVF was created by puncturing the infra-renal aorta with a 25 G needle and extending the puncture through the opposite aortic wall into the IVC, followed by injection of drug solution through the same needle. The solution is maintained intra-caval, *i.e.*, in the venous limb of AVF, until de-clamping. What distinguishes this model from other murine AVF models[17-19] is the lack of sutures or glues that may cause stenosis, acute thrombosis, and possible interference with molecular analysis. The ability to use the same needle that punctures the aorta and IVC to create the AVF to also deliver drugs to the AVF lumen allows simpler delivery with minimal vessel manipulation.

There are some critical steps and points to improve the success rate and consistency of the procedure. The proximal clamp of the aorta and IVC is placed below the left renal vein. If there are large lumbar veins in the upper portion of the infra-renal IVC, then modification of the clamping site to below these vessels is recommended to allow efficient incubation of drug without dilution by retrograde back-bleeding from lumbar veins. The distal clamp of the aorta and IVC is placed at the level of iliac bifurcation, lest it may interfere with the needle puncture of the distal aorta and IVC. Holding the 25 G needle with a curved needle holder enables optimal direction of the needle during vessel puncture. Hemostasis is achieved by covering the arterial entry hole with the adjacent retro-peritoneum. Special attention is paid to provide careful compression while avoiding AVF occlusion and thrombosis as described previously.[1]

The development of gene transfer technology offers the potential to modify surgical results at the molecular level. Reports describing intraluminal gene delivery to a vein have mainly focused on targeted treatment of vein graft failure. In contrast, this model allows for drug delivery for modulation of AVF maturation. Advantages of this model include the use of a murine model allowing examination of varying mouse genetic strains. In addition, a 15-min intraluminal exposure protocol is easily transferable to clinical intra-operative settings and may help assess peri-procedural interventions to prevent juxta-anastomotic stenosis that commonly complicates human AVF surgery. From these initial results, we show that this method yields more site-specific gene expression within the endothelium compared to the traditional external route.

A potential limitation of this study is the effect of variable distension pressure on the IVC. Isolation of the IVC segment is not perfect in this mouse model, *i.e.*, side branches (lumbar veins) were not ligated. Therefore, it is difficult to precisely maintain a consistent level of distension pressure during incubation. Although the IVC is gently distended by manual infusion, higher distending pressures may cause neointimal hyperplasia[20,21] and direct smooth muscle cell damage,[11] leading to interference in analysis. It is also difficult to completely quantitate the efficiency of drug delivery, as some drug may be lost during delivery through the lumbar veins, as well as after clamp removal through the proximal IVC and potentially through the AVF into the aorta. Lastly, this method allows some of the drug to be released systemically after clamp removal; evacuation of the drug and flushing of the site might prevent systemic release, but quantification of the degree of drug washout would need to be confirmed.

In conclusion, this technical modification to the murine AVF model is relatively simple and reproducible and enables targeted endothelial drug delivery to the mouse AVF. Intraluminal injection at the time of AVF creation is technically feasible and can successfully improve viral gene expression in the endothelium compared to adventitial delivery. This AVF-drug delivery model will be a useful modality not only for dissection of the mechanisms that regulate venous AVF limb maturation, but also for therapeutic modulation of AVF maturation.

Disclosures

The authors have nothing to disclose.

Acknowledgements

This work was supported in part by the United States Department of Veterans Affairs Biomedical Laboratory Research and Development Program Merit Review Award I01-BX002336, the National Institute of Health grant R56-HL095498, as well as with the resources and the use of facilities at the VA Connecticut Healthcare System, West Haven, CT.

References

1. Yamamoto, K., Li, X., Shu, C., Miyata, T., & Dardik, A. Technical Aspects of the Mouse Aortocaval Fistula. *J. Vis. Exp.* (77), e50449 (2013).
2. Yamamoto, K., *et al.* The mouse aortocaval fistula recapitulates human arteriovenous fistula maturation. *Am. J. Physiol.: Heart Circ. Physiol.* **305** (12), H1718-H1725 (2013).
3. Escobar-Chávez, J. J., Lòpez-Cervantes, M., Naïk, A., Kalia, Y. N., Quintanar-Guerrero, D., & Ganem-Quintanar, A. Applications of thermo-reversible pluronic F-127 gels in pharmaceutical formulations. *J. Pharm. Pharm. Sci.* **9** (3), 339-358 (2006).
4. Almeida, H., Amaral, M. H., Lobão, P., & Lobo, J. M. S. Pluronic® F-127 and Pluronic Lecithin Organogel (PLO): main features and their applications in topical and transdermal administration of drugs. *J. Pharm. Pharm. Sci.* **15** (4), 592-605 (2012).
5. Karper, J. C., *et al.* Toll-like receptor 4 is involved in human and mouse vein graft remodeling, and local gene silencing reduces vein graft disease in hypercholesterolemic APOE*3Leiden mice. *Arterioscler., Thromb., Vasc. Biol.* **31** (5), 1033-1040 (2011).
6. Redmond, E. M., Hamm, K., Cullen, J. P., Hatch, E., Cahill, P. A., & Morrow, D. Inhibition of patched-1 prevents injury-induced neointimal hyperplasia. *Arterioscler., Thromb., Vasc. Biol.* **33** (8), 1960-1964 (2013).
7. Wong, D. J., *et al.* Ephrin type-B receptor 4 activation reduces neointimal hyperplasia in human saphenous vein *in vitro*. *J. Vasc. Surg.* (2014).
8. Matsumoto, T., *et al.* Hemagglutinating virus of Japan-liposome-mediated gene transfer of endothelial cell nitric oxide synthase inhibits intimal hyperplasia of canine vein grafts under conditions of poor runoff. *J. Vasc. Surg.* **27** (1), 135-144 (1998).
9. Petrofski, J. A., *et al.* Gene delivery to aortocoronary saphenous vein grafts in a large animal model of intimal hyperplasia. *J. Thorac. Cardiovasc. Surg.* **127** (1), 27-33 (2004).
10. Hata, J. A., *et al.* Modulation of phosphatidylinositol 3-kinase signaling reduces intimal hyperplasia in aortocoronary saphenous vein grafts. *J. Thorac. Cardiovasc. Surg.* **129** (6), 1405-1413 (2005).
11. Ohta, S., *et al.* Intraluminal gene transfer of endothelial cell-nitric oxide synthase suppresses intimal hyperplasia of vein grafts in cholesterol-fed rabbit: a limited biological effect as a result of the loss of medial smooth muscle cells. *Surg.* **131** (6), 644-653 (2002).
12. Baldwin, Z. K., *et al.* Modulation of vascular remodeling induced by a brief intraluminal exposure to the recombinant R7020 strain of Herpes simplex-1. *J. Vasc. Surg.* **41** (1), 115-121 (2005).
13. Eslami, M. H., *et al.* Gene delivery to *in situ* veins: differential effects of adenovirus and adeno-associated viral vectors. *J. Vasc. Surg.* **31** (6), 1149-1159 (2000).
14. Globerman, A. S., *et al.* Efficient transgene expression from naked DNA delivered into an arterio-venous fistula model for kidney dialysis. *J. Gene Med.* **13** (11), 611-621 (2011).
15. Yang, B., *et al.* Adventitial transduction of lentivirus-shRNA-VEGF-A in arteriovenous fistula reduces venous stenosis formation. *Kidney Int.* **85** (2), 289-306 (2014).
16. Brahmbhatt, A., *et al.* The role of Iex-1 in the pathogenesis of venous neointimal hyperplasia associated with hemodialysis arteriovenous fistula. *PLoS ONE* **9** (7), e102542 (2014).
17. Karram, T., *et al.* Induction of cardiac hypertrophy by a controlled reproducible sutureless aortocaval shunt in the mouse. *J. Investig. Surg.* **18** (6), 325-334 (2005).

18. Perry, G. J., *et al.* Genetic variation in angiotensin-converting enzyme does not prevent development of cardiac hypertrophy or upregulation of angiotensin II in response to aortocaval fistula. *Circ.* **103** (7), 1012-1016 (2001).
19. Guzman, R. J., Krystkowiak, A., & Zarins, C. K. Early and sustained medial cell activation after aortocaval fistula creation in mice. *J. Surg. Res.* **108** (1), 112-121 (2002).
20. Alexander, J. H., *et al.* Efficacy and safety of edifoligide, an E2F transcription factor decoy, for prevention of vein graft failure following coronary artery bypass graft surgery: PREVENT IV: a randomized controlled trial. *JAMA* **294** (19), 2446-2454 (2005).
21. Khaleel, M. S., *et al.* High-pressure distention of the saphenous vein during preparation results in increased markers of inflammation: a potential mechanism for graft failure. *Ann. thorac. surg.* **93** (2), 552-558 (2012).

