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Title: Murine Model of Thoracic Aortic Dissection Induced by Oral β-Aminopropionitrile and Subcutaneous Angiotensin II Infusion

Authors and Affiliations:

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

Current Protocol Length
Number of Steps: 11
Number of Shots: 25

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Shengkai Zuo:** This study establishes a mouse model of Thoracic aortic dissection or TAD through BAPN and Ang II induction, providing a detailed procedure that offers a valuable tool for exploring TAD pathogenesis and therapeutic approaches **[1]**.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.3.*

What advantage does your protocol offer compared to other techniques?

1.2. **Shengkai Zuo:** The BAPN/Ang II combination provides a method for establishing murine TAD models, which achieve a high TAD incidence rate and recapitulate pathophysiology characteristics that resemble those observed in human TAD **[1]**.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 3.*

What research questions will your laboratory focus on in the future?

1.3. **Shengkai Zuo:** In the future, our research team will integrate clinical and foundational research to elucidate cardiovascular pathogenesis, prioritizing the discovery of novel therapeutic strategies through mechanistic insights and translational innovation **[1]**.

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

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee of Tianjin Medical University

Protocol

2. Angiotensin II (Ang II) Mass Calculation and Dissolution Protocol

Demonstrator: Yi Zhang

2.1. Weigh each mouse and record the maximum body weight to calculate the Angiotensin II *(two)* mass required for the experiment **[1]**. Use the calculation template to determine the Angiotensin II mass for each mouse based on the maximum body weight **[2]**.

2.1.1. WIDE: Talent placing a mouse on a digital scale and recording the body weight.

2.1.2. LAB MEDIA: Table 2. *Video editor: Highlight the 4 rows of the table that are part of the section "Per mouse".*

2.2. Then, calculate the total mass of Angiotensin II required to prepare 130 microliters of Angiotensin II solution per mouse **[1]**. Use the provided template to determine the filling volume of Angiotensin II solution and saline needed for the experiment **[2]**.

2.2.1. LAB MEDIA: Table 2. *Video editor: Emphasize the 3rd row from the bottom (Required Ang II mass per mouse a ng = z ng/µL × 130 µL).*

2.2.2. LAB MEDIA: Table 3.

2.3. Weigh the calculated amount of Angiotensin II in a sterile microtube using an analytical balance **[1]**.

2.3.1. Talent weighing Angiotensin II in a clearly labeled sterile microtube on an analytical balance.

2.4. Add the calculated volume of normal saline into the microtube **[1]**. Using a vortex mixer, dissolve the powder thoroughly until no visible particles remain **[2-TXT]**.

2.4.1. Talent pipetting normal saline into the microtube with Angiotensin II.

2.4.2. Talent placing the microtube containing the solution in a vortex mixer. **TXT: Prepare Ang II solution separately for each mouse based on body weight**

3. Procedure for Osmotic Pump Filling

3.1. Weigh each osmotic pump, including the pump body and flow moderator, using an analytical balance **[1-TXT]**. Attach a filling tube to a freshly opened 1-milliliter sterile

syringe **[2]** and carefully aspirate the prepared Angiotensin II solution **[3]**.

3.1.1. Talent using an analytical balance to weigh the osmotic pump. **TXT: Use this data to calculate the filling ratio**

3.1.2. Talent attaching the filling tube to a new syringe.

3.1.3. Talent aspirating Angiotensin II solution into the syringe.

3.2. Hold the filling tube in an upward position and remove any air bubbles from the syringe **[1]**. Gently insert the end of the filling tube into the top opening of the osmotic pump until the tube cannot be inserted any further **[2]**. Hold the pump upright and slowly press the syringe plunger until Angiotensin II solution appears at the outlet **[3]**, then stop and remove the filling tube carefully **[4]**.

3.2.1. Talent holding the filling tube in an upward position and removing any air bubbles from the syringe.

3.2.2. Talent inserting the end of the filling tube into the osmotic pump's top opening.

3.2.3. Talent holding the pump upright and squeezing the plunger. The solution appears at the outlet.

3.2.4. Talent removing the filling tube.

3.3. Next, insert the flow moderator into the pump opening carefully and slowly **[1]** until no gap remains between the moderator and the top of the pump **[2]**. Weigh the loaded pump using the analytical balance and record the value **[3-TXT]**.

3.3.1. Talent inserting the flow moderator into the pump opening.

3.3.2. A shot showing no visible gap between the moderator and the top of the pump.

3.3.3. Talent weighing the loaded pump using the analytical balance. **TXT: Difference in pump weight before and after infusion is the mass of the loaded Ang II solution**

3.4. Place the filled pumps in sterile saline at 37 degrees Celsius with the moderator head facing up for at least 6 hours before implantation **[1]**.

3.4.1. Talent lowering the filled pumps into a sterile saline bath in an incubator, moderator head facing up.

4. Surgical Procedure for Pump Implantation

4.1. Place the mouse in a prone position on the surgical surface **[1-TXT]**. Use a scalpel to carefully make a 1-centimeter transverse incision in the skin **[2]**.

4.1.1. Talent positioning the mouse in a prone position on the surgical surface. **TXT: Anesthesia:; Induction: 1.5% - 2% isoflurane at a flow rate of 2 L/min**

4.1.2. Talent making a transverse incision in the skin using a scalpel.

4.2. Use one pair of curved forceps to grasp the incisal margin, and another pair of forceps to bluntly dissect the subcutaneous tissue **[1]**, creating a pocket for the osmotic pump **[2]**. Insert the filled pump into the pocket with the flow moderator head pointing toward the caudal end of the mouse **[3]**.

4.2.1. Talent grasping the skin with one curved forceps and gently dissecting underneath with another pair of curved forceps.

4.2.2. A shot of the created pocket for the osmotic pump.

4.2.3. Talent placing the filled pump into the pocket with the moderator head oriented caudally.

4.3. Neatly align the incisal margins **[1]** and close the skin using 6-0 *(six-zero)* non-absorbable sutures **[2]**.

4.3.1. A shot of the aligned incisal margin.

4.3.2. Talent stitching the incision with sutures.

Results

5. Results

5.1. This figure illustrates the incidence of aortic dissection and rupture across all experimental groups **[1]**. Aortic dissection occurred in 100% of mice in the BAPN *(B-A-P-N)* plus Angiotensin II group, with 35% experiencing rupture **[2-TXT]**, surpassing the 55% or 60% incidence observed in the BAPN or BAPN plus saline group **[3]**, while no dissection or rupture occurred in controls **[4]**.

5.1.1. LAB MEDIA: Figure 2A.

5.1.2. LAB MEDIA: Figure 2A. **TXT: BAPN: β-Aminopropionitrile** *Video Editor: Highlight the 1st bar from right (BAPN + Ang II).*

5.1.3. LAB MEDIA: Figure 2A. Video Editor: Highlight the two central bars (BAPN and BAPN + Saline).

5.1.4. LAB MEDIA: Figure 2A. *Video Editor: Highlight the 1st bar from left (Control)*

5.2. Gross aortic morphology showed progressive deformation in BAPN, BAPN plus Saline, and BAPN plus Angiotensin II groups compared to the slender aortas in controls **[1]**.

5.2.1. LAB MEDIA: Figure 2B.

5.3. Maximum thoracic aortic diameters were significantly greater in all BAPN-treated groups **[1]** compared to controls **[2]**, with no significant differences among the treated groups **[3]**.

5.3.1. LAB MEDIA: Figure 2C. *Video Editor: Highlight the red, blue, and green bars.*

5.3.2. LAB MEDIA: Figure 2C. *Video Editor: Highlight the black bar.*

5.3.3. LAB MEDIA: Figure 2C. *Video Editor: Highlight the red, blue, and green bars.*

5.4. Hematoxylin and eosin staining revealed thin, uniform aortic walls in controls **[1]**, while all BAPN-treated groups displayed thickening and inflammatory infiltration **[2]**.

5.4.1. LAB MEDIA: Figure 3 (HE Panel). *Video Editor: Highlight the right image in the Control row in HE Panel.*

5.4.2. LAB MEDIA: Figure 3 (HE Panel). *Video Editor: Highlight the right images in the bottom three rows in HE Panel.*

5.5. Elastin van Gieson staining demonstrated intact elastic fibers in controls **[1]** and progressive fiber fragmentation with false lumen formation in all BAPN-treated groups **[2]**.

5.5.1. LAB MEDIA: Figure 3 (EVG Panel). *Video Editor: Highlight the right image in the Control row in EVG Panel.*

5.5.2. LAB MEDIA: Figure 3 (EVG Panel). *Video Editor: Emphasize the right images in the bottom three rows in EVG Panel and Highlight "F" and the black arrows beside it in all three images.*