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Advanced Abdominal Aortic Aneurysm Modeling in Mice by Combination of Topical Elastase and Oral ß-aminopropionitrile

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Abstract

The topical elastase murine model of abdominal aortic aneurysm (AAA) is enhanced when combined with ß-aminopropionitrile (BAPN)-supplemented drinking water to reliably produce true infrarenal aneurysms with behaviors that mimic human AAAs. Topically applying elastase to the adventitia of the infrarenal aorta causes structural damage to the elastic layers of the aortic wall and initiates aneurysmal dilation. Co-administering BAPN, a lysyl oxidase inhibitor, promotes sustained wall degeneration by reducing collagen and elastin crosslinking. This combination results in large AAAs that progressively expand, form intraluminal thrombus, and are capable of rupture. Refining surgical techniques, such as circumferentially isolating the entire infrarenal aortic segment, can help standardize the procedure for a consistent and thorough application of porcine pancreatic elastase despite different operators and anatomic variations between mice. Therefore, the elastase/BAPN model is a refined approach to surgically inducing AAA in mice, which may better recapitulate human aneurysms and provide additional opportunities to study aneurysm growth and rupture risk.

Introduction

An aneurysm is defined as a pathologic dilation of a blood vessel greater than 50% of the healthy vessel diameter[1]. Despite abdominal aortic aneurysms (AAA) being a commonly encountered condition in the aging population, with an incidence of roughly >5% of males > 65 years of age, there are no directed therapeutic strategies for treating AAA[1]. Current management of AAA is limited to risk factor reduction and surgical repair with either open or endovascular surgery based on aortic diameter or growth rate[2]. The greatest danger of AAA is aneurysm rupture, which is fatal if untreated,

and repair in this emergent setting can carry mortality risks upwards of 90%[1].

The pathophysiology of AAA is complicated, multifactorial, and not fully understood[3]. Features of human AAA include true aneurysmal dilation of the aortic wall with an infiltration of inflammatory cells, the presence of intraluminal thrombus, and progressive dilation that leads to eventual rupture[3,4]. Additionally, AAAs are associated with advanced age, have a 9:1 male:female sex predominance, and most commonly occur in the infrarenal aorta[5]. Modeling all features and behaviors of human AAAs in animals remains an ongoing challenge[6].

Current AAA modeling is primarily conducted in mice, and aneurysms are commonly induced using one of three methods-by angiotensin II (AngII) infusion via a subcutaneously implanted osmotic pump, and by direct application of calcium chloride ($CaCl_2$) or elastase to the aorta[7]. In the latter method, porcine pancreatic elastase (PPE) is applied to a segment of the infrarenal aorta and causes enzymatic degradation of elastin fibers within the elastic lamella of the tunica media. This structural damage results in the weakening of the aortic wall and outward aneurysmal dilation. The use of topical elastase alone, however, produces relatively small infrarenal aneurysms, which fail to progressively enlarge or rupture over time. More recently, Lu et al. improved upon this model by additionally administering β-aminopropionitrile (BAPN), an irreversible inhibitor of lysyl oxidase, to their elastase-treated mice[8]. By preventing the crosslinking of elastin and collagen fibers, BAPN supplementation causes elastase-damaged aortas to progressively dilate to the point of rupture. The elastase/BAPN model additionally has a higher incidence rate of AAA than the topical elastase model, and the aneurysms produced are also larger and contain intraluminal thrombus[8].

In the elastase/BAPN model, the degree of surgical dissection and exposure of the aorta to elastase can impact the success and replicability of this model. In this manuscript, we describe that co-administration of BAPN drinking water and topical elastase application to the aorta following circumferential isolation of the entire infrarenal aortic segment improves replicability, accounts for anatomical differences between animals, and results in a greater AAA induction rate, aneurysm sizes and rupture incidence. In this article, we will describe a standardized approach to reliably inducing advanced abdominal aortic aneurysms in mice using a combination of topical elastase- and BAPN-supplemented water.

Protocol

Animal protocols are approved by the University of Wisconsin-Madison Institutional Animal Care and Use Committee (M005792).

1. Animal maintenance

1. Raise mice on standard maintenance chow. Use adult mice or young adult mice (8-12 weeks old).
   NOTE: Using adults ensures that the animals have reached full maturity and limits any chance that changes in aortic diameter could be related to animal growth. For this study, we utilized male and female C57BL/6J mice aged 22-24 weeks at the time of surgery. Lu and colleagues did not observe significant differences in the aneurysmal response between younger and older mice[8]. Additionally, while most aneurysm modeling is performed

in male mice, this model successfully induces AAAs in both male and female mice[9].

2. Determine the study duration and assign animals to treatment or sham (control) groups. Administer 0.2% BAPN drinking water to treatment group mice and subject them to surgery with topical application of active elastase to the infrarenal aorta. Administer untreated water to control animals and subject them to surgery with the application of denatured elastase to the infrarenal aorta.

2. Initiation of B-aminopropionitrile (BAPN)-supplemented drinking water

1. Two days before surgery, start treatment mice on 0.2% BAPN drinking water. Prepare BAPN water in larger volumes and store it in the dark at 4 °C for up to 28 days. Be sure the BAPN water reaches room temperature before giving it to the mice.

   NOTE: We recommend BAPN water be replaced in cages every 7 days throughout the duration of the study.

3. Day of surgery material preparation

1. Cut surgical gloves into 5 mm x 10 mm strips, which will be used later to help isolate the aorta before treatment with elastase. Prepare a surgical drape by cutting a ~ 1.5 cm x 3 cm sized oval in the center of a surgical drape. Unfold the 2 in x 2 in gauze and cut in half to create strips of gauze roughly 2.5 cm x 10 cm to be used later for retraction of abdominal contents. Autoclave all surgical instruments (see the **Table of Materials**) and set up a sterile surgical field as shown by the example in **Figure 1**.

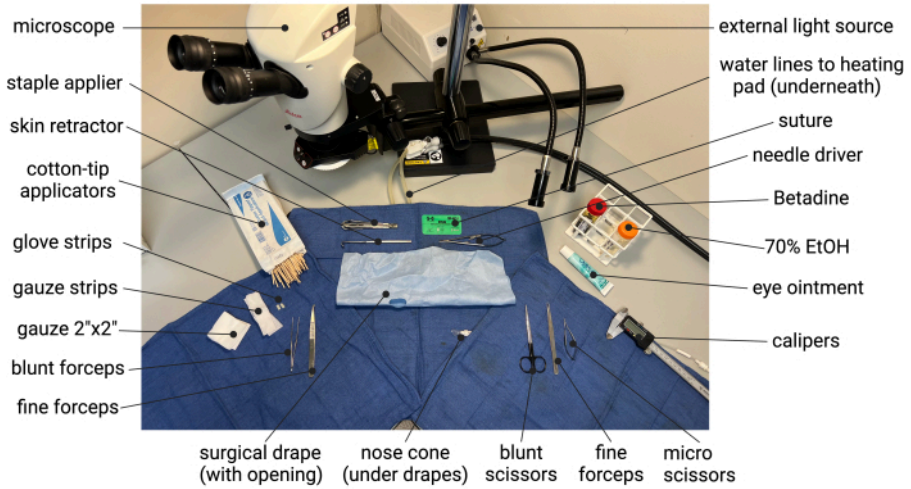

Figure 1: Example of the sterile surgery setup in preparation for the elastase/BAPN murine model of AAA.

Abbreviations: BAPN = ß-aminopropionitrile; AAA = abdominal aortic aneurysm. Please click here to view a larger version of this figure.

2. Prepare a postoperative recovery cage by placing a clean cage under a heat lamp and place saline near the lamp to warm to body temperature (37 °C). Ensure the heat lamp is safely positioned so that the recovery cage and saline are warm but are not beyond 37 °C. Turn on

the water pump to begin circulating warm water through the heating pad.

4. Animal preparation for surgery

1. Place the mice in an induction chamber and anesthetize them with 5% isoflurane at 200 mL/min using a low-flow electronic vaporizer. While anesthetized, weigh each mouse and deliver 0.6 mg/kg of buprenorphine ER and 20 mg/kg of carprofen subcutaneously for analgesia. Use electric hair trimmers to clip the fur on the abdomen, from the lower abdomen to inferior of the xyphoid process. Use gauze or a laboratory wipe to brush away excess hair. Return mice to their cage and wait at least 20 min for analgesia to take effect before proceeding with surgery.
2. After at least 20 min since analgesia was administered, place the mouse in an anesthesia induction chamber and again deliver 5% isoflurane at 200 mL/min using a low-flow electronic vaporizer until the mouse is sedated.
3. Remove the sedated mouse from the induction chamber and place it supine on the surgical field. Apply eye gel and secure the nose cone with surgical tape. Reduce the delivered inhaled isoflurane to a maintenance rate of 1-2% at 50 mL/min. Secure the mouse's front and hind paws with surgical tape.
4. Examine the mouse's lower abdomen for the bladder. Gently apply external pressure to the bladder between the thumb, index, and middle fingers to induce voiding; meanwhile, use a piece of gauze to wick away the urine.
   NOTE: Be careful to not contaminate the surgical field.
5. Begin disinfecting the abdomen by applying an iodine-based or chlorhexidine-based scrub and 70% alcohol with cotton-tipped swabs. Start in the center of the abdomen and work outward in a circular motion 3x. Allow the area to briefly dry between applications.
6. Check for a lack of a toe-pinch response to ensure anesthesia is adequate. Ensure the nose cone and limbs are secured. Place a surgical drape over the mouse, with the opening directly over the surgically prepped abdomen.
   NOTE: Do not drag the drape across the mouse to avoid potential contamination.

5. Surgical induction of AAA

1. Entering the abdominal cavity:
   1. Wash hands and wear clean nitrile or sterile surgical gloves. Before contacting the surgical field, always spray gloves with 70% EtOH and rub gloved hands together until dry.
   2. Use blunt forceps to tent the skin in the midline abdomen. Use surgical scissors to make a small nick in the skin, then extend the incision longitudinally, roughly 2-3 cm in length.
   3. Use forceps to lift the rectus muscles to identify the translucent linea alba. Use scissors to enter the abdominal cavity through the linea alba, then extend along the linea alba proximally and distally.
2. Exposing the abdominal aorta:
   1. Dampen a strip of gauze and two cotton-tipped swabs with warmed saline. Create an abdominal roll by tightly rolling one end of the gauze halfway, leaving a generous tail.
   2. Use a skin retractor to retract the right abdominal wall.

3. Using dampened cotton-tipped swabs, perform a right-medial visceral rotation by gently sweeping the small and large intestines to the left upper quadrant and visualize the aorta and inferior vena cava (IVC). Use an abdominal roll to retract the bowel out of view – tuck the rolled end of the gauze underneath the bowel, and then bring the tail end around and out of the body to gently swaddle the bowel. Apply gentle tension to the tail of the gauze to hold the bowel out of the field of view. Adjust the abdominal roll and skin retractor to gain an optimal view of the retroperitoneal organs, as shown in **Figure 2A**.

   NOTE: The abdominal roll helps to keep the bowel moist and protect it from being inadvertently damaged by surgical instruments. Be sure the gauze remains damp during the procedure to prevent the bowel from drying out. Be careful to not forcefully retract the bowel as this can cause kinking of the superior mesenteric artery and bowel vasculature, which can potentially cause ischemic damage. Moreover, when initially sweeping the small intestine, be cautious of a thin translucent attachment between the large bowel and the inferior liver (hepatocolic ligament), which if not careful, can easily tear off the liver capsule and cause bleeding. If there is tension on this ligament during retraction, divide it sharply with scissors.

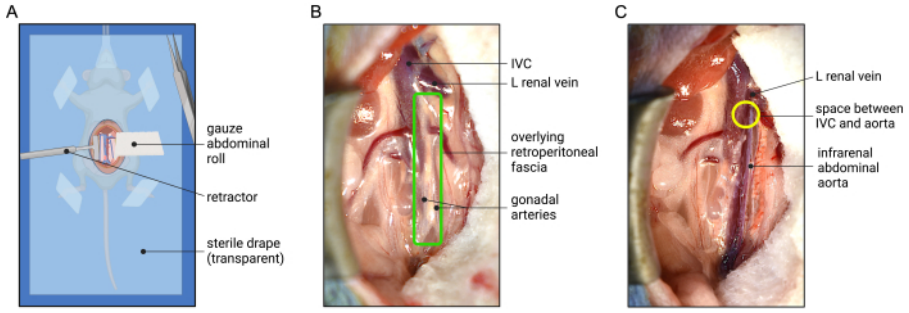

Figure 2: Representation of abdominal retraction and the optimal surgical view for exposure of the mouse infrarenal aorta. (**A**) Placement of a gauze abdominal roll helps to retract the intraabdominal organs, while an opposing retractor assists in providing visualization of the retroperitoneum. A sterile surgical drape **(transparent to show animal orientation)** is placed over the anesthetized animal to help maintain sterility. (**B**) The retroperitoneal fascia (green box) overlies the aorta anteriorly. (**C**) Example of the infrarenal aorta following dissection of the retroperitoneal fascia. Isolation of the aorta from the IVC can be achieved by starting at a potential space between the aorta and IVC located just distal to the left renal vein as it crosses anteriorly (yellow circle). Abbreviation: IVC = inferior vena cava. Please click here to view a larger version of this figure.

3. Circumferential dissection and isolation of the infrarenal aorta:

   1. Confirm that the IVC and infrarenal aorta are in full view. Begin exposing the aorta by first entering and

dividing the retroperitoneal (RP) fascia (**Figure 2B**). Identify the gonadal (testicular or ovarian) arteries that run parallel along the anterior infrarenal aorta (**Figure 2B** and **Figure 3**). Use forceps to bluntly divide the fascia between the gonadal arteries and continue longitudinally to expose the aorta anteriorly (**Figure 2C**).

NOTE: The RP fascia is a thin, translucent layer of connective tissue that contains lymphatics and the splanchnic plexus. It is necessary to dissect through the RP fascia to expose the aorta. However, do not dissect through the connective tissue of the aortic adventitia. A tear in the adventitia (white connective tissue) will expose the media (appears bright red), and the aorta will likely rupture at this site once elastase is applied.

2. Next, start to isolate the abdominal aorta off the IVC. Begin this dissection at a small gap between the IVC and aorta, located just below the inferior edge of the left renal vein as it crosses the aorta (**Figure 2C**). Use the tips of forceps to gently spread apart the connective tissue fibers between the aorta and IVC and continue working circumferentially around the aorta at this level.

   NOTE: The IVC is very thin-walled and closely adheres to the aorta by a fine layer of fibrous connective tissue. Be careful to avoid handling the IVC or cleaning off the IVC as much as possible. Dissecting the right side of aorta off the IVC first (before dissecting the left side of the aorta from the surrounding musculature) will help the aorta to "fall away" from the IVC.

3. Continue bluntly dissecting the plane between the aorta and IVC, working caudally toward the aortic bifurcation. Stop further distal dissection once reaching the aortic bifurcation.

   NOTE: Take extra precaution when dissecting around the inferior mesenteric artery (IMA), which is typically located near the mid-section of the infrarenal aorta and travels laterally across the IVC.

4. Once the right edge of the aorta is separated from the IVC, return proximally to the level of the left renal vein. Dissect the RP fascia off the lateral left edge of the aorta, working circumferentially around until the aorta is fully isolated. See **Figure 3** for relevant anatomy of the retroperitoneal dissection.

   NOTE: Be careful dissecting behind the aorta as there is high variability in the location and number of lumbar veins and arteries. See **Figure 4** for reference of areas at high risk for bleeding with this dissection.

5. Carefully inspect that the aorta is circumferentially isolated from the IVC and the surrounding musculature as much as possible, with careful dissection around the aortic segments tethered by the IMA and lumbar arteries.

6. Place a strip of glove along the right and left edges of the aorta, as demonstrated in **Figure 5A**. Attempt to cover as much of the IVC as possible.

7. Use handheld calipers to measure the widest aortic diameter and record three measurements. Spray the tips of the calipers with 70% EtOH before and after measurements. Avoid directly contacting the aorta with the caliper tips to prevent contamination.

   NOTE: Photos using a calibrated camera-capable microscope can also be utilized.

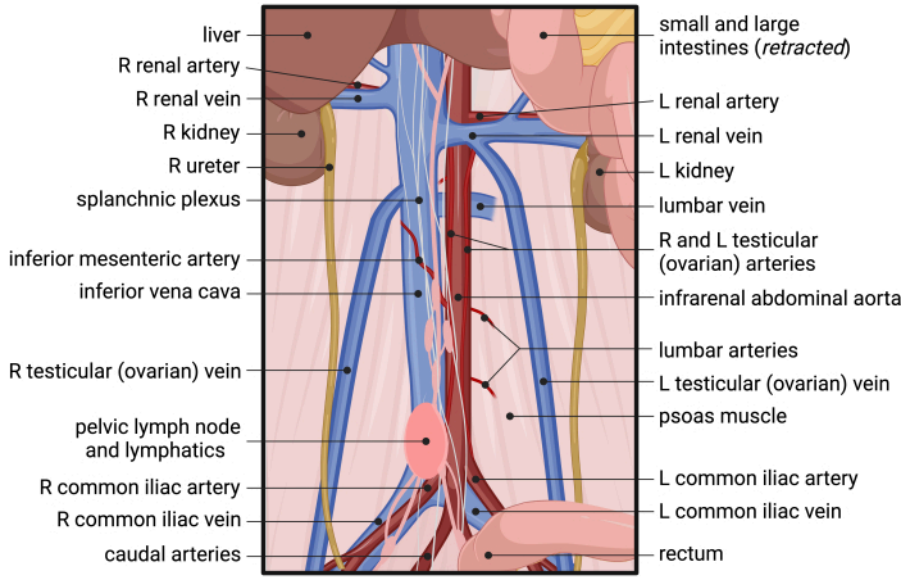

Figure 3: Anatomy of the blood supply to the lower abdomen, pelvis, and retroperitoneum of the mouse.

Abbreviations: R = right; L = left. Please click here to view a larger version of this figure.

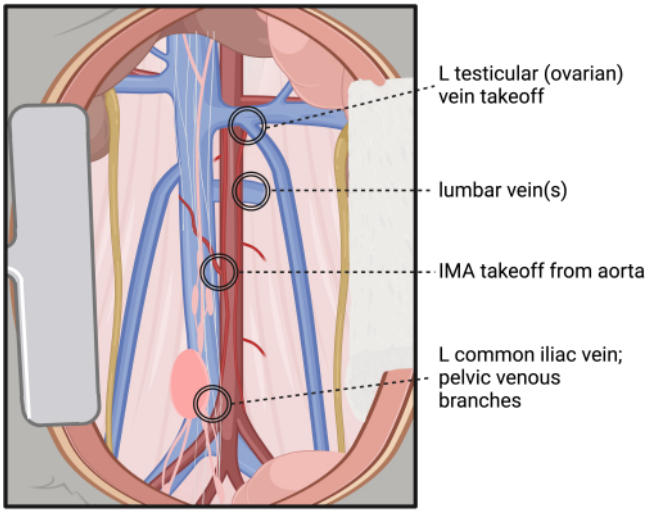

Figure 4: Sites of high risk for injury and hemorrhage during the retroperitoneal dissection and circumferential isolation of the infrarenal aorta. Abbreviations: L = left; IMA = inferior mesenteric artery. Please click here to view a larger version of this figure.

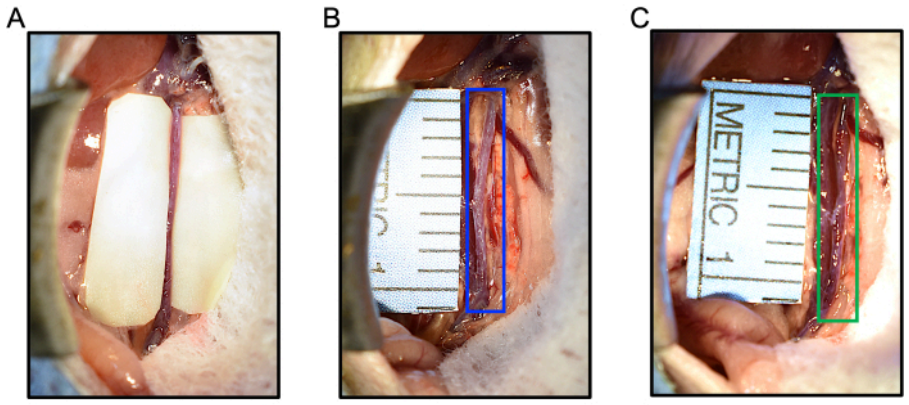

Figure 5: Intraoperative responses to elastase application or sham during the elastase/BAPN murine AAA model. (**A**) Segments of glove are placed along the length of the aorta prior to elastase application to help protect the IVC and bowel from exposure to elastase while keeping the aorta soaked in elastase (**B**) Application of denatured elastase does not cause dilation of the aorta (blue box). The maximal aortic diameter measured 0.627 mm at baseline, then 0.607 mm after 5 min of topical denatured elastase. (**C**) Application of elastase causes aortic dilation after 5 min of treatment. In this example, the aorta (green) dilated to 0.953 mm from 0.607 mm, a 57% increase in diameter. Abbreviations: BAPN = ß-aminopropionitrile; AAA = abdominal aortic aneurysm. Please click here to view a larger version of this figure.

4. Elastase application:
    1. Use a cotton-tipped swab to dab any extra blood or fluid off the aorta.
    2. Next, place a 10 mm x 2 mm piece of dry gauze on top of the aorta. Use a pipette to dispense 5 µL of elastase (or control denatured elastase) to saturate the gauze and aorta. Gently fold the pieces of the glove around the aorta.

        NOTE: To prepare denatured elastase for use in sham or control groups, boil elastase at 100 °C for 30 min.
    3. Allow 5 min for elastase to act on the aorta. During this incubation period, if required, release some of the tension placed by the abdominal roll and the skin retractor.

        NOTE: Due to the batch effect with elastase, we encourage investigators to use the same bottle of elastase for all experiments within a given study. With each new bottle of elastase, we recommend performing a dose-response to ensure there is not an overwhelming number of early ruptures (prior to 4 weeks). The duration of elastase application can also be adjusted between 4 and 6 min depending on the response to elastase.
    4. After 5 min, reset bowel retraction and unfold the pieces of the glove. Irrigate the abdominal cavity with 1 mL of warm 0.9% sterile normal saline, while carefully removing the gauze and pieces of glove off the aorta. Absorb the saline in the abdomen with 10

cm x 10 cm gauze. Repeat irrigating the abdomen for a total of 3 x 3 mL.

5. Use handheld calipers to remeasure the widest aortic diameter post elastase application and record 3x. See **Figure 5B,C** for examples of the aortic dilation to treatment with sham and active elastase.
   NOTE: The averages of the three pre- and post-elastase measurements can be used to calculate the percent change in aortic diameter with treatment. Typically, there is a notable dilation ~30-50% immediately after elastase treatment, which can help to ensure the elastase is functional and the aorta was adequately treated. The diameter of the aorta should not change with the application of denatured elastase or may measure slightly smaller (likely from spasm).

5. Closure of the abdominal cavity:
   1. Carefully remove the abdominal roll from underneath the bowel and out of the body. If needed, apply additional saline to the bowel to prevent sticking to the abdominal roll during removal. Check to make sure the bowel appears pink and adequately perfused.
      NOTE: It is not necessary to try and reposition the bowel back into its original location; attempting so can risk twisting of the bowel or internal hernias.
   2. Reapproximate the abdominal fascia using a running 5-0 non-absorbable monofilament suture. Close the skin with 3-4 skin staples.

6. Postoperative animal care

1. Place the mouse in the recovery cage with a heat lamp. Ensure the temperature of the cage is warm, not hot.
2. Administer a 0.5-1 mL subcutaneous fluid bolus of 0.9% normal saline.
3. Allow the mouse to recover by itself in the warmed cage for ~20 min until active as per institutional protocol, then return to a housing cage.
4. Per institutional protocol, administer carprofen 20 mg/kg at 24 h after surgery on postoperative day 1, and continue daily for 3 days.

7. Aortic measurement and tissue harvest

1. Following euthanasia with isoflurane and cervical dislocation, reopen the abdominal cavity. Extend the incision through the sternum to access the thorax. Excise the right atria and perfuse the left ventricle with 10 mL of cold 1% DPBS solution over 2 min. Resect the lungs, liver, and spleen.
   NOTE: Be careful to not injure the bowel; spillage of enteric contents can impact tissue analysis.
2. Expose the abdominal aorta, and measure the maximal infrarenal aorta diameter, as described above. Continue to dissect the entire aorta and heart. Once the heart and aorta are isolated, cut all arterial branches and common iliac arteries leaving short segments intact on the aorta. Place the heart and aorta on a contrasted background next to a ruler and image.

8. Data analysis and reporting

1. To help account for human error, measure aortic diameters at least 3x each when using handheld calipers, then report the diameter as the averaged value.
2. Define AAA as a 50% increase in the healthy aorta diameter. Be sure to include both the gross aortic

diameters and the percent change in diameter in the study results.

Representative Results

Male and female C57BL/6J mice ages 22-24 weeks were used in this study. Infrarenal aortas were treated with 5 µL of elastase enzyme (6.9 mg protein/mL, 6 units/mg protein) or denatured elastase for 5 min. Elastase-treated male mice demonstrated a 43.4% increase in aortic diameter after 5 min exposure to elastase compared to untreated baseline aortic diameters, while treated female aortas increased by 33.6% (P = 0.0342). Aortic diameters of shams exhibited relatively no change over 5 min exposure to denatured elastase (males 0.5%; females -2.8%). There were no surgically related deaths among the 12 treated and 6 sham mice. Data for the 28-day study is demonstrated in **Table 1**. Of the female treated mice, 3 of 6 died from AAA rupture; one on postoperative day 20 and two on day 25 (**Figure 6**). There were no AAA ruptures among treated males. AAAs (defined as an increase >50% of the baseline aorta diameter or death by AAA rupture) were successfully induced in all treated mice (12 of 12). At 28 days, the average AAA diameter of treated males was 2.86 ± 0.31 mm, with an average change percent change of 257 ± 54%, while AAA diameters of the surviving treated female mice were 3.60 ± 1.87 mm, with an average percent change of 417 ± 286% (**Figure 7**). Sham mice exhibited relatively no change in aortic diameters.

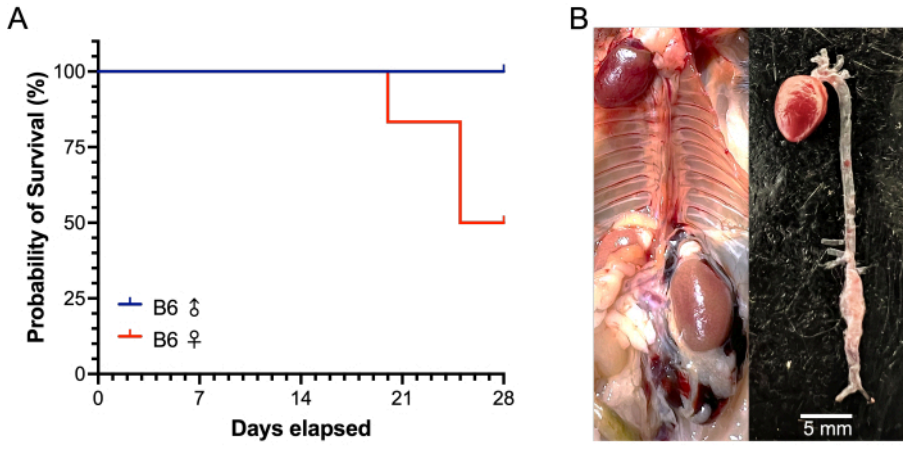

Figure 6: Survival of male and female B6 mice during a 28-day elastase/BAPN model of AAA. (**A**) AAA rupture occurred in 3 of the 6 treated female mice, (one mouse at 20 days, then two mice at 25 days) while there were no ruptures among the 6 treated male mice at 28 days. (**B**) Representative images at necropsy of a female mouse that died from AAA rupture. AAA rupture is demonstrated by a large retroperitoneal hematoma (left) and presence of an infrarenal AAA with a wall defect (right). Abbreviations: BAPN = ß-aminopropionitrile; AAA = abdominal aortic aneurysm. Please click here to view a larger version of this figure.

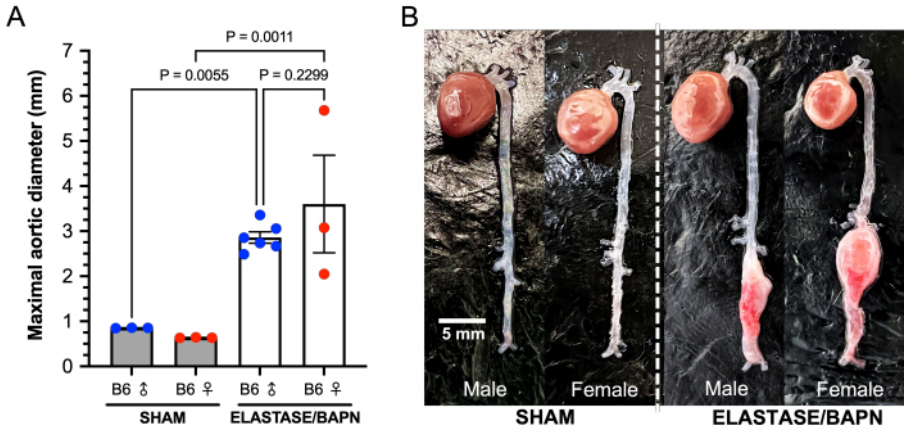

Figure 7: Maximal aortic diameters of elastase/BAPN and sham male and female B6 mice at 28 days. (**A**) Treated mice exhibit significantly larger infrarenal diameters at 28 days compared to shams. (**B**) The combination of elastase and BAPN successfully produces large infrarenal AAAs in both male and female B6 mice. Abbreviations: BAPN = ß-aminopropionitrile; AAA = abdominal aortic aneurysm. Please click here to view a larger version of this figure.

	86 male Sham	86 female Sham	86 male Elastase/8APN	86 female Elastase/8APN
Number of mice	3	3	6	6
Age (weeks)	22.3 ± 0.0	22.7 ± 0.7	23.1 ± 0.2	23.2 ± 0.2
Weight (g; at surgery)	36.3 ± 2.5	23.7 ± 1.2	32.8 ± 1.7*	23.7 ± 0.8
Pre-treatment aortic diameter (mm)	0.89 ± 0.02	0.75 ± 0.04	0.81 ± 0.07	0.73 ± 0.09
Post-treatment aortic diameter (mm)	0.90 ± 0.03	0.73 ± 0.01	1.15 ± 0.03**	0.98 ± 0.12**
Percent change post 5 min treatment (%)	0.5 ± 4.4	-2.8 ± 5.3	43.4 ± 10.2***	33.6 ± 4.5***
AAA incidence (%)	0 / 3	0 / 3	6 / 6	6 / 6
AAA ruptures by 28 days	0 / 3	0 / 3	0 / 6	3 / 6
Survival to 28 days	3 / 3	3 / 3	6 / 6	3 / 6
Maximal aortic diameter at 28 days (mm)	0.85 ± 0.01	0.64 ± 0.01	2.86 ± 0.31*	3.60 ± 1.87**
Percent change aortic diameter at 28 days (%)	-4 ± 2	-16 ± 2	257 ± 54*	417 ± 286**

Table 1: Results of a 28-day model of the elastase/BAPN murine model of AAA. Data are mean ± SD. *P<0.05, **P<0.005, ***P<0.0001 compared to sham of the same sex via one-way ANOVA Fischer's test. Abbreviations: BAPN = ß-aminopropionitrile; AAA = abdominal aortic aneurysm.

Discussion

Understanding the complex pathophysiology of AAA is critical for improving the management of aortic aneurysm disease. While newer strategies are actively developed to improve surgical outcomes, AAAs remain prevalent in our aging society and aneurysm rupture remains a leading cause of death in the United States[10]. Therefore, the unmet needs in AAA detection, prevention, and treatment strategies warrant further foundational aneurysm research[11].

Animal models that accurately and efficiently recapitulate the features and behaviors of human AAAs are essential for mechanistic studies of aneurysm pathophysiology and identifying potential therapeutic targets. While current animal models can mimic the major aspects of the aneurysmal changes that occur in human disease, no single model fully represents the true complexity of human AAAs. Currently, mice are the most widely accepted species for animal AAA modeling. Researchers should consider the various strengths and weaknesses of each murine model for their particular aneurysm study, such as those expertly described in reviews by Daugherty et al. and Busch et al.[12,13].

The use of elastase to induce AAA in rodents was first described by Anidjar et al. in 1990[14]. Perfusing the aorta with porcine pancreatic elastase using a syringe pump creates an initial dilation roughly between 50% and 70%, and the dilated segments favorably demonstrate similar pathologic features

of human AAAs, such as medial degeneration and adventitial inflammation. The classic perfusion model, however, is arguably the most technically challenging aneurysm model, and the aneurysms that are typically formed by the second week begin to gradually resolve thereafter. Bhamidipati et al. in 2012 then demonstrated that adventitial application of elastase could also successfully induce similar aneurysms that are more reproducible in size[15]. A far less challenging model, the topical elastase model became widely adopted in aneurysm research. Additional methodology and advantages of the topical elastase model are discussed in the methods paper by Xue and colleagues[16].

The elastase/BAPN model of murine AAA was developed by Lu and colleagues in 2017[8]. Introducing 0.2% BAPN drinking water improved upon many of the critiques of the classic topical elastase model, now producing aneurysms that continually expand to the point of AAA rupture. In their 2017 study, they demonstrated mice in the elastase/BAPN-treated group had significantly higher AAA formation rates compared to the elastase group (93% vs 65%, $P < 0.01$), which were also more advanced-staged AAAs. Over a 100-day study period, AAAs in the elastase/BAPN group continued to dilate to >800% baseline diameter and formed intraluminal thrombus (53.8%), and 46.2% spontaneously ruptured before the end of the experiment. This model has allowed researchers to investigate factors that may impact aneurysm progression and stability over time.

Berman et al. further explored the elastase/BAPN model by varying the concentration of topical elastase, study duration, timing of BAPN administration, and the impact of animal sex[9]. Treatment with 5 µL of higher concentrated elastase (5 mg/mL or 10 mg/mL) produced larger aneurysms than 2.5 mg/mL over 56 days. The prevalence of intraluminal thrombus formation also depended on the elastase concentration, which occurred in 28.6% of the 5 mg/mL-treated mice, and 62.5% of the 10 mg/mL-treated mice. They also demonstrated the elastase/BAPN model could induce aneurysms in female mice. Although only a few female mice were studied (n=5), they found the aneurysms in females were more prone to rupture (2 of 5 mice) and were significantly larger than male AAAs at 56 days.

In this paper, we aim to provide a method to address one of the largest limitations of surgical modeling, which is the variation in the surgical procedure. Without a clear consensus on the degree of dissection and the area of the aorta treated with elastase, the results of this model could vary dramatically between animals, investigators, and institutions. We have observed numerous anatomical variations between mice, including the number and size of lumbar arteries and veins, and the location of the IMA, takeoff of the left gonadal vein, among others, which can be limiting when attempting to treat only a portion or specific segment of the infrarenal aorta. Here, we demonstrate that circumferentially dissecting the entire length of the infrarenal aorta from the left renal artery proximally to the aortic bifurcation distally helps to provide reproducible degrees of aortic exposure despite anatomical differences while increasing the success of aneurysm induction and providing clear boundaries for the operator. Additionally, the size and more anterior position of the IVC tends to cover a majority of the aorta, which can affect the amount of aorta treated if not isolated from the IVC. While it is necessary to remove the retroperitoneal fascia to expose the aorta, it is important to not fully dissect the connective tissue of the adventitia off the aorta and expose any of the media layers, as this typically results in rupture during the 5 min elastase incubation period. This could serve as an additional internal control to the degree of the dissection

with this model yet can be a frustrating learning curve when adopting this model. Operators will additionally learn higher risk areas (**Figure 4**) that can be easily injured during surgery and lead to uncontrollable hemorrhage.

While it is important that the procedural steps of this model are consistent, the duration of the study and timing of interval ultrasonography can vary depending on the goal of the research. Aortic dilation begins immediately with elastase application, yet studies using this model commonly follow mice for 28 days post surgery[7], as in this example experiment. Extending the study duration should be considered when studying advanced AAAs, long-term growth, intraluminal thrombus formation, or rupture.

Additional perioperative measures, such as maintaining animal body temperature and hydration status can help to improve animal survival of this invasive procedure. The use of a heating pad during surgery and placement in a warm recovery cage can help avoid hypothermia. Saline should be warmed before it is used to irrigate the abdominal cavity. A subcutaneous fluid bolus directly following surgery can account for insensible fluid losses during the operation, and help the animal maintain adequate hydration during the immediate recovery phase. With careful tissue handling and a consistent methodical approach, the elastase/BAPN model can be performed by an experienced operator between 30 min and 45 min per mouse and reliably produce AAA with very low perioperative complications.

Our results demonstrate that the combination of BAPN in addition to circumferential dissection of the infrarenal aorta prior to elastase application produces large, continually expanding AAAs, with larger diameters and rupture incidence at shorter periods. In this experiment, AAAs were successfully induced in all male (6 of 6) and female (6 of 6) mice treated with active elastase. Elastase exposure for 5 min resulted in an immediate increase in aortic diameter by roughly 30-40%, which is helpful in confirming successful and consistent elastase application among treatment groups. Similar to Berman et al., we have shown that this model can induce AAAs in female mice, which also have a greater rupture response than males. Half of the female mice (3 of 6) ruptured within 28 days, compared to 0 of 6 of the males, however, female mice weigh less than males. Male mice demonstrated an increase in AAA diameter by 257% compared to -4% of male controls, while the surviving females showed a 417% diameter increase, compared to -16% of female controls. Aortic diameters were not significantly different between the surviving male and female treated mice at 28 days due to the higher number of ruptures in the female group. We speculate the sham mice exhibit smaller aortic diameters by the end of the study as the aorta tends to dilate slightly during the initial dissection and then, forms scar tissue by 28 days.

The elastase/BAPN model possesses certain limitations. Circumferential dissection of the aorta requires fine surgical skills yet helps improve replicability and the degree of aneurysm induction. Similar to the topical elastase model, there is also a batch effect in elastase enzyme activity, which as mentioned earlier, is therefore important to utilize the same bottle of elastase for all animals in a given experiment. While the incidence of AAA intraluminal thrombus and rupture increases with time and aneurysm severity, these are not guaranteed nor fully predictable in this model.

In summary, the elastase/BAPN model produces large, true infrarenal AAAs in both male and female mice, which progressively expand over time, form intraluminal thrombus, and are capable of rupture. These strengths of this murine model help to better recapitulate some of the behaviors and

characteristics of aneurysms in humans. Although technically difficult, careful and thorough dissection of the aorta can augment the aneurysmal response. Currently, the elastase/ BAPN method is an advanced model for studying infrarenal abdominal aortic aneurysms.

Disclosures

The authors of this manuscript have no conflicts of interest to declare.

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