Video Article

Left Atrial Stenosis Induced Pulmonary Venous Arterialization and Group 2 Pulmonary Hypertension in Rat

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Abstract

The mechanism of mitral stenosis-induced pulmonary venous arterialization and group 2 pulmonary hypertension (PH) is unclear. There is no rodent model of group 2 PH, due to mitral stenosis (MS), to facilitate the investigation of disease mechanisms and potential therapeutic strategies. We present a novel rat model of pulmonary venous congestion-induced pulmonary venous arterialization and group 2 PH caused by left atrial stenosis (LAS). LAS is achieved by constricting the left atrium using a half-closed titanium clip. After the LAS surgery, a rat model with a transmitral inflow velocity greater than or equal to 2.0 m/s on echocardiography gradually develops pulmonary venous arterialization and group 2 PH over an 8- to 10-week period. In this protocol, we provide the step-by-step procedure of how to perform the LAS surgery. The presented LAS rat model mimics MS in humans and is useful for studying the underlying molecular mechanism of pulmonary venous arterialization and for the preclinical evaluation of therapies for group 2 PH.

Video Link

The video component of this article can be found at https://www.jove.com/video/58787/

Introduction

The purpose of this article is to demonstrate the step-by-step procedure of how to perform the LAS surgery in rats. Surgically induced LAS closely mimics MS and cor triatriatum in humans, which involve the creation of a mechanical obstruction in the left atrium[1]. Obstruction of the left ventricular (LV) inflow often causes a congestion of the pulmonary venous circulation, and patients gradually develop PH. The World Health Organization classifies PH due to left heart diseases as group 2, which is the most prevalent group of PH[2,3,4]. The diagnosis of PH in patients with left heart diseases is associated with a greater than a sevenfold increase in the 1-year standardized mortality[4]. Currently, there is no approved therapy for group 2 PH apart from treating the underlying left heart diseases (*e.g.*, surgically replacing the stenotic mitral valve). However, even effective mitral valve replacement does not resolve PH fully in up to half of the patients with MS[5]. This persistent PH is due to adverse pulmonary vascular remodeling, which is poorly understood. Hence, animal models are important to enhancing our understanding of the underlying molecular mechanisms of adverse pulmonary vascular remodeling in group 2 PH.

There are a few animal models of group 2 PH. Coronary artery ligation[6,7] and transverse aortic banding[8,9,10] in rodents are the most commonly used group 2 PH animal models. The major disadvantage of these models is the involvement of LV, which makes the outcome of group 2 PH studies difficult to interpret. In contrast, the LV remains intact in the LAS model. Furthermore, the LAS model is clinically relevant because it results in the slow and progressive development of PH over a 10-week period[11]. In humans, MS is considered significant if the transmitral Doppler flow velocity is greater than 2.0 m/s[11], and we also use this number as a cut-off to determine whether the LAS surgery has produced significant stenosis. Furthermore, although the LAS model generates mild or moderate PH, it demonstrates characteristic histologic changes, similar to those in human patients, namely the development of intrapulmonary venous arterialization[11]. The LAS rat model is a novel and clinically relevant group 2 PH model with preserved LV function. It is suitable for studying the pathophysiology of persistent pulmonary vascular remodeling, identifying molecular targets, and testing novel therapies for group 2 PH.

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Protocol

The LAS experimental protocol has been approved by the Jikei University School of Medicine Animal Care Committee and the University Research and Ethics Committee (protocol #2015-118).

1. Pre-operative Preparation

1. After arriving at the animal facility, provide 5-week-old male Sprague Dawley rats between 150 to 200 g with 1 week to acclimate to their new home prior to the operation.
2. Prepare the following equipment before the surgery by autoclaving: 1) a small animal respirator, 2) an anesthetic machine, 3) an intubation kit (composed of a pair of hemostat forceps, a tongue depressor, and an 18 G angiocatheter), 4) surgical instruments (which include a pair of curved forceps, a pair of straight forceps, a needle driver, a chest retractor, a pair of scissors, a 5-0 monofilament suture, a clip applicator, medium-large clips, and a 23 G chest tube).
3. Have sterile Q-tips and gauze ready to deal with bleeding.
4. Use a heating pad to maintain the animal body temperature around 37 °C during the surgery.

2. Anesthesia and Endotracheal Intubation

1. Anesthetize the rat in an induction chamber with 5% isoflurane mixed with 2 L/min room air.
2. Prior to intubation, shave the rat's chest hair with a hair shaver and apply hair removal cream to remove fine hair.
3. Check the pedal reflex to confirm successful anesthesia prior to intubation.
4. Hook the front teeth with a string and secure the string with two pins.
5. Open the rat's mouth with the hemostat forceps and insert the tongue depressor into the mouth.
6. Lift the tongue depressor to visualize the vocal cord.
   NOTE: It is helpful to shine a strong light over the head region of the rat to help visualize the vocal cord.
7. Insert the 18 G angiocatheter as an endotracheal tube into the trachea, and then, quickly, connect the catheter to the respirator.
8. Set the tidal volume to 10 µL per gram with a respiratory rate of 100 breaths/minute.
9. Maintain anesthesia with 2% isoflurane mixed with 2 L/min room air.

3. Preparation of the Surgical Site

1. Prepare the surgical site with alternating scrubs of chlorhexidine and alcohol x3.
2. Give buprenorphine 0.01 mg/kg subcutaneously.
3. Cover the rat with a sterile drape.
4. Check the pedal reflex to confirm a successful intubation and maintenance of anesthesia.

4. Left Atrial Stenosis Surgery

1. Mark the incision site 2 cm below the rat's left armpit with a rule.
2. Make a 2 cm left lateral chest wall incision with a pair of scissors.
3. Separate the intercostal muscles between the fourth and the fifth rib, using the straight and the curved forceps, until entering the chest cavity.
4. Insert the chest retractor into the chest cavity. Continue to use the straight and curved forceps to separate the intercostal muscle to obtain a direct visualization of the thymus and the heart.
5. Lift the thymus with a pair of straight forceps. Remove the thymus covering the heart with a pair of scissors. Avoid cutting or poking into any major blood vessels.
6. Carefully pass a 5-0 monofilament suture through the surface of the left ventricle, right below the left atrial appendage. Avoid passing the needle through the major coronary arteries.
7. After the suture is in place and there is no significant bleeding, tie a loose knot.
8. Pull the suture thread up and forward to lift the heart out of the chest.
9. Once the heart is lifted out of the chest, quickly apply a medium-large clip to the left atrium, just above the mitral valve.
   NOTE: The clip is half-way closed, with the tip of the clip pinching the left atrium, causing left atrial stenosis.
10. Quickly put the heart back into the chest. Ensure the heart is not outside of the chest for longer than 30 s.
11. Remove the stay suture used to lift the heart.
12. Close the chest with a 5-0 monofilament suture, using a simple interrupted pattern.
13. Insert a 23 G chest tube attached to a 10 mL syringe into the chest, and then, proceed with closing the chest wall muscle and skin with simple interrupted sutures.
14. Draw out any air, blood, and pleural effusion *via* the inserted chest tube, using the attached 10 cc syringe, and then, pull the tube.
15. Close the skin layer with a 5-0 monofilament suture, using a simple interrupted pattern.
16. Give buprenorphine 0.01 mg/kg subcutaneously.
17. Turn off the isoflurane.
18. Disconnect the respirator after spontaneous respiration is observed.
19. Keep the rat intubated and allow it to recover on the heating pad until it wakes up.
20. Safely extubate the rat after one or more of the following signs is/are observed: the rat starts moving its four limbs, it regains its righting reflex, it regains its gag reflex, or it displays spontaneous voiding.

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5. Post-operative Care

1. Every 8 - 12 h, give buprenorphine 0.01 mg/kg subcutaneously. Carprofen 5 mg/kg is given subcutaneously on a daily basis for 2 days and, then, as needed, if the rat is not moving around well and looks like it is in pain.
2. Give 5 mL of normal saline subcutaneously right after the surgery, as the rat may have difficulty drinking from the water spigot, immediately postoperative.

6. Confirmation of the Success of the Left Atrial Stenosis with Echocardiography

1. Perform a transthoracic echocardiography 2 weeks after the surgery to determine the LV inflow velocity.
2. Anesthetize the rat following the steps outlined in section 1.
3. After the induction of anesthesia, maintain anesthesia using a nose cone with 2% isoflurane mixed with 2 L/min room air.
4. Shave the rat's chest wall with a hair shaver and use hair removal cream to remove any remaining hair.
5. Place the ultrasound probe at the apex of the heart, which is around the fifth intercostal space on the left side of the chest. Move the probe around in this region until a good four-chamber view is obtained.
6. Measure the LV inflow velocity using the pulsed-wave Doppler mode just above the mitral valve annulus.
7. An LV inflow velocity greater than 2.0 m/s is required for the development of moderate pulmonary hypertension at 8 - 10 weeks post-LAS surgery.

7. Sham Operation

1. Except for applying the clip (step 4.9), perform all the steps above to create age-matched, sham-operated control (SOC) rats.

Representative Results

The effectiveness of the LAS is confirmed using echocardiography, 2 weeks postoperative. Rats with an LV inflow velocity greater than 2.0 m/s, measured with a four-chamber view, are considered to have developed significant stenosis (**Figure 1**) and reliably develop moderate PH and pulmonary venous arterialization 8 - 10 weeks post-LAS surgery.

Ten weeks post-LAS surgery, the rats in the LAS group show left atrial enlargement (**Figure 2B**), pulmonary congestion (**Figure 2E**), right ventricular (RV) pressure overload (**Figure 2F**), and an increased pulmonary venous flow (**Figure 2F**,**G**) compared to rats in the SOC group (**Figure 2A**-**E**). There is also an increased RV systolic pressure in the LAS group *versus* the SOC group (**Figure 3**). A histologic examination of a lung cross section stained with elastic-Van Gieson (EVG) shows increased pulmonary artery (PA) and pulmonary vein (PV) medial thickness, and an increased PV dimension in the LAS group *versus* the SOC group (**Figure 4A**-**D**). Furthermore, alpha-smooth muscle actin (αSMA) immunostaining shows an increased number of smooth muscle cells in the PA and the PV of the LAS group *versus* control rats (**Figure 4E**,**F**). Thus, the LAS model increases muscularization in both the PA and the PV of the LAS rat.

Table 1 summarizes the operative parameters, comparing the SOC group to the LAS group. Specifically, the RV-to-body-weight ratio and lung-to-body-weight ratio are significantly increased in the LAS group *versus* the SOC group. Hemodynamic parameters, including RV systolic pressure, RV end-diastolic pressure, and estimated LA pressure, are significantly increased in the LAS group compared to the SOC group (**Table 1**).

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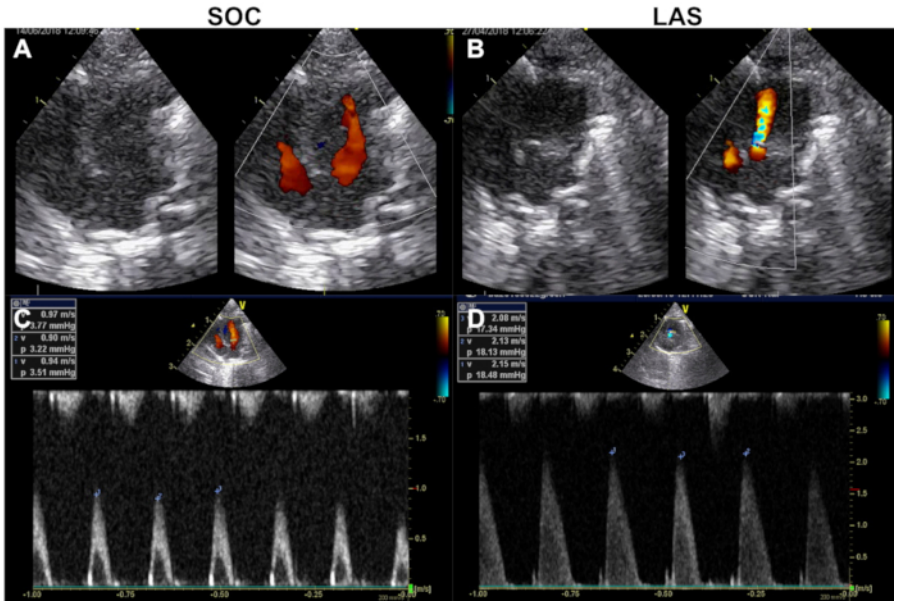

Figure 1: Representative echocardiogram comparing the left ventricular inflow velocity of sham-operated control (SOC) *versus* left atrial stenosis (LAS) rats. (**A**) Four-chamber view and corresponding color Doppler echo of an SOC rat. (**B**) Four-chamber inflow velocity and corresponding color Doppler echo of an LAS rat. (**C**) Peak left ventricular inflow velocity of an SOC rat (0.94 m/s) *vs.* (**D**) an LAS rat (2.12 m/s). Please click here to view a larger version of this figure.

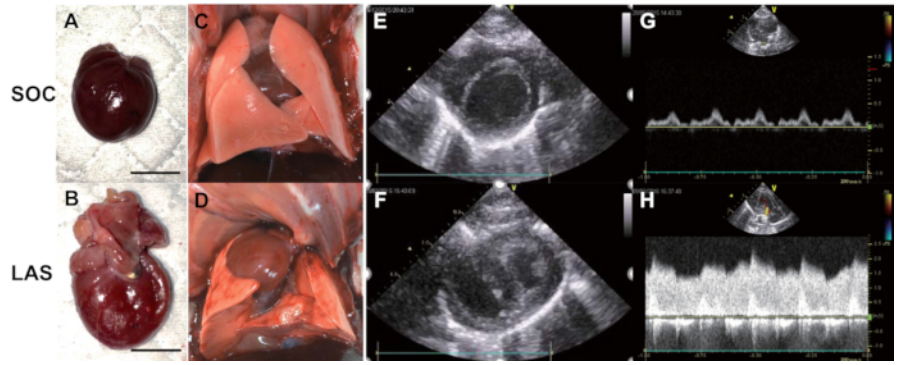

Figure 2: Representative macroscopic and echocardiographic findings in sham-operated control (SOC) *versus* left atrial stenosis (LAS) rats 10 weeks after surgery. (**A**) Macroscopic findings of the heart from an SOC rat *versus* (**B**) the heart from an LAS rat, which show left atrial dilatation. The black scale bar represents 1 cm. (**C**) Macroscopic findings of the lung from an SOC rat *versus* (**D**) the lung from an LAS rat, which show pulmonary congestion. (**E**) Echocardiographic short-axis view of an SOC rat *versus* (**F**) an LAS rat, which shows interventricular septum flattening with increased right ventricular free wall thickness. (**G**) Pulmonary venous flow of the SOC rat *versus* (**H**) the LAS rat, which showed increased PV inflow. This figure is reproduced and modified from Fujimoto *et al.*[11] with permission. Please click here to view a larger version of this figure.

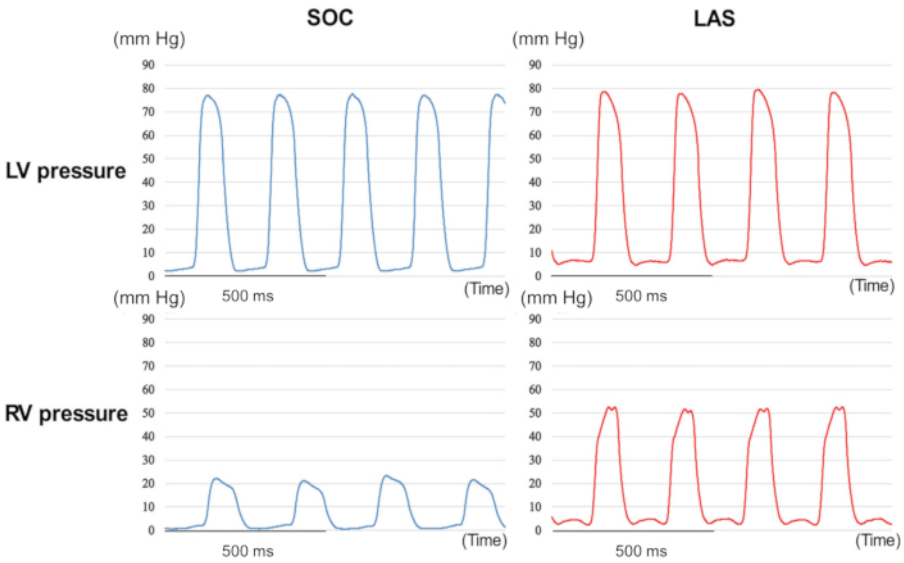

Figure 3: Representative hemodynamic recording of a sham-operated control (SOC) rat *versus* a left atrial stenosis (LAS) rat, showing no difference in left ventricular (LV) pressure but an increase in right ventricular (RV) pressure in the LAS rat. The figure is reproduced and modified from Fujimoto *et al.*[11] with permission. Please click here to view a larger version of this figure.

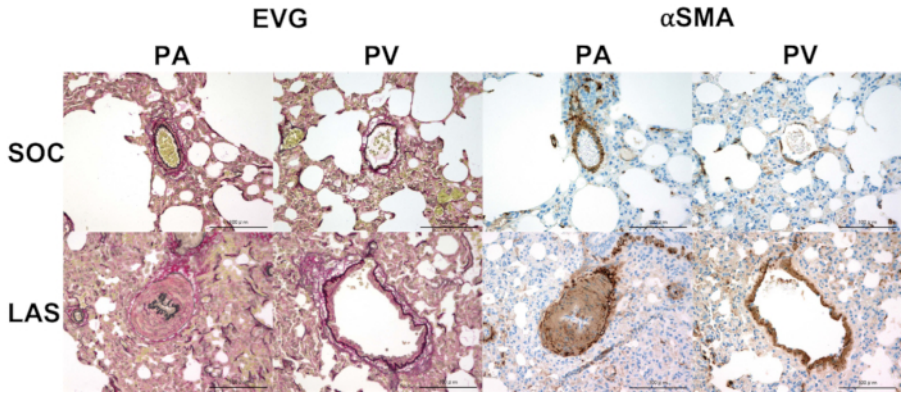

Figure 4: Representative histological changes in sham-operated control (SOC) *versus* left atrial stenosis (LAS) rats, 10 weeks after surgery. Lung cross section stained with elastic-Van Gieson (EVA) shows (**A** - **B**) increased pulmonary artery (PA) and (**C** - **D**) pulmonary vein (PV) thickness and an increased dimension of the PV in the LAS group. Alpha-smooth muscle actin (αSMA) immunostaining shows an increased number of positively stained cells in the vessel walls of the (**E** - **F**) PA and the (**G** - **H**) PV in the LAS group. The scale bars represent 100 µm. This figure is reproduced and modified from Fujimoto *et al.*[11] with permission Please click here to view a larger version of this figure.

Operative Parameters	SOC group (n=5)	Column1	LAS group (n=5)	Column2	Column3
	Median	IQR	Median	IQR	P-value
BW operation (g)	195	190-205	194	190-208	0.98
BW sacrifice (g)	416	410-420	452	390-505	0.65
RV weight/BW	0.39	0.38-0.43	0.54	0.50-0.59	<0.01
LV weight/BW	1.91	1.85-1.95	1.98	1.78-2.20	0.69
RV weight/LV weight	0.2	0.19-0.22	0.27	0.27-0.28	<0.01
Lung weight/BW	0.37	0.36-0.41	0.47	0.42-0.51	<0.01
Cardiac catheterization					
RVSP (mmHg)	18	16-20	40.6	30-50	<0.01
RVEDP (mmHg)	1.6	1.0-2.0	3.4	3.0-4.0	<0.01
LVSP (mmHg)	84	60-80	77.6	70-80	0.72
LVEDP (mmHg)	2.8	2.0-3.0	7.6	7.0-8.0	0.013
RVSP/LVSP	0.22	0.15-0.27	0.52	0.54-0.60	0.021
Estimated LA pressure (mmHg)	7.9	6.8-8.4	28.1	22.8-27.0	<0.01

Table 1: Operative and cardiac catheterization parameters and estimated left atrial pressure in the sham-operated control and left atrial stenosis groups. Abbreviations: SOC = sham-operated control; LAS = left atrial stenosis; IQR = interquartile range; BW = body weight; RVSP = right ventricular systolic pressure; RVEDP = right ventricular end diastolic pressure; LVSP = left ventricular systolic pressure; LVEDP = left ventricular end diastolic pressure; LA = left atrium. This table is reproduced and modified from Fujimoto *et al.*[11] with permission.

Supplemental Figure 1: Landmarks for clip placement and tightness of clip closure. (**A**) One end of the clip is placed next to the base of the pulmonary artery. (**B**) Another end of the clip is placed just above the coronary sinus, halfway across the left ventricle. (**C**) The clip should be halfway closed, so the ends just touch each other. Please click here to download this figure.

Discussion

The LAS rat is a novel group 2 PH model that has already received substantial interest from researchers in the field[12,13]. Comparing to the two existing group 2 models, namely the pulmonary vein stenosis (PVS) model[14], using piglets, and the supracoronary aortic banding (SAB) rat model[8,9,10], the LAS rat model has several advantages. Compared to the PVS piglet model, the LAS rat model costs less to generate and the surgical procedure in rat is less complicated than in piglet. Compared to the SAB rat model, which is the most commonly used group 2 PH animal model, the pathophysiology of group 2 PH in the LAS model is less complicated than in the SAB model, as aortic banding first causes left ventricular failure before developing pulmonary congestion and PH. It is likely that the LAS and SAB models in rodents will be complementary tools to better understand the etiology of group 2 PH.

Two most critical steps in LAS surgery are the placement of the stay suture and the application of the metal clip. Regarding the placement of the stay suture, the choice suture is crucial. Avoid suturing with a cutting needle. Use a monofilament suture, as it produces less drag and friction when passing through the left ventricle. Regarding the application of the metal clip, it is important to identify the surface landmarks. One end of the clip is ideally placed next to the base of the pulmonary trunk and the other end placed just above the coronary sinus, halfway across the LV (**Supplementary Figure 1**). The clip should be halfway closed, so the ends just touch each other (**Supplementary Figure 1C**).

The LAS rat model has several limitations. First, the LAS model is only able to generate moderate PH with PASP around 40 mmHg[11]. We have explored the use of tighter atrial clips, but the operative mortality increased significantly as a consequence. Second, the fast-beating heart made it difficult to accurately place the clip at the desired landmarks. As a result, the success rate is around 50%, due to either a loose band or incorrect clip placement. A modified clip applier with a stopper would improve the consistency of the clip tightness. Third, with the currently available technology, it is still difficult to obtain direct pulmonary arterial pressure and pulmonary capillary wedge pressure measurements in a rat model. Finally, the fidelity of molecular mechanisms in rat PH models to human PH remains questionable, and it remains an area of active investigation.

Despite these limitations, the LAS rat is a clinically relevant, economical, and reproducible small animal model that is suitable for studying the pathophysiology and molecular mechanism of group 2 PH and pulmonary venous arterialization. It can also serve as a workhorse for the preclinical testing of novel therapies developed to treat group 2 PH.

Disclosures

The authors have nothing to disclose.

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