Mouse cardiac surgery: comprehensive techniques for the generation of mouse models of human diseases and their application for genomic studies

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Tarnavski, Oleg, Julie R. McMullen, Martina Schinke, Qing Nie, Sekwon Kong, and Seigo Izumo. Mouse cardiac surgery: comprehensive techniques for the generation of mouse models of human diseases and their application for genomic studies. Physiol Genomics 16: 349–360, 2004. First published December 16, 2003; 10.1152/physiolgenomics.00041.2003.—Mouse models mimicking human diseases are important tools in trying to understand the underlying mechanisms of many disease states. Several surgical models have been described that mimic human myocardial infarction (MI) and pressure-overload-induced cardiac hypertrophy. However, there are very few detailed descriptions for performing these surgical techniques in mice. Consequently, the number of laboratories that are proficient in performing cardiac surgical procedures in mice has been limited. Microarray technologies measure the expression of thousands of genes simultaneously, allowing for the identification of genes and pathways that may potentially be involved in the disease process. The statistical analysis of microarray experiments is highly influenced by the amount of variability in the experiment. To keep the number of required independent biological replicates and the associated costs of the study to a minimum, it is critical to minimize experimental variability by optimizing the surgical procedures. The aim of this publication was to provide a detailed description of techniques required to perform mouse cardiac surgery, such that these models can be utilized for genomic studies. A description of three major surgical procedures has been provided: 1) aortic constriction, 2) pulmonary artery banding, 3) MI (including ischemia-reperfusion). Emphasis has been placed on technical procedures with the inclusion of thorough descriptions of all equipment and devices employed in surgery, as well as the application of such techniques for expression profiling studies. The cardiac surgical techniques described have been, and will continue to be, important for elucidating the molecular mechanisms of cardiac hypertrophy and failure with high-throughput technology.

DESPITE MAJOR REDUCTIONS in mortality rates for cardiovascular disease during the past 30 years, this disease continues to be the most common threat to life and health. The lifetime risk of developing coronary heart disease after the age of 40 is 49% in men and 32% in women. An estimated 8% of the United States population (~20 million people) have some form of heart disease (12). In 1999, cardiovascular disease claimed 958,775 lives in the United States. More than 2,600 Americans die of cardiovascular disease each day, an average of 1 death every 33 s (1). By 2020 it is predicted that cardiovascular disease will annually claim 25 million deaths worldwide, and coronary heart disease will surpass infectious disease as the world’s number one cause of death and disability (4).

The substantial decline in the mortality rate due to cardiovascular disease was attributed, in part, to the use of innovative preventive and therapeutic measures, which came about from extensive cardiovascular research. Historically, a large portion of cardiovascular research has been performed using laboratory animals (5, 11, 14, 23, 32). A vast number of animal models have been developed to imitate human diseases and investigate physiological and pathological processes. The tremendous achievements in molecular biology and genetics within the last decade have opened up new horizons for the management of heart disease. The demand for animal models has increased because a high proportion of genes are common in animals and humans (4).

In the last decade, animal surgery in cardiovascular research has largely shifted from the rat to the mouse. In the past, the major advantage of using the rat in surgery was due to its larger size (~10 times bigger than the mouse). However, the appearance of more sophisticated microdissecting microscopes and diligent microsurgical instruments has now made surgery in the mouse as feasible as in the rat (23). Mouse models have gained popularity for a number of reasons. These include small size, rapid gestation period (21 days), large litter size, and relatively low maintenance costs. Moreover, the mouse genome has been extensively characterized, and gene-targeted “knockout” and transgenic overexpression experiments are performed using mice, rather than rats (23). It is now clear that similar genes and signaling pathways regulate the development of the heart and vasculature in mice and humans. Molecular genetics will most likely play a critical role in revolutionizing the ability to diagnose and treat patients with cardiovascular diseases (4).

To date, there are no schools or courses available for animal surgeons to attain and develop the necessary technical skills to perform surgical techniques in mice, and very few publications exist describing these techniques. Consequently, the number of laboratories that are proficient in performing cardiac surgical procedures in mice has been limited. To perform surgical procedures in mice, investigators are usually limited to brief descriptions in journal articles, and then they must work out the details of the surgical model by trial and error. Even for those with considerable surgical experience, this empirical approach is usually very time-consuming, not always successful, and often involves the loss of a number of animals. Furthermore, the outcome of these interventions can be highly variable, resulting in the use of high numbers of animals to attain statistically significant results.

Microarray technologies measure the expression of thousands of genes simultaneously, allowing for the identification of genes and pathways that may potentially be involved in the disease process. The statistical analysis of microarray experi-
mments is often confounded by the number of samples that are being studied and the amount of variation within the experiment. Biological variation is influenced by genetic or environmental factors and is likely to be greater in surgical models compared with transgenic models due to the variability in the surgical procedure. To keep the number of required independent biological replicates and the associated costs of the study to a minimum, it is critical to minimize experimental variability by optimizing the surgical procedures.

The aim of this publication was to provide a comprehensive description of techniques required to perform mouse cardiac surgery, such that reproducible results are attained, allowing for the use of these models for high-quality genomic studies. Specifically, a detailed description of the intubation of the mouse and three major surgical procedures used in cardiovascular research have been provided: 1) aortic constriction (left ventricular pressure-overload model), 2) pulmonary artery constriction (right ventricular pressure-overload model), and 3) myocardial infarction (MI) including ischemia-reperfusion. We guide the investigator step-by-step through the whole procedure from the initial handling of the mouse for anesthesia to its full recovery after surgery. These guidelines do not attempt to replace surgical training with a skilled animal surgeon, but instead attempt to enable trained researchers to optimize their procedures to maximize their success and to minimize technical variability. All procedures follow the “Guide for the Care and Use of Laboratory Animals” of the Institute of Laboratory Animal Research, Commission on Life Sciences, National Research Council. Emphasis has been placed on technical procedures with the inclusion of thorough descriptions of all equipment and devices employed in surgery, as well as the application of such techniques for genomic studies.

MATERIALS AND METHODS

Animal Ethics

All surgical procedures described in this publication were performed in accordance with the National Institute of Health standards and approved by the Institutional Animal Care and Use Committee of Beth Israel Deaconess Medical Center.

Strain of Mice

Different strains of mice were utilized depending on genetic manipulations and conditions investigated. The most common strains used were FVB/N and C57BL/6. Surgical procedures were performed on transgenic, nontransgenic, knockout, and wild-type mice. For experiments described in this publication adult male mice (11–12 wk) were used. At this age, developmental growth of the heart is complete. The weight of mice at this age ranged between 25 and 30 g.

Anesthesia

The surgical procedures described are relatively short; thus it is not necessary to withhold food and water from mice prior to surgery. Pentobarbital sodium (70 mg/kg) was chosen as the anesthetic because it provides an adequate depth of anesthesia for 30–40 min. For longer procedures such as ischemia-reperfusion, an additional dose of 30 mg/kg is necessary during the course of the surgery. The anesthetic is injected intraperitoneally with a short 27-gauge 1/2-inch needle. The mouse should sit for 5–7 min in an empty cage while the anesthesia takes effect. It is important not to disturb the animal prematurely, since this will agitate the mouse and affect the quality of the subsequent procedure.

The most useful indicator to ensure that an adequate depth of anesthesia has been attained is the toe-pinch reflex. The toe of the hindlimb should be pinched firmly between the operator’s fingernails. If the mouse attempts to withdraw its limb, then it is not sufficiently anesthetized and a top-up dose should be given (~10–20% of the initial dose). When there is no response to the toe pinch, medium-deep anesthesia has been attained.

Presurgical Preparation

The animal’s chest is shaved with an animal hair clipper (Harvard Apparatus, size 40 clipper blade). The mouse is then positioned on an operating table for the subsequent intubation. A simple small Styrofoam platform (a cover from a commercial freezer container) serves well as an operating table (a platform with ladder-shaped edges is optional, Fig. 1A). The noninvasive method, described by Brown et al. (5), is similar to the one described here in principal but requires some extra equipment. The method described in this publication is simple, reliable, and requires no more than proper restraint of the mouse as described above, curved forceps (used as a laryngoscope, Roboz catalog no. RS-5228), and a light source for the transillumination of the neck. The intubation tube is made from a 20-gauge intravenous catheter (Fisher catalog no. 9706397), cut at exactly 25 mm (1 inch) in length, attached to a connector (10 mm piece of PE-190 tubing; 1.19 mm ID, 1.70 mm OD). The extender should be kept to the right of the mouse (for the subsequent procedure.

The platform with the mouse should be brought to the very edge of the table with the head of the mouse directed toward the operator. The curved forceps (Roboz catalog no. RS-5228) and intubation tube with the extender should be kept to the right of the mouse (for the right-handed operator). Between surgeries the intubation tube is kept in alcohol for disinfection. Before use, the tube should be shaken vigorously to remove any remaining fluid within the tube, to avoid aspiration by the mouse. A drop of 1% lidocaine is put on the tip of the tube to numb the throat and reduce the gag reflex. A power light with flexible horns [Microvideo Instruments (MVI), Avon, MA; catalog no. MVI-DGN] is used to illuminate the neck of the mouse.
To enhance the view during the intubation and operation, we use binocular lenses with 1.75× magnification (SCS, Stony Point, NY; catalog no. H10L1.75×). The operator should then kneel down so that the head of the mouse is at eye level. The tongue of the mouse is held with the curved forceps held in the operator’s right hand, it should be moved to the left, then using the left hand the tongue is held with thumb and index finger and moved up slightly. The forceps (still held in right hand) are put under the tongue to hold it firmly to the lower jaw (the forceps should be kept strictly horizontal with the ends turned up and opened 1–2 mm). The vocal cords and trachea (as a light hole closing like a valve) should then be visualized. The operator should carefully move the forceps to his/her left hand preserving the same position of the tongue. With the operator’s free right hand, the catheter of the intubation tube is gently inserted into the trachea until the connector starts going into the oral cavity (Fig. 1 C). The extender is then gently detached using forceps, and the connector should stay on the tube. Restraint and intubation of the mouse takes ~5 min.

Ventilation

For artificial ventilation a mouse ventilator 687 series (Harvard Apparatus) is used. The tidal volume and ventilation rate are calculated from formulas provided by the company

\[ \text{Vt} = 0.0062 \times \text{Mb}^{0.86} \]

Where Vt is tidal volume, and Mb is animal mass in kg.

Ventilation rate (breaths/minutes) = \( 53.5 \times \text{Mb}^{-0.26} \)

A list of tidal volumes and ventilation rates for mice of particular body weights is shown in Table 1 (R. Toubeau, Harvard Apparatus, personal communication). Periodic maintenance of the ventilator is important, because the plunger in the pump gets worn, resulting in a decreased tidal volume.

A modified Y-shaped connector (Fisher catalog no. 15-320-10A) is used to attach the mouse to the ventilator (Fig. 1 D). The lower edge of the connector has been cut off to reduce the dead space. A short 5-mm piece of PE-240 tubing is inserted into that end to be used as an adapter. The connector is attached to the ventilator with regular intravenous infusion system tubing. The Y-shaped connector is attached to the Styrofoam platform with a piece of tape (5 mm in width). All pieces of tape holding the mouse in position, except those on the upper legs, should be removed and the mouse positioned to the proximity of the Y-shaped connector. The mouse is positioned and secured on its right side with the chest rotated and exposed to the operator at a 45° angle to the plane of the table (Fig. 2). The connector of the intubation tube (beveled) should then be inserted into the adapter of the Y-shaped connector (the operator should be careful while making this connection so as not to leave a large dead space or, on the other hand, not to block an airway). The operator should now

<table>
<thead>
<tr>
<th>Mass, g</th>
<th>Vt, ml</th>
<th>Ventilation Rate, breaths/min</th>
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<tbody>
<tr>
<td>20</td>
<td>0.12</td>
<td>148</td>
</tr>
<tr>
<td>30</td>
<td>0.18</td>
<td>133</td>
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<td>40</td>
<td>0.24</td>
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<td>50</td>
<td>0.30</td>
<td>117</td>
</tr>
<tr>
<td>60</td>
<td>0.36</td>
<td>111</td>
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Vt, tidal volume (does not account for system dead space).
visually confirm rhythmic movements of the chest synchronized with the ventilator. The ventilator is set up to 133 breaths/min and a tidal volume of 0.2 ml (accounts for system dead space) for a 30-g mouse (Table 1); 100% oxygen (2 liters/min) is loosely connected (via intravenous catheter) to the inflow of the ventilator to provide a supplement to room air. After intubating the mouse, the hind legs and tail should be fixed to the platform with strands of tape 5 mm in width and secured with wider pieces of tape (Fig. 2). All the steps described above are used for all of the open-chest surgeries. At this point the operator can proceed with the surgery itself.

If an accidental extubation should occur during surgery, then the chest immediately by placing a suture on the skin. After that the mouse should be re-intubated. Attempting to re-intubate the mouse with the chest open will usually result in loss of the animal.

General Considerations for Surgery and Tools

Procedures involving the measurement of physiological parameters such as blood pressure require body temperature to be strictly maintained between 36–37°C. By contrast, the majority of surgeries described here can be performed at room temperature (20–25°C). The body temperature of the mouse may fall to 33–34°C after anesthesia, but this is not a problem for short surgical procedures (10–15 min). For longer procedures (i.e., ischemia-reperfusion model), the body temperature should be strictly monitored.

For the operations described, six basic surgical tools are used: 1) curved forceps (Roboz catalog no. RS-5228) used for the intubation, 2) straight forceps (Roboz catalog no. RS-5130), 3) curved forceps (Roboz catalog no. RS-5101) used for the operations, 4) scissors with large rings (Roboz catalog no. RS-5850), 5) chest retractor (Roboz catalog no. RS-6510; the two proximal pairs of teeth (closest to the junction) should be removed with the other two pairs left in place], and 6) needle holder (Roboz catalog no. RS-6410). Occasionally some other tools are used, and these are described further in the text. The only optics necessary for the surgical procedures described is a binocular lens (SCS Limited catalog no. H10L1.75×) with a 1.75× magnification. For illumination a power light with flexible horns (MVI catalog no. MVI-DGN) is used.

As routinely practiced in small animal surgery, the operations are carried out under aseptic conditions. Surgical tools are autoclaved once a day prior to surgery. The same instruments are used for 5–10 mice; however, between operations they are sterilized in a hot bead sterilizer (Harvard Apparatus catalog no. HA61-0183). We use 6–0 nylon for the chest cavity and skin closure, 6.0 Vicryl (absorbable suture) for suturing the muscles, and 7–0 silk for manipulations on the heart.

Before surgery the operating field is disinfected with Betadine solution and 70% alcohol three times in alternation. To prevent contamination of the surgical site during the operation, a large transparent sterile plastic drape should be placed over the mouse, leaving only the operating field exposed. A set of sterile gloves is used for each individual mouse. For hemostasis it is usually sufficient to use a piece of gauze or cotton applicators, although in some instances cautery is necessary, as indicated in the text.

Operation I: Ascending Aortic Banding (Left Ventricular Pressure-Overload Model)

Aortic banding is a very popular method to induce left ventricular (LV) hypertrophy in mice and mimics human aortic stenosis with development of pressure-overload-induced LV hypertrophy. The aortic band can be placed on either the ascending, transverse, or descending portion of the aorta depending on the study design. In this publication we describe constriction of the ascending aorta because it provides a more direct and rapid source of pressure overload on the LV with a significant degree of hypertrophy after 48 h (6). The method has been described (with some modifications) in a number of scientific papers (9, 11, 14), but technical details were very limited.

The landmark for the incision is the level of the left axillary (“armpit”, with paw extended at 90°). Before the incision, a local anesthetic (0.1 ml of 0.1% lidocaine) is injected subcutaneously. A transverse 5-mm incision of the skin is made with scissors 2 mm away from the left sternal border, 1–2 mm higher than the level of the armpit (Fig. 2A). The operator should try to visualize the superficial thoracic vein that runs under the skin at the lateral corner of the incision. Both layers of thoracic muscles should be cut, taking caution to avoid the vein; damaging this vein is not life-threatening but can result in profuse bleeding. Cotton applicators are sufficient to stop the bleeding. Through the thin and semitransparent chest wall, the ribs and inflating lung should be visible. The chest cavity is opened with scissors by a small incision (5 mm in length) at the level of the second intercostal space (in this space the very tip of the lung is visible) 2–3 cm from the left sternal border. It is important not to approach the sternal border any closer than this, because the internal thoracic artery (running along the sternal border of the inner surface of the thoracic cavity) can be easily damaged, and intense bleeding may occur. If the artery is damaged, cautery may be used to stop the bleeding. While opening the chest wall, extreme care should be taken not to damage the underlying lung. The chest retractor is gently inserted to spread the
wound 4–5 mm in width, taking care to avoid the lungs getting caught in the teeth of the retractor. The view will be obstructed by the thymus and fat, which should be pulled away with forceps to the left arm of the retractor. With two forceps the pericardial sac should be gently pulled apart and attached to both arms of the retractor. Note that the pericardial sac and thymus are intimately connected to the left superior vena cava which runs immediately next to the left side of the heart (mice and rats have two superior venae cavae in contrast to humans). While being mobilized, the pericardial sac should not be pulled apart with too much force, since it may rupture the wall of the left superior vena cava. The great vessels and upper part of the left atrial appendage can then be visualized. With the curved forceps the ascending portion of the aorta is bluntly dissected from the pulmonary trunk on the right (extreme caution should be taken, since bleeding from any of the great vessels is fatal). From the left side, the curved forceps are placed under the ascending aorta. The tips of the forceps should appear on the right side between the aorta and the pulmonary trunk (a slight poking movement is required to go through the connective tissue between the aorta and pulmonary trunk). Then, 7–0 silk is grasped by forceps and moved underneath the aorta, and a loose double knot is made (7–10 mm diameter loop). For aortic constriction of 11- to 12-wk-old mice, we use a 25-gauge needle with OD 0.51 mm. The size of the needle depends on the amount of stenosis and degree of hypertrophy/failure desired. The needle should be blunted and bent to make an L-shape in advance. To hold the needle in position, a “needle holder” construct was made (Fig. 3A). The needle is attached to a 1-ml syringe, which is held on a retort stand by means of two rods (Harvard Apparatus catalog no. AH 50-4415 and no. AH 50-4407), connected by a two-ball joint connector (Harvard Apparatus catalog no. AH 50-4431). The needle is delivered through the loose double knot from the left side and placed directly above and parallel to the aorta. The loop is then tied around the aorta and needle and secured with the second knot (this should be done very quickly, to minimize ischemia and a buildup of pressure) (Fig. 4). The needle is immediately removed to provide a lumen with a stenotic aorta. Two more knots are made to secure the tie. The chest retractor is then removed, and the thymus is moved back to its normal position. Since the lungs were partially compressed by the retractor, they should be reinflated by shutting off the outflow on the ventilator for 1–2 s using a finger. This step is important, since collapse of the lungs will result in respiratory distress.
and poor recovery after surgery. The chest cavity is closed by bringing together the second and third ribs with one 6–0 nylon suture (taking care not to suture the lungs). While making a knot, slight pressure is applied on the chest with the needle holder to reduce the volume of free air in the chest cavity. All layers of muscle and skin are closed with 6–0 continuous absorbable and nylon sutures, respectively. The wound is treated with betadine. With practice, the whole procedure takes ~15 min. An important control for aortic banding as well as the subsequent operations described below is the sham surgery. For the sham operation the mice undergo a similar procedure but the interventricular septum is left intact and no ligature is placed. Great care must be taken while performing this dissection, as its location and fragile nature makes it very vulnerable to injury. The lungs are then reinflated and the chest is closed as described above.

The subsequent postoperative care is identical for all the operations and has been described in detail in a later section (see Postoperative Care).

**Operation II: Pulmonary Artery Banding (Right Ventricular Pressure Overload Model)**

The pulmonary artery banding model mimics pulmonary coarctation in humans. Pulmonary arterial stenosis is usually a congenital syndrome. It can be presented as an isolated form but is commonly associated with other cardiovascular abnormalities such as tetralogy of Fallot. It may also be seen as a sequela of congenital rubella or Williams, Noonan, or Alagille syndrome (12).

In cardiovascular research pulmonary arterial banding has been used as a model of right ventricle (RV) hypertrophy and failure (27). In small animals, the pulmonary banding model has not been as widely used in research as the aortic banding model. This may be explained by the much lower prevalence of RV hypertrophy and failure compared with the left in humans (22), as well as certain difficulties in performing the surgery itself. To our knowledge, there is only one report in the literature describing the technique of pulmonary artery banding in mice. This method was described in 1994 by Rockman et al. (27), but it lacked sufficient technical details to be reproduced by other investigators.

The major challenges which arise during this surgery are due to 1) the extremely thin fragile walls of the pulmonary trunk and 2) the inability of the RV to withstand stress while the pulmonary artery is being manipulated. The blood pressure in the RV is very low compared with that in the left. Thus any dissection underneath the pulmonary trunk using forceps leads to blockage of blood flow to the lungs, and this results in immediate respiratory and cardiac distress. Consequently, the animal may not recover. To overcome this complication, special techniques are required (see below).

In theory, pulmonary artery banding is technically similar to aortic banding. However, some unique tools and devices are employed to aid in the success of this surgery. The access to the great vessels is facilitated in the same manner as described for aortic banding. After mobilization of the pericardium, the pulmonary trunk (partially covered by the left atrium) should be visualized. The position of the atrium creates an additional complication, as its location and fragile nature makes it very vulnerable during the dissection. The pulmonary trunk should be bluntly dissected with curved forceps from the aorta (on the left) and left atrium (on the right). Great care must be taken while performing this dissection. The dissection should be relatively superficial, and no attempt should be made to go underneath the pulmonary trunk at this stage. To create a tunnel underneath the pulmonary trunk, an L-shaped 28-gauge blunted needle is used (an insulin syringe with the needle attached serves as a convenient tool). The needle should be placed from the side of the pulmonary trunk closest to the left atrium and gently pushed underneath the pulmonary trunk so that the end of the needle appears between the pulmonary and aortic trunks. A poking movement is required to rupture the connective tissue between the trunks. The shape of the needle allows a tunnel to be created underneath the pulmonary trunk without compromising the pulmonary blood flow, which as noted above, would not be possible with the use of forceps. Throughout the dissection, special attention should be paid to the atrium and the walls of the pulmonary artery, as these structures are very fragile and bleeding is invariably fatal. The next step involves placing the ligature behind the pulmonary trunk. This step is aided by the use of a special device. A provisional patent application has been filed by the inventor, Dr. P. Hu. The device named “a wire and snare” consists of an intravenous catheter with a needle and port. The device was designed to perform minimally invasive transverse aortic banding and atrial septectomy in rodents (12). Inquiry regarding the device should be addressed to Dr. P. Hu or University of Utah Technology Transfer Office. With the help of this device the 7–0 silk suture is moved behind the pulmonary trunk. The suture is then caught with forceps between the pulmonary and aortic trunks, and the catheter is removed. A loose double knot is made (7–10 mm diameter loop). For moderate stenosis the suture is tied against a 25-gauge needle. A more severe stenosis can be created in 23- to 25-g mice using a 26-gauge needle (0.46 mm OD) (27). The needle held in position using the “needle holder construct” (see Operation I: Ascending Aortic Banding) is introduced into the loop, above and parallel to the pulmonary trunk. For easier access, the operating table with the mouse can be temporarily rotated 90° in a clockwise direction. The loop is then tied around the pulmonary trunk and needle (and secured with a second knot) swiftly so as not to severely compromise pulmonary blood flow). The needle is immediately removed to provide a lumen with a stenotic pulmonary pulmonary artery. At this point the heart rate noticeably slows as the RV has difficulty withstanding the buildup in pressure. To improve the heart rate and override this condition, the tail of the mouse is pinched with forceps. This stimulus usually accelerates the heart rate instantly. If the RV cannot compensate for the increase in pressure, then the heart rate may not improve, usually resulting in loss of the animal. This is an unavoidable complication of this surgery. Two more knots are made to secure the tie. The chest retractor is removed, and the thymus is moved back to its physiological position. The lungs are reinflated, the chest cavity is closed with 6–0 nylon suture, and the muscles and skin are closed layer by layer with 6–0 absorbable and 6–0 nylon sutures, respectively. The entire procedure takes ~15–20 min. For the sham operation, dissection of the pulmonary trunk is performed using the L-shaped needle but a ligature is not placed or tied. The lungs are then reinflated, and the wound is closed as previously described.

**Evaluation of ventricular pressure created by aortic and pulmonary banding, 1) LV pressure measurement for the evaluation of ascending aortic banding.** Assessment of the pressure in the LV is important for ensuring that the aortic banding operation was successful and that the LV pressure is similar in different experimental groups. Because of the position of the band, it is not feasible to pass the catheter into the ventricle retrogradely from the right carotid artery. In this case the method of choice is the direct pressure measurement via the LV apex. The mouse is anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) given at 0.1 ml intraperitoneally. The mouse is intubated and fixated in the same manner as described for surgeries. The animal is ventilated with 100% oxygen (as a supplement) at a tidal volume of 0.2 ml at 133 breaths/min (Harvard Respirometer, model 687). The temperature should be maintained at 36–37°C using a temperature controller connected to a rectal probe (Fine Scientific Tools, TR-100). The rectal probe is inserted after the mouse has been intubated and ventilated. The chest is opened via an incision through the left 4th intercostal space, and the pericardial sac is carefully pulled apart to visualize the apex of the heart. A pulse-sample 7–0 silk suture is placed on the apex, and a superficial nick is made with an 18-gauge needle inside the purse-string. A 1.4-Fr high-fidelity pressure catheter (Millar catheter) is placed through the nick into the ventricular chamber. Before tying the purse string around the catheter, it is important to ensure that the
catheter is correctly positioned. While recording pressure signals at 2 kHz, the catheter should be gently moved back and forth to ensure the catheter is not hitting the ventricular wall or the signal is not being dampened by trabeculae. Pressure signals are then recorded at 2 kHz for 10 min, stored to disk, and analyzed using PowerLab software (Chart 4.1.2, ADInstruments). Anesthetics and open-chest surgery are known to depress heart rate (15, 35). Thus it is important to monitor heart rate during the pressure recordings to ensure pressure measurements were acquired under similar experimental conditions.

2) RV Pressure Measurement for the Evaluation of Pulmonary Artery Banding. This technique is similar to the one described for the LV, but the surgical access is different. An incision is made in the left third intercostal space down to the level of the sternum (cautery is used at this point to stop bleeding). Three 5-0 silk sutures are placed on the ribs (two on the second and one on the third rib) to spread the edges of the wound apart. The whole heart can now be visualized covered from the top with thymus. Thymus and the pericardium are removed to facilitate the view. The front wall of the RV should be visualized in the wound. A superficial incision is made at the midportion of the RV with an 18-gauge needle prior to insertion of a 1.4-Fr high-fidelity pressure catheter. Since the wall of the RV is much thinner than that of the left, a common complication can be that the RV is punctured during this process (i.e., before inserting the catheter while making the superficial incision). Bleeding can be stopped with cotton applicators. The catheter is secured in place with a purse-string 7-0 silk suture. Pressure signals are recorded at 2 kHz for 10 min, stored to disk, and analyzed using PowerLab software (Chart 4.1.2, ADInstruments).

Operation III: Myocardial Infarction Model and Ischemia-Reperfusion

In the United States ~1 million patients annually suffer from acute MI, and in nearly all cases this results from coronary atherosclerosis (4). In a variety of animal models this condition has been mimicked by ligation of the left coronary artery. Reperfusion of the ischemic heart has become an important therapeutic intervention for MI. However, the process of reperfusion, although beneficial in terms of myocardial salvage, also results in cell death and scar formation due to a process known as reperfusion injury (4). The underlying mechanisms involved in ischemia-reperfusion are not well understood.

The murine model of MI and ischemia-reperfusion has been widely described in the literature (2, 10, 13, 21, 26, 34), and the procedure was described in reasonable detail by Michael et al. (26). However, the technique of correctly visualizing the left anterior descending (LAD) coronary artery and temporarily occluding the LAD during reperfusion can be very difficult. In the current publication we have placed more emphasis on technical approaches and devices utilized in this surgery which significantly aid in the success and reproducibility of this surgical technique.

1) Myocardial infarction. The landmark for the incision is the left armpit. Lidocaine, 0.1 ml of 0.1% solution, is injected subcutaneously. An oblique 8-mm incision is made 2 mm away from the left sternal border toward the left armpit (1–2 mm below it) (Fig. 2B). The muscles are separated as previously described (see Operation I: Ascending Aortic Banding) with caution taken to avoid damaging blood vessels. The rib cage and moving left lung are then visualized. The 4th intercostal space represents the area between those ribs where the lowest part of the lung is observed. The chest cavity is then opened taking caution not to damage the lung. The chest retractor is inserted and opened gently to spread the wound 8–10 mm in width. The heart partially covered by the lung is then visualized. The pericardium is gently picked up with curved and straight forceps, pulled apart, and placed behind the arms of the retractor. This resulting in bleeding the lung up slightly, mobilizing it and providing better exposure of the heart. The LAD coronary artery should then be visualized as a pulsating bright red spike, running in the midst of the heart wall from underneath of the left atrium toward the apex. Certain experience is needed to properly visualize the artery, since it can easily be confused with the vein (correct lighting is critical for this step, it should be focused but not too bright). If the LAD artery cannot be visualized, the left atrium can be lifted so that the origin of the LAD artery from the aorta is located.

The position of the ligation depends on the volume of infarction desired. For most studies, the LAD artery is ligated 1–2 mm below the tip of the left auricle in its normal position, which induces roughly 40–50% ischemia of the LV (Fig. 5). Once the site of ligation has been determined, the curved forceps are used to gently press on the artery a little below the subsequent ligation (this will enhance the view of the artery and stabilize the heart). Next, with a tapered needle a 7-0 silk ligature is passed underneath the LAD coronary artery (Fig. 5A). For easier and smoother passage the needle should be bent in advance to make the curvature rounder. When placing the ligature under the artery, it is important not to enter the cavity of the ventricle with the needle, but at the same time not to be too superficial, as the ligature will cut through the wall of the ventricle. The ligature is then tied with three knots. Occlusion is confirmed by the change of color (becoming pale) of the anterior wall of the LV. The retractor is removed, and the lungs are reinfalated by shutting off the ventilator outflow as previously described (see Operation I: Ascending Aortic Banding). The chest cavity is closed by bringing the superficial incision, and the 5th ribs with one or two 6-0 nylon sutures (with pressure applied to the chest wall to reduce the volume of free air). The muscles and skin are closed layer by layer with 6-0 absorbable and nylon sutures, respectively. The duration of the whole procedure takes ~12–15 min. The sham-operated mice undergo the same procedure without tying the suture but moving it behind the LAD artery. The chest is closed as described above.

2) Ischemia-reperfusion. The goal of this operation is to create transient ischemia of the LV by the temporary occlusion of the LAD coronary artery. We routinely occlude the LAD coronary artery for 60 min. An immediate complication of this surgery is cardiac arrhythmia, which may occur after the restoration of blood flow (reperfusion). To prevent arrhythmia, two intraperitoneal doses of lidocaine may be given at specific time points during the procedure (see below). For the ischemia-reperfusion model it is essential to monitor body temperature for two reasons. First, the procedure is considerably longer than that described for the other operations; thus the body temperature of the mouse will fall dramatically during this period. Second, infant size is dependent on the time of the occlusion as well as body temperature. Thus it is critical that both are kept constant during this procedure. Body temperature should be maintained at 36–37°C with a heating pad monitored by a rectal probe, connected to a temperature controlling device (Fine Science Tools). The rectal probe is inserted after the mouse has been intubated and ventilated.

In the ischemia-reperfusion operation the occlusion is made around fine PE-10 tubing (0.28 mm ID, 0.61 mm OD; VWR catalog no. 63018-623) to minimize the damage of the artery and facilitate the removal of the ligating suture. All of the steps are the same as described for the MI model up to the point at which a 7-0 silk ligature is passed underneath the LAD coronary artery. Then a loose double knot is made to leave a 5- to 6-mm diameter loop, and the PE-10 tubing is placed into the loop. The correct positioning of the tube on the beating heart can be difficult. To aid in this procedure, we designed a construct. A 5- to 6-cm piece of PE-10 tubing is attached to a 30-gauge needle, which has been bent to a 60° angle. The needle (with the tubing) is placed on a 1-ml syringe and is attached to a stand by two rods and a two-ball joint connector (Fig. 3B). The tubing is then introduced into the loop from the distal side directly above and parallel to the LAD artery until it touches the left auricle (1–2 mm above the base of the suture). The loop is then tied around the LAD artery and tubing and secured with two additional knots (the operator should be careful not to cut through the ventricle wall). Next, the tubing is cut with durable curved scissors (Roboz catalog no. RS-5983) 1–2 mm
distal from the suture to leave a piece 3–4 mm in length. Occlusion is confirmed by the dramatic change in color of the anterior wall of the LV (it becomes very pale). The first dose of lidocaine (6 mg/kg) is then given intraperitoneally. The chest retractor is removed, the lungs are reinflated, and the wound is temporarily closed with two interrupted 6–0 nylon sutures on the skin. An additional dose of pentobarbital sodium (30 mg/kg) should then be given intraperitoneally. The mouse remains on the ventilator for the duration of the 60-min LAD artery occlusion.

The second dose of lidocaine (6 mg/kg) is injected intraperitoneally immediately before the reperfusion. The temporary sutures are removed, and the chest cavity is opened with the chest retractor. The pericardium is moved apart to expose the heart. Holding the tubing with curved forceps, the ligating suture is gently cut with a no. 15 sterile surgical blade, and the suture and PE-10 tubing are removed. Reperfusion is confirmed by return of the pink-red color of the anterior wall of the LV (it may take 15–20 s). Next, the lungs are reinflated, and the chest cavity, muscles, and skin are closed layer by layer with 6–0 absorbable and nylon sutures, respectively. The duration of the whole procedure amounts to ~85–90 min.

When performing ischemia/reperfusion and making comparison of infarct size, it is important to verify that the area at risk was similar between groups. The area at risk is determined by the site of placement of the ligature around the coronary artery. During surgery, the ligature suture can be a slipknot, left in place during reperfusion after removal of the PE-10 tubing. For assessment of the area at risk/infarct size just prior to euthanasia, the slipknot and suture that was left in place can be retied and the heart perfused with ink to delineate the area at risk.

Postoperative Care

Postoperative care for all the surgeries described is critical to ensure full recovery after surgery. The measures taken are directed to alleviate pain, provide supplementary heat to prevent hypothermia, and control respiratory depression (33). Immediately after the operation, 0.5 ml of 37°C saline can be given intraperitoneally, and a dose of analgesic (buprenorphine, 0.1 mg/kg) is given subcutaneously and then every 8 h for the next 48 h. The mouse is moved to another ventilator in a designated recovery area, and 100% oxygen is con-

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**Fig. 5. Myocardial infarction (MI).** A: the needle with 7–0 silk suture is passed underneath the LAD coronary artery for subsequent ligation. The visibility of the artery has been digitally enhanced. B: the position of the suture on the LAD coronary artery (illustrated by the red cross). C: 24 h after MI operation: position of the suture, and the size of the area at risk (pale zone) after injection of 2% Evans Blue into the left renal vein.
Table 2. Ventricular pressure measurements in mice subjected to ascending aortic constriction and pulmonary artery constriction (severe stenosis: 26 gauge)

<table>
<thead>
<tr>
<th></th>
<th>LV Overload, Ascending Aortic Banding</th>
<th>RV Overload, Pulmonary Artery Banding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Band</td>
</tr>
<tr>
<td>Number</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>229±8</td>
<td>276±29</td>
</tr>
<tr>
<td>Ventricular SP, mmHg</td>
<td>68.9±2.7</td>
<td>136.9±11.4*</td>
</tr>
<tr>
<td>Ventricular DP, mmHg</td>
<td>0.9±0.6</td>
<td>5.1±1.3*</td>
</tr>
</tbody>
</table>

All pressure measurements were made using a 1.4-Fr high-fidelity pressure catheter (Millar catheter). LV, left ventricular; RV, right ventricular; SP, systolic pressure; DP, diastolic pressure. Statistical analysis was performed using an unpaired t-test. *P < 0.05 compared with sham.

RESULTS

Peri-Operative Survival

In the current study peri-operative survival rates for aortic constriction, pulmonary artery constriction (severe stenosis: 26 gauge), MI, and ischemia-reperfusion were 93.0% (n = 250), 81.2% (n = 100), 96.5% (n = 250), and 96.2% (n = 100), respectively. The lower survival rate for pulmonary artery constriction reflects the inability of the RV to compensate for the increased ventricular pressure immediately after placement of the band in some mice.

Aortic Banding and Pulmonary Artery Banding

Ascending aortic and pulmonary artery banding are important tools for examining the responses of transgenic and knockout mice to a pathological stress. The pressures developed in the ventricles using aortic and pulmonary artery banding are presented in Table 2. The peak systolic pressures and heart rates were relatively low in the present study. This can be explained by the effects of anesthesia and the invasive technique used to measure LV pressures in open-chest mice. It is noteworthy that our values for LV pressures are similar to those reported in a previous study using similar open-chest techniques (15). Mice subjected to pulmonary artery banding display a significant increase in RV weight after 4 days, 1 wk, and 2 wk compared with sham-operated mice (Table 3), and dilation of the RV is obvious on histological examination (Fig. 6).

Using the surgical procedures described in this publication, we previously found that ascending aortic banding resulted in an increase in the heart weight/body weight ratio of 40–45% in nontransgenic mice compared with sham-operated mice (for sham, normalized heart wt/body wt = 1.00 ± 0.02, n = 10; for aortic band, normalized heart wt/body wt = 1.43 ± 0.05, n = 13 (25, 30)). Furthermore, aortic banding was associated with interstitial fibrosis and re-expression of fetal genes, e.g., atrial natriuretic peptide and brain natriuretic peptide, none of which was detected in sham-operated mice (25, 30). Utilizing this aortic banding model, we were able to demonstrate that mice expressing a dominant negative mutant of phosphoinositide-3 kinase (dnPI3K) are more susceptible to a pathological stress than nontransgenic littermates (25). At baseline, cardiac function (assessed by echocardiography) of sham-operated dnPI3K transgenics and nontransgenic mice was normal (dnPI3K fractional shortening, 51 ± 5%, n = 5; nontransgenic fractional shortening, 53 ± 2%, n = 10); however, after aortic banding the cardiac function of dnPI3K transgenics was depressed compared with nontransgenic mice (dnPI3K, 33 ± 3%, n = 8; nontransgenic, 49 ± 4%, n = 13; P < 0.05) (25).

Myocardial Infarction

MI is associated with extensive remodeling and thinning of the LV after 48 h, and extensive collagen deposition is apparent in the infarcted area after 1 wk (Fig. 7A). The collagen fibers stain blue with the Masson’s trichrome stain. Assessment of determining infarct size has been well described in the literature (3, 7, 16, 17, 28, 36) and will not be described here. To gain valuable information from genomic studies, it is critical that variation between mice subjected to the same procedure is kept to a minimum. Within-group comparisons from three independent biological replicates should ideally have higher correlation coefficients compared with samples from between groups, e.g., sham operation vs. MI. A large variation in phenotype will result in low correlation coefficients. Using hearts from mice subjected to MI or the sham operation, we calculated Pearson correlation coefficients from microarray data. Within-group comparison of microarray data derived from three independent biological replicates of mice subjected to MI or the sham operation resulted in mean correlation coefficients of 0.973 ± 0.021 and 0.989 ± 0.005, respectively. These correlation coefficients are similar to those

Table 3. Postmortem analysis of mice subjected to pulmonary artery banding (severe stenosis: 26 gauge)

<table>
<thead>
<tr>
<th>Time point</th>
<th>Sham</th>
<th>4 days</th>
<th>1 wk</th>
<th>2 wk</th>
<th>2 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Body wt (BW), g</td>
<td>26.6±0.3</td>
<td>18.4±1.2*</td>
<td>23.6±0.2</td>
<td>24.3±1.9</td>
<td></td>
</tr>
<tr>
<td>Tibial length (TL), mm</td>
<td>15.5±0.1</td>
<td>15.5±0.2</td>
<td>15.7±0.1</td>
<td>15.4±0.2</td>
<td></td>
</tr>
<tr>
<td>RV wt, mg</td>
<td>24.2±1.6</td>
<td>36.0±2.0*</td>
<td>39.1±8.5*</td>
<td>38.3±1.3*</td>
<td></td>
</tr>
<tr>
<td>RV/BW, mg/g</td>
<td>0.91±0.06</td>
<td>1.99±0.18*</td>
<td>1.66±0.37*</td>
<td>1.59±0.09*</td>
<td></td>
</tr>
<tr>
<td>RV/TL, mg/mm</td>
<td>1.56±0.11</td>
<td>2.33±0.15*</td>
<td>2.49±0.53*</td>
<td>2.49±0.06*</td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis was performed using one-way ANOVA followed by Fisher’s PLSD. *P < 0.05 compared with sham.
observed for within-group comparisons of microarray experiments from transgenic mice (mean correlation coefficient = 0.987 ± 0.009) and nontransgenic mice (mean correlation coefficient = 0.985 ± 0.003). In contrast, the mean correlation coefficient for the comparison between sham and MI at the 1 wk time point, which is clearly associated with marked differences in phenotype, was relatively low (correlation coefficient = 0.698 ± 0.088). Within-group and between-group variation of the MI data set are also presented visually on a color correlation matrix (Fig. 7B). Based on the color of the grids illustrating normalized expression, there is good reproducibility for within-group comparison and significant differences between sham and MI.

Minimizing within-group variation allows for the identification of a greater number of genes which may be critical for the phenotype observed, i.e., sham vs. MI. At 48 h postsurgery, 1,395 genes were differentially expressed between sham-operated mice and those subjected to MI (infarcted area) at a \( P \) value of 0.01. The raw data generated from GeneChip arrays were analyzed using Affymetrix Microarray Analysis Suite as described (http://www.cardiogenomics.org).

Further Applications

Further applications of the surgical methods can be found at http://www.cardiogenomics.org (select ”Animal Models of Cardiomyopathy” then “Current Animal Models”), including details about experimental procedures, histology images, and microarray data.

DISCUSSION

Microarray analysis is becoming a standard tool for detecting genes that are differentially expressed in various disease states. The most recent generation of microarrays can evaluate...
the expression of up to 34,000 genes. Because of the vast amount of data that is being generated in such studies, statistical quantification is a standard requirement for analysis and interpretation, rather than the qualitative description of observations. Microarray experiments are generally costly and time-consuming (19); therefore, many investigators choose to perform only a limited number of independent biological replicates. Microarray studies are confounded by a number of sources of variation, ranging from measurement error, technical variation induced during the RNA extraction, probe labeling, hybridization, and to biological variation. It has been shown that the cumulative contribution of these sources of variation in a cDNA microarray experiment can cause the correlations between samples obtained from individual inbred mice to be as low as 30% (8). Biological variation is one of the largest contributing factors to variation in microarray experiments. Therefore, it is critical that experimental variation is kept to a minimum so that small sample sizes can provide statistically significant results. We show here that the application of the described methods leads to consistent results. We show here that the applicability of the described methods leads to consistent results.

The motivation for writing this paper was to provide a detailed description of surgical techniques used in cardiac surgery so that other investigators could generate these models and attain reproducible results with low experimental variation. A number of investigators have performed some of these surgical procedures, but because of journal space constraints it has largely been impractical for authors to provide descriptions in sufficient detail for these techniques to be successfully duplicated. Consequently, the large majority of papers utilizing mouse cardiac surgery have come from a relatively small number of laboratories, and other laboratories have looked for simpler alternatives (18).

Here we have described three major surgical procedures used in cardiovascular surgery, i.e., aortic constriction, pulmonary artery banding, and MI and ischemia-reperfusion. We have also described the construction of some devices which we developed or improved and found critical for obtaining reproducible results. We are not suggesting that our methods are more successful than some of those previously described by pioneers in this field (9, 26, 27). However, such publications lack information regarding the complications and pitfalls which can be associated with each of the techniques described. Yet, knowledge of these complications is critical for the final outcome of the surgery, as well as for obtaining reproducible results. The recommendations described here are based on optimizing the survival rate and obtaining consistent results from each of the models. Ultimately, this is cost efficient and reduces the number or animals required for such studies.

The mouse models described in this paper have been critical for evaluating the differential expression of genes in cardiac pathological states and are likely to be important for the development of more effective therapeutics for the treatment of patients with heart disease.

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GRANTS

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