Video Article

Intravascular Delivery of Biologics to the Rat Kidney

Federico Franchi[1], Xiang Yang Zhu[2], Tyra A. Witt[1], Lilach O. Lerman[2], Martin Rodriguez-Porcel[1]

[1]Divisions of Cardiovascular Diseases, Department of Internal Medicine, Mayo Clinic

[2]Divisions of Nephrology and Hypertension, Department of Internal Medicine, Mayo Clinic

Correspondence to: Martin Rodriguez-Porcel at rodriguez.m@mayo.edu

Abstract

The renal microvascular compartment plays an important role in the progression of kidney disease and hypertension, leading to the development of End Stage Renal Disease with high risk of death for cardiovascular events. Moreover, recent clinical studies have shown that renovascular structure and function may have a great impact on functional renal recovery after surgery. Here, we describe a protocol for the delivery of drugs into the renal artery of rats. This procedure offers significant advantages over the frequently used systemic administration as it may allow a more localized therapeutic effect. In addition, the use of rodents in pharmacodynamic analysis of preclinical studies may be cost effective, paving the way for the design of translational experiments in larger animal models. Using this technique, infusion of rat recombinant Vascular Endothelial Growth Factor (VEGF) protein in rats has induced activation of VEGF signaling as shown by increased expression of FLK1, pAKT/AKT, pERK/ERK. In summary, we established a protocol for the intrarenal delivery of drugs in rats, which is simple and highly reproducible.

Video Link

The video component of this article can be found at https://www.jove.com/video/54418/

Introduction

The renal microvasculature is involved in a wide spectrum of kidney diseases. Depending on the pathophysiology of disease, the endothelial cells may present structural or functional impairment, which may play a pivotal role in propagating kidney damage by creating an ischemic microenvironment. This renal microvascular dysfunction may catalyze the onset of a progressive deterioration of renal function over time, leading to chronic kidney disease (CKD), end-stage renal disease, hypertension and cardiorenal syndrome. In fact, untreated hypertension may have implications in renal arterioles, causing nephrosclerosis or glomerulosclerosis with significant reduction in vascular volume fraction, increase in vascular resistance and development of tubulointerstitial fibrosis[1].

Loss of renal microvasculature may be due to altered vascular homeostasis induced by local angiogenic/anti-angiogenic factors imbalance. This correlates with attenuated Vascular Endothelial Growth Factor (VEGF) signaling as well as elevated thrombospondin-1[2-4]. Thus, using different animal models (mice, rats and pigs), the therapeutic effect of exogenous administration of VEGF has been recently investigated in some forms of renal disease, showing reduced interstitial fibrosis and stabilized renal and cardiac function[3-5]. This effect is likely due to actions of VEGF on endothelial cells of the microvascular bed and inflammatory monocyte phenotype switching[6].

For some preclinical studies, the use of rodents, the most commonly used laboratory animals, provides a good animal model for high throughput studies due to relatively low costs and ease of handling. Moreover, the use of genetically-altered rats as models of human diseases, such as hypertension, has become more and more frequent in the scientific community. Therefore, the aim of this protocol is to describe a useful intrarenal VEGF delivery technique in rats that is easy to perform and highly reproducible. Moreover, the same method can be used to selectively deliver other drugs.

Protocol

The experiments were performed on female Sprague-Dawley rats, weighing 250-300 g. All animal procedures complied with the standards stated in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy of Sciences, Bethesda, MD, USA) and were approved by the Mayo Clinic College of Medicine Institutional Animal Care and Use Committee (IACUC).

1. Preparation

1. Autoclave all surgical instruments before surgery. If multiple surgeries on different rats are planned in the same day, rinse instruments after each animal procedure and then sterilize using a hot bead sterilizer.
2. Anesthetize the rat with 4% isoflurane in 1 L/min O_2.

3. Transfer the rat to a controlled heating pad to preserve the body temperature at 37 °C. Maintain anesthesia with 1-2% isoflurane in 1 L/min O_2.
4. Administer the analgesic drug (Buprenorphine Sustained Release 0.6 mg/kg) subcutaneously.
5. Apply ointment to the eyes to prevent drying during the procedure.
6. In order to compensate for loss of body fluids due to laparotomy, it is important to administer 10 ml/kg of 0.9% normal saline subcutaneously preoperatively.
7. Shave the abdominal area and clean the skin with povidone-iodine and 70% ethanol pads.

2. Surgical Procedure

1. Ensure that the depth of sedation is adequate by monitoring physical reflexes, such as withdrawal from toe pinch, palpebral reflex, jaw tone, and respiration rate/pattern.
2. Perform a laparotomy through a small midline incision (2-2.5 cm in length) using a surgical scalpel blade No. 10.
3. Pull the intestine and colon to the right side of the abdomen by using cotton swabs and cover them with sterile gauze soaked in to 0.9% normal saline to maintain the organs moist.
4. Gently retract upward the spleen, liver, stomach and pancreas to expose the aorta and the left kidney artery.
5. With the help of a surgical microscope, carefully separate the abdominal aorta above and below the left kidney and the left renal artery from the veins, the fat and the surrounding connective tissue with blunt dissecting curved forceps and sterile cotton swabs.
    1. Use the forceps with a repeated open-close motion (blunt dissection) along the length of the vessels to remove the connective tissue and the cotton swabs with a lateral rolling motion to remove the fat.
    NOTE: The dissection of the peri-aortic region is a very delicate step as nerves and lymphatic vessels might be damaged. Make sure to keep the arteries moist with saline during the dissection procedure.

6. Place a 4-0 silk suture underneath the aorta.
7. Using microvascular clips, clamp the aorta above (just below the superior mesenteric artery) and below the renal artery bifurcation.
8. Puncture the aorta at the level of the left kidney artery bifurcation with a 24 G intravenous catheter and advance the catheter into the renal artery.
    NOTE: this is a critical step as puncture through the renal artery may occur.
9. Connect a syringe filled with the drug solution or saline (up to 500 μl) to the catheter and perfuse the kidney.
10. Immediately after perfusion, clamp the left renal vein and the left ureter with a microvascular clip and remove the catheter. Then, place a piece of absorbable hemostat gelatin sponge, with a small drop of tissue adhesive, over the punctured area of the aorta and gently apply pressure with a cotton swab.
11. At the same time, release the clamp from the abdominal aorta, below the left renal artery bifurcation. After 5 min, release the clamp from renal vein and ureter.
12. Carefully release the clamp from the aorta, above the left renal artery bifurcation, and allow for kidney reperfusion. The total renal ischemia should last no longer than 7 min.
13. Ensure that no active bleeding occurs and closely observe the area for 10 more min.
14. Close the abdominal incision in two layers (muscle and skin), using 4-0 absorbable sutures and a continuous pattern to prevent infection. In addition to the continuous pattern suture technique, another option would be to use a simple, interrupted technique, especially for body wall closure to prevent dehiscence.
15. Apply topical antibiotic ointment over the incision area to prevent infections.
16. Transfer the rat into a bedding-free observation cage on a warm pad until complete recovery with a temperature range set at 35-37 °C. Loose bedding should be covered (*e.g.* with a drape or paper towel) or removed from the cage until animals are fully recovered to prevent suffocation or aspiration of bedding.
17. After surgery, observe the animals continuously until breathing spontaneously, then hourly for a few hours. Re-dose the analgesic Buprenorphine SR 72 hr later if signs of discomfort are observed, such as lethargy, hunched and scruffy, grimace, not resuming normal activities.
18. After completion of all studies, euthanize animals with the inhalation of an overdose of CO_2 and harvest the renal tissues for *ex vivo* analyses such as histology and Western Blotting[5].

Representative Results

We injected two different doses of recombinant rat VEGF (rrVEGF, 0.17 μg/kg and 5 μg/kg) or PBS. The animals were euthanized 8 hr post-surgery to examine the activation of the VEGF pathway. The surgical procedure did not affect the morphology of the perfused kidney (**Figure 1A**) when compared to control (**Figure 1B**), as shown by H&E staining. While Sirius red staining did not show any increase in extracellular matrix deposition in response to the ischemic time and the infusion of the rrVEGF (**Figure 1C**) when compared to control (**Figure 1D**). By Western Blot analysis, we observed a small but significant increase in the expression of proteins involved in the VEGF pathway, such as VEGF, FLK1, pAKT/AKT, pERK/ERK (**Figure 2**).
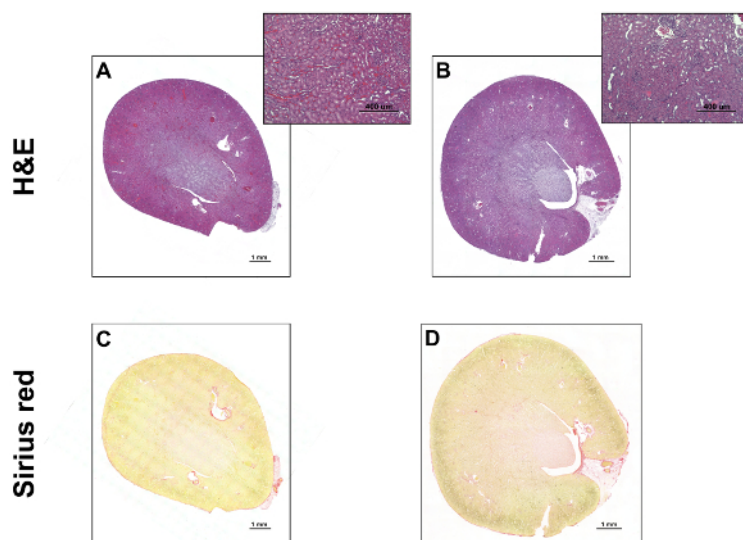
Figure 1: Representative Images of Histological Staining of Renal Tissue. Hematoxilin and Eosin (H&E) staining of kidney sections shows no anatomical changes in rats that received VEGF (5 µg/kg, **B**) compared to sham animals (**A**) via intrarenal injection. Whereas, sirius red staining shows no significant differences in collagen deposition in rats that received VEGF (5 µg/kg, **D**) compared to sham animals (**C**). Scale bar represents 1 mm and magnified images represent 400 µm. Please click here to view a larger version of this figure.
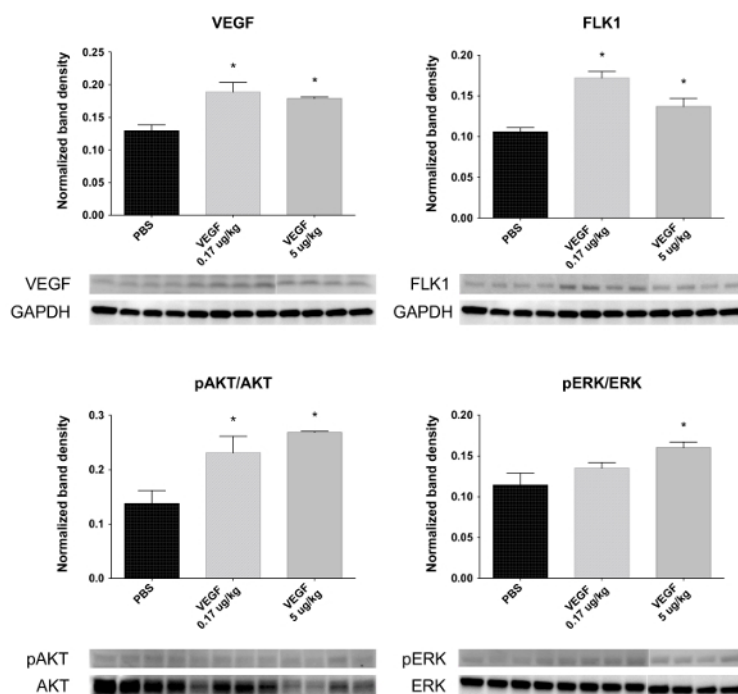

Figure 2: Activation of VEGF Pathway. Western Blot analysis of VEGF, FLK1, phosphoAKT/AKT and phosphoERK/ERK showing the upregulation of VEGF pathway after rrVEGF infusion in the kidney (n = 4). Data are presented as mean ± SEM. *$p < 0.05$ vs. PBS. Please click here to view a larger version of this figure.

Discussion

The increasing incidence of chronic kidney disease raises the need for novel therapeutic approaches that can promote functional kidney recovery[7,8]. Traditional therapies include the systemic administration of anti-inflammatory, anti-fibrotic drugs[9]. However, these strategies are frequently characterized by unwanted side effects due to off-target distribution of the injected drug. Therefore, in this manuscript, we describe a simple procedure for delivering drugs into the renal artery of rats. This protocol will be useful for testing drugs in rodent models of hypertension, renal artery stenosis or any disease that simulate clinical renovascular diseases. However, in preclinical studies, the use of small animal models, though convenient and sometimes beneficial, may bear several disadvantages that need to be taken into consideration. Importantly, the small

size of the organs in rodents requires a higher degree of surgical skills, which may be achieved with some practice. Thus, following previously tested critical steps of a surgical technique, may facilitate and aid the learning process.

A pivotal aspect of the procedure described is the dissection of the renal artery from the renal vein and the surrounding adipose/connective tissue. The use of cotton swabs helps avoid damage to nerves and lymphatic and/or blood vessels, in particular to the thin-walled veins. Another important and delicate step is the cannulation of the renal artery: because of the very small diameter of the vessel, the catheter might puncture through the artery. For this reason, working under a surgical microscope and finding a good angle for the insertion of the catheter is fundamental: the needle should be as much as possible parallel to the renal artery. At the same time, the observation of a distinct, slow color change in the kidney, caused by the infusion of the drug suspension, is essential to be certain that the catheter has been positioned correctly.

One of the most important issues that might affect the outcome of this surgical procedure is the duration of the ischemia. A prolonged and complete restriction of blood supply to the kidney may cause serious damage to the renal tissue, characterized by increase of reactive oxygen species (ROS) levels and activation of fibrogenic mechanisms and inflammatory mediators within the renal parenchyma[10-12]. Thus, it is crucial to minimize the time of ischemia in an effort to prevent irreversible interstitial fibrosis as well as cell death. Selective necrosis of the immediate postglomerular portion of a tubular segment sensitive to inhibition of mitochondrial respiration has been previously described after only 15 min of ischemia in rats[13]. Therefore, we recommend that the total renal ischemia should not exceed 7 min in duration.

Finally, for an optimal recovery, it is very important to keep the animal very well hydrated and warm for the entire duration of the surgery and immediately post op, as laparotomy procedures may result in body fluids loss as well as hypothermia, both of which may increase post op mortality.

In this manuscript, we are showing results obtained from healthy rats to demonstrate the feasibility and the safety of the surgical procedure. We believe that a greater up-regulation of the VEGF pathway would be observed in a disease model. Furthermore, it is important to highlight that, although with the described method biologics can be delivered directly into the renal artery, other organs might be affected via venous outflow and subsequent systemic circulation. However, clamping the vein soon after the infusion for about 5 min, should minimize any potential systemic side effect. In conclusion, the protocol described is safe, and useful for large scale drug testing in rat preclinical studies where high throughput is required before translation to more clinically relevant animal models.

Disclosures

References

1. Dejani, H., Eisen, T. D., & Finkelstein, F. O. Revascularization of renal artery stenosis in patients with renal insufficiency. *Am. J. Kidney Dis.* **36** (4), 752-758 (2000).
2. Kang, D. H., *et al.* Impaired angiogenesis in the remnant kidney model: I. Potential role of vascular endothelial growth factor and thrombospondin-1. *J. Am. Soc. Nephrol.* **12** (7), 1434-1447 (2001).
3. Kang, D. H., Hughes, J., Mazzali, M., Schreiner, G. F., & Johnson, R. J. Impaired angiogenesis in the remnant kidney model: II. Vascular endothelial growth factor administration reduces renal fibrosis and stabilizes renal function. *J. Am. Soc. Nephrol.* **12** (7), 1448-1457 (2001).
4. Kang, D.-H., *et al.* Role of the microvascular endothelium in progressive renal disease. *J. Am. Soc. Nephrol.* **13** (3), 806-816 (2002).
5. Chade, A. R., & Kelsen, S. Reversal of renal dysfunction by targeted administration of VEGF into the stenotic kidney: a novel potential therapeutic approach. *Am. J. Physiol.- Renal Physiol.* **302** (10), F1342-50 (2012).
6. Eirin, A., *et al.* Changes in Glomerular Filtration Rate After Renal Revascularization Correlate With Microvascular Hemodynamics and Inflammation in Swine Renal Artery Stenosis. *Circ.-Cardiovasc. Interv.* **5** (5), 720-728 (2012).
7. Chade, A. R. Distinct Renal Injury in Early Atherosclerosis and Renovascular Disease. *Circulation.* **106** (9), 1165-1171 (2002).
8. Seddon, M., & Saw, J. Atherosclerotic renal artery stenosis: review of pathophysiology, clinical trial evidence, and management strategies. *Can. J. Cardiol.* **27** (4), 468-480 (2011).
9. Lao, D., Parasher, P. S., Cho, K. C., & Yeghiazarians, Y. Atherosclerotic renal artery stenosis--diagnosis and treatment. *Mayo Clin. Proc.* **86** (7), 649-657 (2011).
10. Sharfuddin, A. A., & Molitoris, B. A. Pathophysiology of ischemic acute kidney injury. *Nat. Rev. Nephrol.* **7**, 189-200 (2011).
11. Noiri, E., *et al.* Oxidative and nitrosative stress in acute renal ischemia. *Am. J. Physiol.- Renal Physiol.* **281** (5), F948-F957 (2001).
12. Koesters, R., *et al.* Tubular Overexpression of Transforming Growth Factor-β1 Induces Autophagy and Fibrosis but Not Mesenchymal Transition of Renal Epithelial Cells. *Am. J. Pathol.* **177** (2), 632-643 (2010).
13. Shanley, P. F., Rosen, M. D., Brezis, M., Silva, P., Epstein, F. H., & Rosen, S. Topography of focal proximal tubular necrosis after ischemia with reflow in the rat kidney. *Am. J. Pathol.* **122** (3), 462-468 (1986).