Video Article

A Model of Cardiac Remodeling Through Constriction of the Abdominal Aorta in Rats

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Abstract

Heart failure is one of the leading causes of death worldwide. It is a complex clinical syndromethat includes fatigue, dyspnea, exercise intolerance, and fluid retention. Changes in myocardial structure, electrical conduction, and energy metabolism develop with heart failure, leading to contractile dysfunction, increased risk of arrhythmias, and sudden death. Hypertensive heart disease is one of the key contributing factors of cardiac remodeling associated with heart failure. The most commonly-used animal model mimicking hypertensive heart disease is created via surgical interventions, such as by narrowing the aorta. Abdominal aortic constriction is a useful experimental technique to induce a pressure overload, which leads to heart failure. The surgery can be easily performed, without the need for chest opening or mechanical ventilation. Abdominal aortic constriction-induced cardiac pathology progresses gradually, making this model relevant to clinical hypertensive heart failure. Cardiac injury and remodeling can be observed 10 weeks after the surgery. The method described here provides a simple and effective approach to produce a hypertensive heart disease animal model that is suitable for studying disease mechanisms and for testing novel therapeutics.

Video Link

The video component of this article can be found at https://www.jove.com/video/54818/

Introduction

Heart failure is a complex clinical syndrome, the symptoms of which include fatigue, dyspnea, exercise intolerance, and fluid retention in peripheral tissues. It is the leading cause of death in developed countries[1]. Aside from inherited cardiomyopathy caused by mutations in sarcomere proteins or ion channels[2], myocardial dysfunction can be caused by a variety of medical conditions, including hypertension, valvular heart diseases, obesity, and diabetes[3]. Changes in myocardial structure, electrical conduction, and energy metabolism lead to inadequate cardiac pumping capacity to meet circulatory demands, which ultimately results in heart failure[3,4]. Investigating the mechanisms underlying heart failure, therefore, is critical in the field of cardiovascular research. Identifying molecular mechanisms leading to heart failure progression can eventually aid in the discovery of novel therapeutic targets or useful biomarkers[1]. It is therefore important to develop heart failure animal models that share key clinical features with heart failure in humans[5].

Cardiac hypertrophy and remodeling plays a critical role in the development of heart failure. Hypertensive heart disease is the key contributing factor of cardiac hypertrophy and the maladaptive remodeling seen in human patients[1]. To mimic these human conditions, animal models are often established through surgical procedures. In particular, the transverse or abdominal aorta can be constricted to increase the resistance against the left ventricle, which ultimately leads to a pressure overload in the heart. This phenomenon usually results in cardiac hypertrophy, a physiological compensation of the cardiomyocytes to meet the functional demand of the cardiovascular system. However, the functional demand overrides the normal physiological compensatory mechanisms, leading to cardiac fibrosis and contractile impairment. Transverse aortic constriction (TAC) surgery often involves complicated procedures, including thoracotomy, mechanical ventilation, and separation of the thymus and fat tissue from the aortic arch. In contrast, abdominal aortic constriction requires simpler experimental techniques[6-8]. The abdominal aorta, between the left and right renal arteries, is constricted during the surgery. Cardiac hypertrophy and remodeling can be observed several weeks after the abdominal aortic constriction surgery[6-8]; they produce robust hypertensive heart disease similar to that generated by the transverse aortic constriction surgery[9,10]. Here, we describe a protocol to conduct abdominal aortic constriction in rats using an efficient, highly-reproducible, and minimally-invasive method. The abdominal aorta adjacent to the renal arteries is constricted by a 0.72 mm loop formed by a 4-0 silk thread. Ten weeks after the surgery, cardiac hypertrophy and remodeling can be observed. The rat model of abdominal aortic constriction-

induced cardiac hypertrophy provides a platform for studying disease mechanisms and pathophysiology, as well as the development of potential therapeutics.

Protocol

All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996). This protocol was approved by and in accordance with the guidelines set forth by the Institutional Animal Care and Use Committee at National Taiwan University.

1. Animal Surgery

1. Prepare a 22 G syringe needle by blunting the tip of the needle on a honing stone. Using pliers, plicate the needle to a right angle.
2. Before surgery, prepare the required surgical instruments and materials, as well as a recovery cage. Autoclave all instruments and surgical supplies before use.
3. Maintain the rats around 200 g and keep them under 12 hr light/dark cycles at a controlled temperature (21 ± 2 °C) with free access to food and water. Include at least 6 rats in each group. Anesthetize the rats with pentobarbital (75 mg/kg in 0.5 ml, i.p.) or another appropriate anesthetic agent. Confirm the depth of anesthesia by testing the tail reflex.
   NOTE: The absence of tail reflex is an indicator of adequate anesthesia.
4. Place a rat in supine position on a surgery platform with a heating pad to maintain the body temperature. Shave the abdominal region of the rat with an animal hair clipper and hair remover lotion to avoid surgical contaminations. Scrub the cleanly-shaven abdomen with betadine or another cleansing reagent before surgery.
   NOTE: It is important to maintain a sterile field throughout the procedure.
5. Make a 2 cm incision along the midline of the abdomen with a scalpel. Using normal saline, keep the abdominal organ moist during the surgery. Displace the digestive organs carefully to the side using cotton balls to expose the inferior vena cava that lies in the posterior peritoneal region. Identify the abdominal aorta, which lies juxtaposed to and usually on the left of the inferior vena cava.
   NOTE: The abdominal aorta is the vessel that pulsates in time with the heart rate.
6. Pierce the peritoneum with a pair of forceps to uncover the vessels beneath. Gently isolate the abdominal aorta adjacent to the renal arteries and pass an 8 cm-long 4-0 silk suture underneath the abdominal aorta between the origins of the right and left renal arteries.
7. Make a loose double knot with the suture; leave a 3 mm diameter loop, and place the blunted and bent 22 G needle inside the loop. Tighten the knot around the aorta and the needle, and then remove the needle immediately to achieve a 0.7 mm diameter constriction.
8. Close the abdominal cavity with 6-0 absorbable suture material. Suture the muscle or skin incisions with simple interrupted sutures. To prevent infection, scrub the surgical site with an iodine tincture.
9. Observe the animal carefully until it regains sufficient consciousness, as indicated by free movement and food intake. Do not leave an animal unattended until it has regained sufficient consciousness to maintain sternal recumbency. To prevent post-surgery pain, treat the rat with acetaminophen (300 mg/kg in 0.5 ml, i.p.).
   NOTE: Users should administer analgesics as approved by institutional policies.

2. Tissue and Blood Sample Collection

1. At 10 weeks post-surgery, weigh the rat and anesthetize it with pentobarbital (75 mg/kg in 0.5 ml, i.p.). Before surgery, confirm the depth of anesthesia by testing its tail reflex. Place the rat on a metal tray.
2. Make a 2 cm incision along the midline of the neck using a scalpel. Displace the muscles carefully with forceps to expose the trachea. Observe carefully to identify the carotid arteries, which are parallel to the trachea and pulsate in time with the heart rate.
3. Collect the blood from the carotid artery into a blood collecting tube coated with EDTA. Immediately centrifuge the blood for 15 min at 2,000 x g and collect the plasma. Store the blood plasma at -80 °C until needed.
4. Make a 5 cm incision at the thoracic region, around the midline of xyphoid process. Pierce the diaphragm with sharp forceps. Using a pair of scissors, cut and remove the rib cage along the mid-clavicular lines on both sides to expose the heart. Excise the heart carefully along the cardiac and vascular borders. Remove the heart gently without grabbing the tissue.
5. Mount the heart on a modified Langendorff perfusion apparatus by tying the aortic trunk to the perfusion needle. Perfuse the heart with Krebs buffer (containing 110 mM NaCl, 2.6 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, and 11 mM glucose [pH 7.4]) to wash out the blood. Weigh the heart and calculate the heart-weight-to-body-weight ratio. Fix the heart with 4% paraformaldehyde on the ice. Remember to wear a mask, since paraformaldehyde vapor is toxic.

3. Tissue Fibrosis Quantification

1. Place the paraformaldehyde-fixed heart tissue on a tissue sectioning device and cut 2 mm thick sections. Place the tissue sections in an embedding cassette. Dehydrate the tissue through a series of graded alcohol baths (50%, 75%, 95%, and 100% for 1 hr each).
2. Infiltrate the tissue with xylene for 1 hr and finally in wax for 1 hr. Place the infiltrated tissue into an embedding cassette and embed with paraffin wax. Store the embedded tissues in paraffin blocks at room temperature until microtoming.
3. Slice the embedded tissue into 4 μm thick sections. Place the sections in a 45 ºC water bath. Dip a glass slide into the water bath at an angle and gradually approach the paraffin section edges to allow partial attachment to the slide.
4. Move the slide in and out of the bath to remove potential air pockets under the tissue section and to facilitate better attachment. Dry the slides at 37 ºC for 1 hr and store them at room temperature for histological staining.
5. Put the slide into a tank. Deparaffinize the slide with xylene for 30 min and rehydrate it in sequentially diluted alcohol (95%, 75%, and 50% for 3 min each) and finally in distilled water. Use adequate picrosirius red solution to completely cover the tissue sections for 1 hr. Rinse the slides in a 0.5% acetic acid solution for two changes, and then perform two rinses in absolute alcohol.
6. Air-dry the slide and mount the slide in synthetic resin with a coverslip. Photograph the slide in a visible light field.

NOTE: The red area in the photograph shows a picrosirius red positive zone under a microscope at 200X magnification. Calculate the percentage of the picrosirius red positive zone over the total area, which indicates the extent of fibrosis[11].

4. Blood Troponin Quantification

1. Quantify plasma troponin levels using an enzyme-linked immunosorbent assay (ELISA). Assay each sample in duplicate. Load 50 µl of blood plasma and 50 µl of an antibody cocktail into the appropriate wells. Seal the plate and incubate for 1 hr at room temperature on a plate shaker at 400 rpm.
   NOTE: Cardiac troponin in the plasma is a marker for cardiac injury.
2. Aspirate the liquid and wash each well with 250 µl of wash buffer three times. After the last wash, invert the plate and blot it against clean paper towels to remove excessive liquid.
3. Add 100 µl of tetramethylbenzidine substrate to each well and incubate for 10 min in the dark on a plate shaker at 400 rpm. Add 100 µl of stop solution to each well. Shake the plate on a plate shaker for 1 min to mix. Record the optical density (OD) at 450 nm.
   NOTE: The troponin concentration is proportionate to the OD value.

Representative Results

10 weeks after the abdominal aortic constriction surgery, the resulting cardiac pathology was analyzed. The cardiac histology was measured by calculating the ratio of the heart weight to the body weight and by detecting the amount of collagen in the heart. Cardiac injury was confirmed by measuring plasma cardiac troponin concentration.

As shown in **Figure 1A**, the cardiac size was enlarged after abdominal aortic constriction surgery, as demonstrated by a higher heart-weight-to-body-weight ratio (**Figure 1B**), an indicator of cardiac hypertrophy. By using picrosirius red staining, fibrotic myocardium stained with an increased collagen content (red) can be distinguished from normal areas (yellow, **Figure 2**). Cardiac fibrosis was increased after abdominal aortic constriction surgery (**Figure 2B**) as compared to controls (**Figure 2A**). The results correlate with plasma troponin concentrations (**Figure 3**). An increased troponin concentration indicates that cardiac remodeling and injury during pressure overload has occurred. Taken together, abdominal aortic constriction results in evident cardiac injury, marked cardiac hypertrophy, and tissue remodeling.
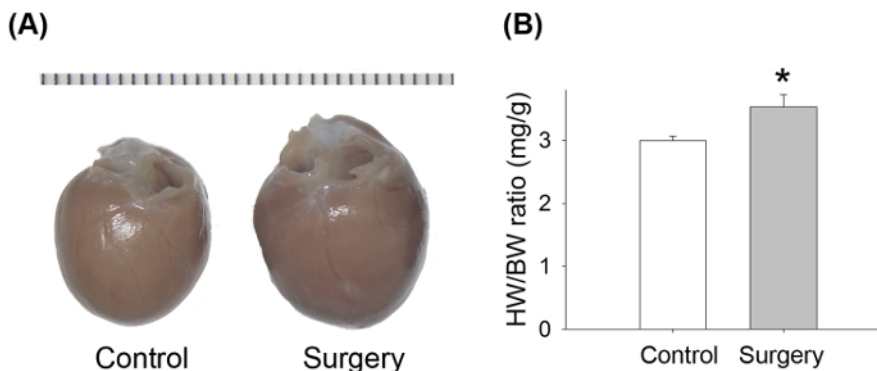

Figure 1: Cardiac Hypertrophy during Pressure Overload. (**A**) Representative heart and (**B**) heart-weight-to-body-weight ratios are shown 10 weeks after abdominal aortic constriction surgery. Data represent the mean ± SEM of six independent experiments performed. Differences between groups were assessed by Student's t-test. *$p < 0.05$ versus control. Please click here to view a larger version of this figure.

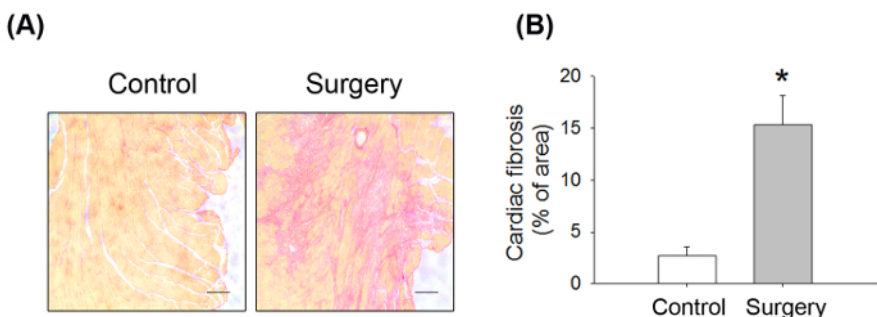**Figure 2: Cardiac Fibrosis during Pressure Overload.**
Picrosirius red staining revealed increased collagen expression. (**A**) Representative picrosirius red staining (Scale bar = 200 µm) and (**B**) percentage of fibrotic area 10 weeks after abdominal aortic constriction surgery. Data represent the mean ± SEM of six independent experiments performed. Differences between groups were assessed by Student's t-test. *$p < 0.05$ versus control. Please click here to view a larger version of this figure.
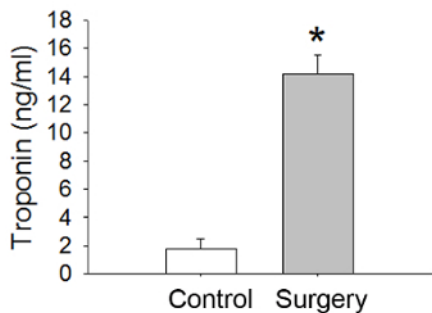
Figure 3: Cardiac Injury during Pressure Overload.
Blood plasma troponin concentrations were measured 10 weeks after abdominal aortic constriction surgery. Data represent the mean ± SEM of six independent experiments performed. Differences between groups were assessed by Student's t-test. *p <0.05 versus control. Please click here to view a larger version of this figure.

Discussion

Hypertensive heart disease, a major health problem that contributes greatly to morbidity and mortality, can lead to cardiac hypertrophy and heart failure[5]. The pathogenesis and progression of hypertensive heart disease in humans is complex, so an appropriate animal model is critical to investigate the underlying mechanisms and to test novel therapeutics that aim to improve cardiac structure and function[5]. The abdominal aortic constriction model, which simulates chronic heart disease, is an effective method for cardiovascular research. The abdominal aorta adjacent to the renal arteries is constricted to induce cardiac remodeling, which eventually leads to cardiac injury. The extent of cardiac remodeling is evaluated by calculating the ratio of heart weight to body weight, performing picrosirius staining for the measurement of collagen expression, and conducting an ELISA-based method for the detection of plasma cardiac troponin levels. The abdominal aortic constriction method allows more reproducible results simulating cardiac remodeling in rat models.

Surgical models of heart disease are advantageous for closely mimicking the pathophysiology of hypertension and aortic stenosis[1]. Most of the currently-available surgical techniques to induce cardiac hypertrophy are conducted through transverse aortic constriction[12,13], which is a common experimental procedure used to create a pressure overload. The sudden onset of hypertension that is achieved causes an approximately 50% increase in left ventricle mass within 2 weeks[5], making the model an excellent choice to examine the molecular mechanisms leading to cardiac hypertrophy. However, transverse aortic constriction requires complex procedures and a high level of surgical skill. The stress associated with open-chest surgery and mechanical ventilation results in high surgical mortality. Moreover, the acute onset of severe hypertension, characteristic of this model, lacks direct clinical relevance[5]. In contrast, abdominal aortic constriction is less technically demanding. The onset and progression of cardiac hypertrophy is gradual, making this model clinically relevant to hypertensive cardiac diseases[1,3]. In addition, renal hypoperfusion by abdominal aortic constriction consequently activates the renin-angiotensin system, and therefore, the same surgical technique can be used as an animal model of kidney hypoperfusion injury[15].

After surgery, cardiac pathology, including the appearance of fibrosis in the heart and changes in cardiac function, develops. In the early stages of hypertensive heart disease, myocardial contractility is enhanced by cardiac hypertrophy to compensate for the pressure overload[10]. In the later stages of hypertensive heart disease, cardiac function decompensates with fibrosis, which leads to heart failure[10]. We did not elaborate on the functional measurements of the heart, including pressure-volume loop analysis[16] and echocardiography[11]. These approaches are invasive or non-invasive methods that are useful for understanding the changes in cardiac function. The time period after surgery can be varied to produce different degrees of cardiac remodeling. The longer the aorta is constricted, the greater the extent of cardiac dysfunction as a result of remodeling. The picrosirius red staining to measure fibrotic area and the ELISA measurements of plasma troponin levels are useful for the assessment of the degree of cardiac remodeling in order to set the endpoints after the surgery.

A key aspect of the surgery to induce cardiac hypertrophy is the clear identification and constriction of the abdominal aorta. Precision in the placement of the constriction site improves consistency of hypertrophy induction time, since variation in constriction placement may affect the length of time required for hypertrophy induction. The closer the stricture is to the heart, the shorter the induction time needed, although the harder it is to isolate the aorta. The abdominal aorta, between the origins of the right and left renal arteries, is a suitable site for performing constriction. The use of a rat model for the abdominal aortic constriction method has great advantages for the study of cardiac hypertrophy and heart failure. A limitation of this approach is that the surgical incision leads to tissue damage and to the secretion of inflammatory cytokines, which are different from the human cardiac hypertrophy and heart failure resulting from hypertension. Furthermore, the use of anesthetics and analgesics should be cautious, since some of these agents are reported to offer cardioprotective effects[17].

The method described here provides a simple and effective approach to produce cardiac remodeling and injury in rats. Our technique is easy to perform, and the results are robust and reproducible. Once our surgical approach is mastered, this procedure will prove to be a useful platform for the investigation of disease mechanisms and the development of therapeutics in cardiac hypertrophy and remodeling.

Disclosures

The authors have nothing to disclose.

Acknowledgements

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