Submission ID #: 60787
Scriptwriter Name: Nilesh Kolhe
Project Page Link: https://www.jove.com/account/file-uploader?src=18540083

Title: Targeting the rat's small bowel: long-term infusion into the superior mesenteric artery

Authors and Affiliations:

Alessandro Borrello[1], Amedea Luciana Agnes[1], Eleonora Pellegrino[1], Sabina Magalini[1], Daniele Gui[1]

Department of Emergency Surgery, Università Cattolica del S. Cuore, Roma, Italy

Corresponding Authors:

Alessandro Borrello alebor93@gmail.com

Email Addresses for All Authors:

alebor93@gmail.com
amedeaagnes@gmail.com
eleonora.pellegrino93@gmail.com
Sabina.magalini@unicatt.it
daniele.gui@unicatt.it

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

    Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 19
Number of Shots: 39

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Amedea Luciana Agnes:** Superior mesenteric artery cannulation in the rat is difficult and is not often used as an experimental model. This technique makes it possible to perform a "functional canulation" and opens a field of experimentation with first passage effects of intraarterial drugs.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Amedea Luciana Agnes:** This method avoids bleeding and thrombosis while consenting the canulation of the superior mesenteric artery and allowing for long-term infusion of drugs in a relatively free animal, who can eat and move.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

OPTIONAL:

1.3. **Amedea Luciana Agnes:** Pharmacological research on drugs active on the intestine may greatly benefit from this technique. There is no alternative to study the effect of a direct single passage of the drug.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

1.4. The studies described in this manuscript were approved by the local animal Ethics Committee (Università Cattolica del Sacro Cuore, Roma) and were conducted in accordance with the Italian Ministry of Health.

Protocol

2. Preparation for the Surgical Procedure

2.1. Begin by cutting the larger cannula **[1-TXT]**, then cut the smaller cannula **[2-TXT]** and insert it 1 centimeter into the larger cannula **[3]**.
- 2.1.1. WIDE: Experimental shot of talent cutting the larger cannula. **TXT: 0.93 mm O.D, 0.5 mm I.D.**
- 2.1.2. Talent cutting the smaller cannula. **TXT: 0.4 mm O.D, 0.25 mm I.D.**
- 2.1.3. Talent inserting smaller cannula into larger cannula.

2.2. Fix the two cannulas together with cyanoacrylate glue, avoiding occlusion of the lumen **[1]**. Afterwards, connect the free extremity of the larger cannula to a Luer stub adapter mounted on a 1-milliliter syringe filled with saline solution **[2]**. *Videographer: This step is important!*
- 2.2.1. Talent attaching the cannulas by cyanoacrylate glue.
- 2.2.2. Talent connecting larger cannula to stub adapter.

2.3. Sharpen the free tip of the smaller cannula with scissors to facilitate the insertion of the catheter into the branch of the superior mesenteric artery **[1]**. Check the patency of the cannula by flushing with saline solution **[2]**.
- 2.3.1. Talent sharping the tip of smaller cannula.
- 2.3.2. Talent flushing the cannula.

2.4. Shave the fur from the surgical regions of the anaesthetized rat, including the abdomen for the branch of the superior mesenteric artery, or SMA, cannulation and the back of the neck for the cannula exit **[1]**.
- 2.4.1. Talent shaving the surgical area of rat.

2.5. Clean the surgical regions aseptically using povidone-iodine solution **[1]** and place the animal in a supine position on a wooden board, immobilizing the four limbs **[2]**.
- 2.5.1. Talent disinfecting the surgical area.
- 2.5.2. Talent positioning the rat on a wooden board.

3. Cannulation of a Proximal Branch of the SMA

3.1. Use a scalpel blade to open the abdominal wall with a straight 3-centimeter incision on the midline of the mesogastric region through all the abdominal planes into the peritoneum **[1]**.
- 3.1.1. Talent making an incision.

3.2. Place gauzes soaked with saline solution around the laparotomy incision, using sutures to keep the surgical incision open **[1]**. Use cotton swabs to identify and expose the small intestine **[2]** and extract the mesentery out of the laparotomic cut and lay it downward on the gauzes **[3]**.
  3.2.1. Talent keeping the gauzes around incision.
  3.2.2. Talent exposing small intestine. NOTE: This and next shot together
  3.2.3. Talent extracting out of the cut.

3.3. Identify the SMA, by feeling pulsation **[1]**. Using cotton swabs, separate the mesenteric fat and uncover the SMA and 2 to 3 of its proximal branches **[2]**. *Videographer: This step is important!*
  3.3.1. Talent identifying the SMA by pulsation.
  3.3.2. Talent uncovering the SMA.

3.4. Choose a proximal branch of the SMA that is sufficiently large to allow the surgical maneuvers of cannulation **[1]**. Tie this branch 3 to 4 centimeters downstream from its origin to allow its expansion by keeping the suture ends long enough to be manipulated later **[2-TXT]**.
  3.4.1. Talent choosing branch of SMA.
  3.4.2. Talent knotting the branch. **TXT: With 4-0 silk suture**.

3.5. Place a rigid support under the branch of the SMA and hold the extremity of the smaller cannula using forceps, then pull the suture ends with the other hand to strain the vessel and to facilitate the entry of the catheter **[1]**.
  3.5.1. Talent keeping rigid support and facilitating the entry of catheter into vessel.

3.6. Hold the tip of the cannula at a 20-degree angle from the plane of the vessel in the direction opposite to the blood flow, then lightly press the tip to penetrate the artery wall and insert the cannula **[1]**. *Videographer: This step is difficult and important!*
  3.6.1. Talent penetrating the cannula into the artery wall. NOTE: This and next shot together

3.7. Continue the insertion of the cannula for 1 centimeter in the arterial branch close to the origin from the SMA **[1]**. Fix the cannula to the artery with a surgical knot **[2]** and verify its correct functioning by flushing 1 milliliter of sterile saline solution or by blood sampling **[3]**.
  3.7.1. Talent inserting the cannula in the arterial branch.
  3.7.2. Talent fixing the cannula to the artery by knotting the string.
  3.7.3. Talent verifying the knot.

3.8. Make a 1-centimeter incision of the posterior region of the neck and accommodate a spherical valve **[1]**, then pass the cannula from the laparotomy access to the valve placed in the neck through subcutaneous tissues **[2]** and close the distal extremity of the cannula with a catheter plug to avoid air inflow **[3]**. *Videographer: This step is important!*
  3.8.1. Talent making an incision.
  3.8.2. Talent passing the cannula towards the valve.
  3.8.3. Talent closing the cannula.

3.9. Replace the small bowel in the abdominal cavity and close the abdominal wall **[1]**, then secure the valve to the neck skin with stitches **[2]**.
  3.9.1. Talent closing the abdominal wall.
  3.9.2. Talent applying stiches.

4. Post-operative Management

4.1. Dress the rat with a jacket to protect the button valve **[1]**. Protect the exposed part of the cannula with a steel rod during infusion and secure it to the valve **[2]**.
  4.1.1. Talent covering the rat with jacket.
  4.1.2. Talent protecting the exposed part of the canula.

4.2. Connect the distal extremity of the cannula to an elastomeric pump filled with 50 milliliters of sterile saline solution and proceed with infusion for 24 hours **[1-TXT]**.
  4.2.1. Talent connecting the cannula to pump and continuing the infusion. **TXT: 100 mL volume max, 5.0 mL/h flow rate**

4.3. After the infusion, disassemble the animal's external infusion system by removing the pump, the jacket, the steel rod, and the valve from the rat **[1]**. Close and cut the cannula as it comes out of the neck, leaving the extremity under the skin of the neck after wound suture **[2]**.
  4.3.1. Talent disassembling the external infusion system.
  4.3.2. Talent closing the cannula.

Results

5. Results: Rat SMA-branch Cannulation

5.1. In this study, 15 rats were subjected to this procedure and average daily food **[1]** and water intake increased progressively until normal after 3 days **[2]**. The weight gain was also regular and gradually increasing until the end of the observation period **[3]**.
5.1.1. LAB MEDIA: Figure 3A.
5.1.2. LAB MEDIA: Figure 3B.
5.1.3. LAB MEDIA: Figure 3C.

5.2. After 24 hours, there was saline residue in just 2 balloon pumps while the other 13 were completely empty **[1]**. Furthermore, after infusion period, 12 cannulas were still functional for both blood sampling and saline infusion, while 3 cannulas were not patent anymore **[2]**.
5.2.1. LAB MEDIA: Table 1. *Video Editor: Emphasize on elastomeric rows and column.*
5.2.2. LAB MEDIA: Table 1. *Video Editor: Emphasize on canula rows and column.*

5.3. At autopsy, 100 percent of cannulas were located in the SMA branch and no rats had signs of bowel ischemia or intrabdominal bleeding **[1]**.
5.3.1. LAB MEDIA: Figure 4B.

5.4. An angiography was obtained in 5 rats by injecting iodinated contrast medium into the mesenteric cannula. The mesenteric arterial circle, particularly the SMA and its main branches were observed without contrast medium spreading in the abdomen, confirming that the cannula was well-placed and fixed to the branch of the SMA **[1]**.
5.4.1. LAB MEDIA: Figure 4A. *Video Editor: Emphasize on red circle.*

Conclusion

6. Conclusion Interview Statements

6.1. **Alessandro Borrello:** Patient and meticulous isolation of the branch to canulate, ligature of the vessel down-stream, passing the suture underneath the vessel and choosing the most appropriate position to puncture and insert the canula are the most important aspects of this protocol. Errors are uncorrectable.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.6.*