T400-Series Surgical Protocol

Mouse Renal Artery: Acute and Chronic Blood Flow Measurement

APPLICATION BASICS

Site:	Renal artery
Species:	Mouse
Body Weight:	20 - 50 grams
Duration:	Chronic
Vessel Diameter:	0.35 - 0.55 mm
Length:	0.25 mm

PROBE

Size:	0.5 mm
Reflector:	JN
Connector:	4-pin
Cable Length:	6 cm
Catalog #:	MC-0.5PSL-JN-WC06-CA4S-GC

FLOWMETER

TS420 Perivascular Module

Measurement of Renal Arterial Blood Flow in the Mouse Protocol©
Courtesy of Michael F. Callahan, Dept. of Orthopaedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC.

For the complete application PowerPoint presentation see: www.transonic.com

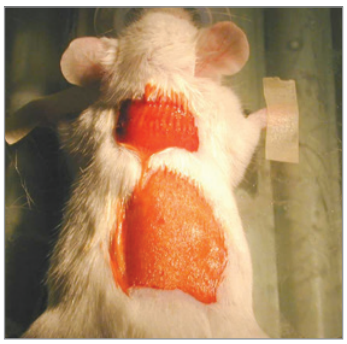

Fig. 1: Shaved, prone mouse on a warming pad prepared for surgery.

Preparation

1. The mouse is prepared for renal Flowprobe implantation by placement in a prone position on a warming pad to maintain core temperature (Fig. 1).
2. The surgical site is shaved and prepared with Betadine. Note: the midscapular area is also shaved and prepared for exteriorizing the Probe connector.
3. The mouse is anesthetized with a mixture of ketamine:xylazine (50:10 mg/kg). For the duration of the implantation procedure, the mouse is anesthetized with inhaled isoflurane (1 - 2%).

Fig. 2: Make the incision 1 cm lateral to back midline. Cut through the skeletal muscle to the hilus of the kidney.

Surgical Implantation of Flowprobe

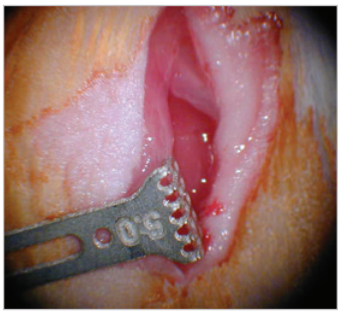

Fig. 3: Place a miniature retractor at the base of the incision to reveal the kidney beneath the muscle layer.

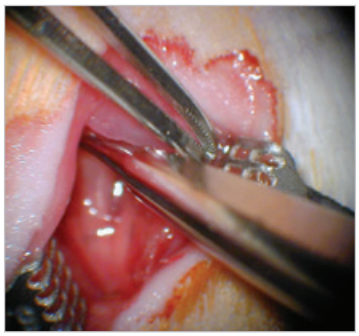

Fig. 4: Extend incision to see the renal artery. Place a second retractor and pack the kidney with gauze to keep it out of the surgical field.

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Mouse Renal Artery: Acute and Chronic Blood Flow Measurement Cont.

Surgical Implantation of Flowprobe cont.

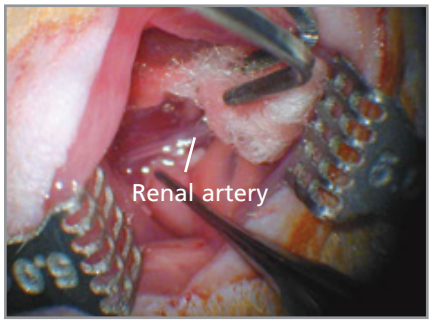

Fig. 5: Here the renal artery lies on top of the delicate renal vein. With a ventral laparotomy, the renal artery lies under-neath the vein making for a much more difficult dissection.

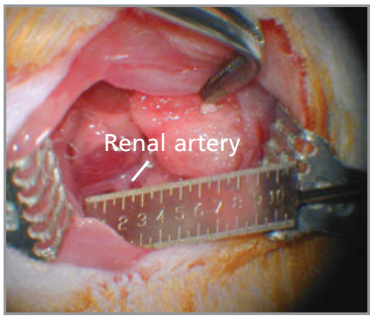

Fig. 6: This renal artery length is approximately 1.75 mm between branches, just adequate for the 0.5PSB Flowprobe.

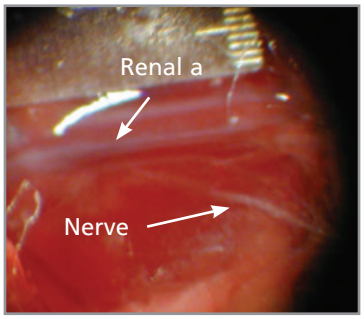

Fig. 7: Under increased magnification, the diameter of the renal artery is 0.35 mm. Note the nerve below the artery and take care to avoid damage to the nerve during dissection.

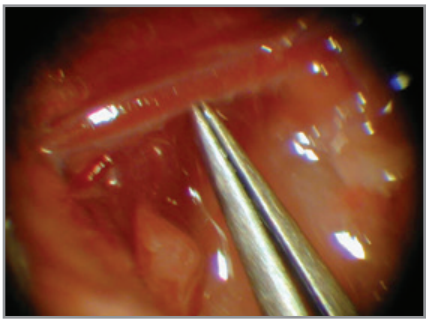

Fig. 8: Separate the renal artery from the renal vein by carefully grabbing the adventitia of the artery using very fine Dumont vessel dilators (D-5aZ) or by carefully passing microblunted 45° Dumont forceps under the vessel as shown.

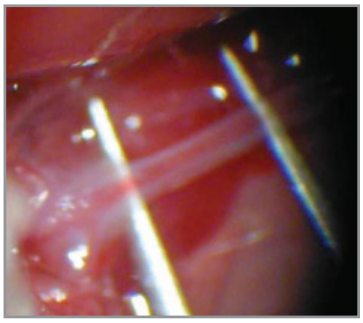

Fig. 9: Apply slight pressure against the renal artery to allow the 45° Dumont forceps to spread and dissect the adventitia away from the artery itself. Do not apply any kind of dissecting force against the renal vein.

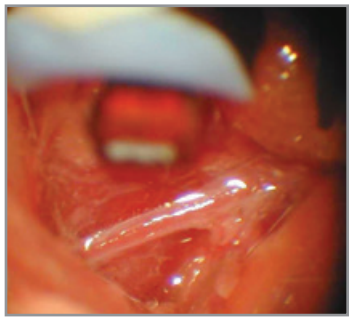

Fig. 10: Carefully go around the renal artery until a long enough segment is freed from the underlying vein to accommodate the Flowprobe reflector.

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Mouse Renal Artery: Acute and Chronic Blood Flow Measurement Cont.

Acute Measurement Protocol

Flow measurements may be recorded for acute studies by using acoustic coupling gel (SurgiLube) to displace the air in the Flowprobe. Use a blunt tipped syringe or angiocath to insert the gel, being careful not to impact the delicate Probe reflector.

Connect the MA-0.5PSB or MA-0.5PSL Probe to the TS420 Flow Module and check that the "Test" signal indicates "Good." A "Low" signal may indicate an air bubble. Select "Measure" to record flow.

Do not grasp the Probe by its fragile reflector.

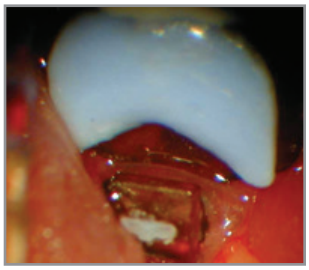
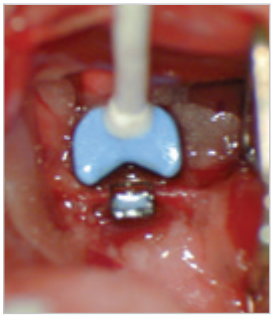


Fig. 11: Place the Flowprobe around the vessel so that the reflector hook is not tugging on or deforming the vessel and the Probe head is not placing pressure on or blanching the kidney. The vessel may be gently lifted into the Flowprobe with blunted D5aZ forceps.

Acute style MA-0.5PSB Probe. For acute experiments Nanoprobes are configured with a handle for easy maneuvering and stabilization.

Chronic Measurement Protocol



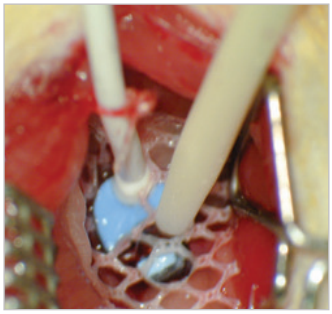


Fig. 12: The MC-0.5PSL lateral cable configuration is preferred for chronic implant. The Probe is maneuvered by gently grasping the blue Probe body with D5aZ or curved forceps. Position the Probe with the cable rostrally, so the Probe is "floating" on the renal a. Anchor the cable with a suture to the psoas muscle. A 3/4" square of Mersilene (dacron) mesh is placed over the Probe and acoustic gel is deposited into the lumen of the Probe to provide a barrier before placing the sealing agent into the retroperitoneal space. The ultrasonic pathway must remain unobstructed. This can be checked by monitoring the Probe test signal on the Flowmeter.

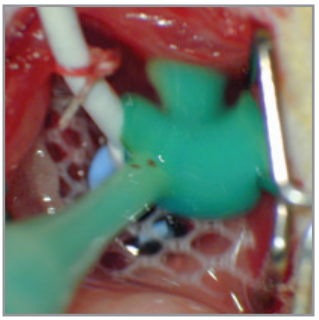


Fig. 13: Inject Kwik-Sil into the retroperitoneal cavity beginning at the corner and around the Probe to hold the Probe, cable and kidney in place.

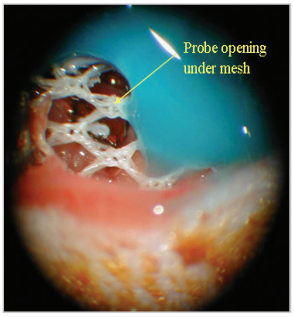


Fig. 14: Kwik-Cast is shown. Kwik-Sil has a shorter cure time (1 minute) and is less likely to obstruct the ultrasound path, which will block the ultrasound signal, and prevent flow measurement.

Kwik-Sil (clear) and Kwik-Cast (blue) are both 2-part agents used to attach electrodes in nerve recording.

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Mouse Renal Artery: Acute and Chronic Blood Flow Measurement Cont.

Chronic Measurement Protocol cont.

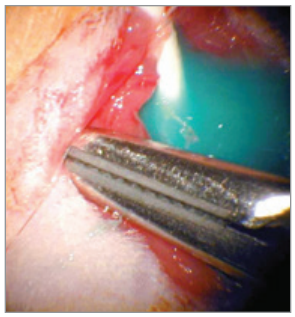


Fig. 15: Allow the cast to harden before moving the tissues. Anchor the Probe cable to the psoas major muscle with 5-0 Vicryl suture.

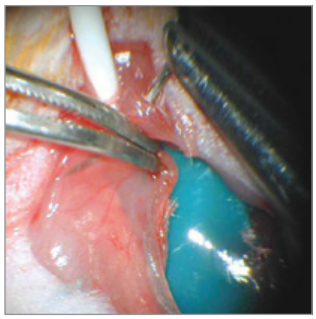
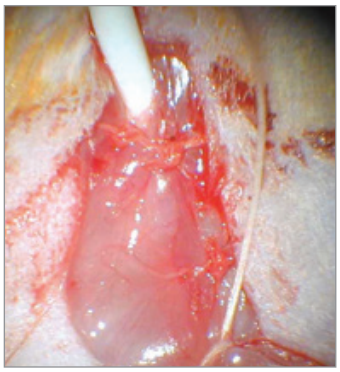


Fig. 16: Continue to close the skeletal muscles over the renal Flowprobe preparation.



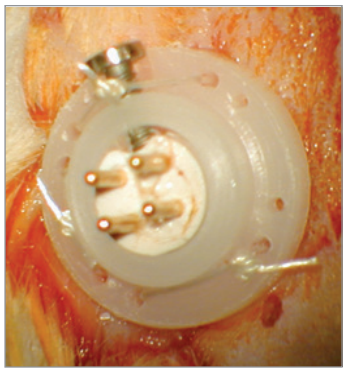


Fig. 17: Close the skin over the incision with 5-0 Vicryl sutures. The Flowprobe maintains the best position if the cable is not disturbed during subcutaneous preparation. To do this, cut and close the skin over the cable to the Probe connector at the midscapular region, leaving the Probe's CA4 connector exposed. Use a 3/4" square of Mersilene Mesh under the skin at the connector and suture the skin closed around cable. Install a skin button cuff over the CA4 connector as shown suturing through the skin and the mesh to improve long term stability of the implant.

REFERENCES

Brands MW et al, "Interleukin 6 knockout prevents angiotensin II hypertension: role of renal vasoconstriction and janus kinase 2/signal transducer and activator of transcription 3 activation," Hypertension 2010; 56(5): 879-84; 819-21.

Iliescu R et al, "Renal blood flow and dynamic autoregulation in conscious mice," Am J Physiol Renal Physiol 2008; 295(3): F734-40.

Fig. 18: The mouse is shown with the skin button positioned after surgery. The wound is cleaned and the animal is allowed to recover before measurements are made. Generally, it takes 3-5 days to achieve a stable signal as fibrotic tissue helps to encapsulate the Probe, though the flow signal may be available as soon as 1 day post surgery.

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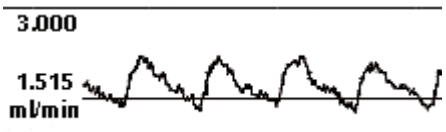
Mouse Renal Artery: Acute and Chronic Blood Flow Measurement Cont.

Chronic Measurement Protocol cont.

Fig. 19: Blood flow measurements can be continuously monitored in the conscious mouse via a tether connection to the skin button and suitable low torque electrical swivel (Dragonfly Inc.).



This renal blood flow recording was made 1 day after implant in the conscious mouse.

ACKNOWLEDGEMENTS

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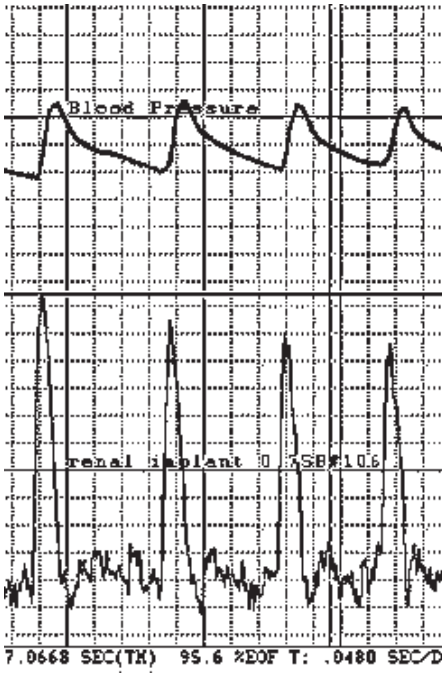

Renal blood flow recording ~1.5 years after implant in the conscious mouse during femoral catheter implantation (isoflurane anesthesia).

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