Video Article

A Mouse 5/6th Nephrectomy Model That Induces Experimental Uremic Cardiomyopathy

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Abstract

Chronic kidney disease (CKD) is a great risk factor for cardiovascular disease events and mortality, and progressively develops to the clinical phenotype called "uremic cardiomyopathy". We describe here an experimental CKD mouse model, named 5/6th partial nephrectomy (PNx) with pole ligation, which developed uremic cardiomyopathy at four weeks post-surgery. This PNx model was performed by a two-step surgery. In step-one surgery, both poles of the left kidney were ligated. In step-two surgery, which was performed 7 days after the step-one surgery, the right kidney was removed. For the sham surgery, the same surgery procedures were performed but without pole ligation of the left kidney or removal of the right kidney. The surgical procedures are easier and less time-consuming, compared to other methods. However, the remnant functional renal mass is not as easily controlled as the renal artery ligation. Four weeks after surgery, in comparison with the sham-operated mice, the PNx mice developed impaired renal function, anemia, cardiac hypertrophy, cardiac fibrosis, and decreased heart systolic and diastolic function.

Video Link

The video component of this article can be found at https://www.jove.com/video/55825/

Introduction

CKD, also known as chronic renal failure, is a progressive loss of kidney function over time that eventually develops into permanent kidney failure. CKD, from early stage renal disease states to end-stage renal disease (ESRD), is a great risk factor for cardiovascular disease events and mortality, and progressively develops to the clinical phenotype called "uremic cardiomyopathy"[1,2,3]. The uremic cardiomyopathy in patients with CKD or ESRD is associated with cardiovascular abnormalities, mainly caused by overload of left ventricular (LV) pressure and/or volume, leading to LV hypertrophy (LVH), LV dilation, and LV systolic dysfunction[4,5,6]. Cardiac fibrosis is another common pathological process of uremic cardiomyopathy that reduces cardiac compliance resulting in LV diastolic dysfunction. Severe cardiac fibrosis can lead to sudden cardiac death even in those without cardiac symptoms[7].

The 5/6th PNx is a commonly used CKD animal model for animal studies involving renal failure, uremic cardiomyopathy and hypertension. PNx is achieved by ablation of 5/6 renal parenchyma. The rat model was initially developed with the two most common protocols employed being surgical resection or infarction. The rat PNx model is an extremely useful model to study uremic cardiomyopathy with substantial elevations in blood pressure, cardiac hypertrophy and impaired diastolic function. Later, mouse PNx models, operated with the similar techniques as the rat model, were developed due to the wide availability and ease of making genetic manipulations in the mouse system.

It is well-documented that systemic oxidant stress is a constant feature of both clinical and experimental uremic cardiomyopathy[8,9]. Furthermore, oxidant stress contributes to the uremic syndrome[10], and plays a critical role in the pathogenesis of the cardiac abnormalities seen in uremic cardiomyopathy[11,12,13]. To this point, we have demonstrated that the rodent 5/6th PNx model causes physiological, morphological, and biochemical features of uremic cardiomyopathy[14,15,16,17]. In the mouse PNx model described here, PNx-operated mice developed significant oxidative stress, at least partially mediated by Na/K-ATPase signaling function, which is critical in PNx-mediated experimental uremic cardiomyopathy. Attenuation of the Na/K-ATPase signaling not only reduces oxidative amplification, but also ameliorates the phenotypical changes in PNx-mediated experimental uremic cardiomyopathy[18].

Protocol

All animal care and experiments were approved by the Marshall University Institutional Animal Care and Use Committee (IACUC) in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. Male C57BL/6 mice (10-12 weeks old) were

housed in a pathogen free animal facility in designated rooms equipped with cages that supply purified air under a 12 h light/dark cycle. Food and water were supplied *ad libitum*.

1. Surgery Preparation

NOTE: The surgical instruments and materials are obtained from different sources that are not specific to surgery operations. Instruments and materials from other sources can also be used for the same purpose. See the **Table of Materials** for a list of surgical instruments.

1. **Preparation for the surgery**
    1. Place the following within reach of the operating table: warming pad, halogen lamp, oxygen tank and isoflurane tank, isoflurane vaporizer, hair clipper, hair removal cream, scales, sterilized operating set, sterilized surgical linen, cotton swabs, 70% alcohol, polyhydroxydine solution (containing 1% iodine), 0.9% NaCl solution, 1 mL syringes and needles (30 G), 3-0 and 4-0 silk sutures, buprenorphine and penicillin, antibiotic ointment, and lubricant eye ointment.
    2. Clean a cage and place a heat pad under the bedding for mice housing after surgery.

2. Step-one Surgery: Pole Ligation of Left Kidney

NOTE: Maintain sterile conditions during the surgery. Cover the autoclaved surgical instruments with a sterile pad. Keep more than one set of surgical instruments in hand for more than one surgery to prevent cross contamination during the surgery. If the instruments need to be used again, in the case of multiple surgeries, disinfect the instruments with betadine solution and 70% ethanol, and then sterilize in a germinator glass bead sterilizer for 5 min. Disinfect the operating area with 70% ethanol. Wear a gown, mask (to cover nose and mouth), cap (to cover head), and a pair of sterile gloves. Change gloves after each surgery.

1. Wash and disinfect hands and wear sterile surgical gloves. Wear a head cap and a mask.
2. Disinfect the table with 70% alcohol. Apply lubricant eye ointment to both eyes of the mouse to prevent eye dryness while under anesthesia.
3. Place the mouse in an induction chamber of an isoflurane vaporizer system and induce anesthesia with a mixture of 2.5% isoflurane with 100% oxygen (0.8 - 1.2 L/min) for 1 - 2 min.
   NOTE: The efficacy of anesthesia was determined by the absence of pain reflex elicited by pinching the tail, as well as by the absence of palpebral reflex by touching the eyelid.
4. Inject penicillin (40,000 unit/kg body weight, IM) and buprenorphine (0.02 mg/kg body weight, SC) to prevent infection and pain during the surgery.
5. Place the mouse on a warming pad (initially set at 42 °C) with a rectal thermometer to maintain and monitor the body temperature around 37 °C during the procedure. Place the mouse nose into the nose cone and ventilate with a mixture of 0.8-1.2% isoflurane with 100% oxygen (0.8-1.2 L/min) during the surgery.
6. Place the mouse on the right side. Shave the hair of surgical area with a hair clipper and then remove with the hair removal cream. Disinfect the site with polyhydroxydine solution (containing 1% iodine) followed by 70% ethanol. Align autoclaved surgical linen on the surgical area.
7. Make a ~1.0 cm lumbar incision and separate the muscle and fascia to expose the left kidney using the scalpel and forceps (**Figure 1a**).
8. Pull out the left kidney very gently by pulling on the perirenal fat with small blunt forceps. In the process, in order not to disturb the adrenal gland, move the adrenal gland upwards from the kidney with forceps.
9. Using blunt forceps, gently tease the connective tissue and adrenal gland from the superior end of the kidney towards the middle pylorus of the kidney where blood vessels enter and exit. Identify and isolate the ureter.
   NOTE: The ureter lies around the inferior half of the kidney embedded in the connective tissue and clearly visible under light.
10. Gently grab the connective tissue and separate from the kidney tissue to ensure that the ureter is not ligated.
11. Before polar ligations, use a sterile ruler to identify the superior and inferior 1/3 part of kidney. The mouse kidney usually is around 1.25 - 1.5 cm long.
12. Ligate using a 3-0 silk suture string and with proper force, approximately 0.4 cm from the superior portion towards the middle of pylorus and similarly, 0.4 cm from the inferior portion towards the pylorus (**Figure 1a**, dotted lines).
13. After ligating both poles (**Figure 1b**), allow a 2-min observation time to make sure that there is no bleeding.
    NOTE: In the process, it is very important to avoid ligation of the adrenal gland, renal artery, and ureter. If the left ureter is ligated, the mouse will die after removal of the right kidney in the step-two surgery. The ligation force can restrict blood flow to the poles without causing intra-renal bleeding. Within 1 - 2 min after ligation, the poles are discolored due to the limit of blood flow to the poles.
14. Push the left kidney back to the original place. Close the muscle and skin with 4-0 and 3-0 silk suture strings, respectively. Apply antibiotic ointment to the surgical area for better recovery and disinfection.
15. After ligation, house the mouse separately in a cage with a heat lamp over the cage so that the bedding area is close to 37 °C (monitored with a thermometer), before placing the animals in individual cages.
    NOTE: Monitor the mouse until it has regained sufficient consciousness to maintain sternal recumbency.
16. Allow easy access to water and food during recovery. Administer penicillin and buprenorphine every 12 h for the first 3 days post-surgery to prevent infection and pain.
    NOTE: The mouse should recover from the trauma of the surgery within 2 - 3 days and should be back to normal eating, drinking and moving. At this time, house the mouse individually for full recovery. The following must be monitored: drinking, eating, walking patterns, awkward gait, hunched back, ocular and/or nasal discharge, and aggressive behavior. Euthanize the mouse if it shows any sign of seizure, coma, untreatable infection, difficulty to walk, loss of gait, unable to eat and drink, and loss of more than 15% pre-operation weight.

3. Step-two Surgery: Removal of Right Kidney

1. Seven days later, expose the right kidney as described for the left kidney in steps 2.1 to 2.10, except place the mouse on its left side.
2. Place the right kidney on the linen and clear surrounding fat and connective tissue with blunt forceps.

3. Identify the renal artery and vein, and then place two ligatures (3-0 silk) each with a single loose knot around the vessels. Gently tease the connective tissue and adrenal gland from the superior end of the kidney towards the middle pylorus of the kidney using blunt forceps. Gently displace the ureter from the inferior end and tie all the vessels together (3-0 silk) without causing bleeding.
4. Move the two loose ligature knots along the vessels. One towards the abdominal aorta side, and the other one towards the kidney side.
5. First tie off the ligature knot (towards the abdominal aorta side) with double knots; a solid knot will change the color of the right kidney. Tie off the ligature knot (towards the kidney side) with double knots. Leave enough space between these two double knots so as to cut the vessels between the two knots without cutting the ligature knots.
6. Cut the renal vessels between the two knots and remove the right kidney. Check for possible bleeding of the renal vessels.
7. Dry the right kidney using sterile gauze and weigh it.
8. Close the muscle incision with 4-0 silk suture string and then close the skin incision with 3-0 silk suture string.
9. House the mouse separately in a clean solitary cage with a heat lamp over the cage (step 2.15) with easy access to water and food for at least 24 h to recovery. Administer penicillin and buprenorphine every 12 h for 3 days to prevent infection and pain.
   NOTE: The mouse should recover from the trauma of the surgery within 2 - 3 days, and should be back to normal eating, drinking and moving.
10. After surgery, monitor the mice twice daily for three days, and then once a day for the duration of the experiment. Follow the post-surgical monitoring and treatment procedures described in step 2.16.

4. Sham Surgery

1. For the sham surgery, perform the same surgery procedures, including exposure of the kidney, dissection of tissue, and wound closure, but without the pole ligation of left kidney or removal of right kidney.

5. Evaluation of Experimental Uremic Cardiomyopathy

1. One day before sacrifice, perform a transthoracic echocardiography and capture images[18].
   NOTE: Here, the transthoracic echocardiography was performed with a mouse handling table and rectal thermometer. The echocardiographic images were captured using an 18-38 MHz operating frequency transducer attached to an imaging system. Relative wall thickness (RWT), myocardial performance index (MPI), and left ventricle mass index (LVMI) were calculated as we described[18].
2. At the time of sacrifice, measure the body weight. Measure hematocrit (HCT) with a HCT centrifuge as per manufacturer's instructions. Prepare the plasma samples using heparin-coated tubes by centrifuging the blood samples for 10 min at 1,500 x g at 4 °C in a tabletop refrigerated centrifuge. Measure cystatin C, creatinine and BUN using kits following the manufacturer's instructions. Perform the measurements in duplicate.
3. At the end of the experiment, anesthetize the mice with ketamine (90 mg/kg, IP) and xylaxine (10 mg/kg, IP) and sacrifice by thoracotomy. Measure the heart weight after removing it from the chest. Prepare cardiac (LV) and kidney homogenates, as well as determination of Type-1 collagen (COL-1), phosphorylation of c-Src and protein carbonylation, as described before[18,19].

Representative Results

The data indicated that this modified 5/6th PNx model by pole ligation is a simple and effective model to investigate uremic cardiomyopathy. At four weeks post-surgery, this PNx model presents impaired renal function, anemia, cardiac hypertrophy, cardiac fibrosis, and decreased heart systolic and diastolic function. The results are summarized below.

At four weeks post-surgery, the PNx mice developed impaired renal function in addition to cardiac morphological and biochemical changes that are consistent with the phenotype of human uremic cardiomyopathy. These PNx-mediated changes include the following observations as compared to the sham-operated mice. First, as expected, the PNx surgery caused significant impairment of renal function. As shown in **Figure 2**, the PNx surgery significantly increased plasma levels of Cystatin C (**Figure 2a**, n = 16), creatinine (**Figure 2b**, n = 16), BUN (**Figure 2c**, n = 16), when compared to the sham surgery. Second, there was PNx-stimulated anemia demonstrated by the significantly lower HCT in the PNx mice (**Figure 2d**, n=8). Third, the PNx-mediated cardiac hypertrophy and diastolic dysfunction were demonstrated by an increased heart weight/ body weight ratio (**Figure 3a**), and transthoracic echocardiography (ECHO) analysis showing the significant increases in posterior wall thickness (PWT), anterior wall thickness (AWT), RWT (**Figure 3b**), MPI (**Figure 3c**), and LVMI (**Figure 3d**). There was no significant change of ejection fraction (EF, %). Fourth, the PNx-mediated cardiac fibrosis was demonstrated by PNx-induced increases in type I collagen expression in the LV homogenates (**Figure 4a**) and left remnant kidney homogenates (**Figure 4b**). Fifth, oxidant stress plays an important role in experimental uremic cardiomyopathy. In this PNx model, PNx significantly stimulated direct protein carbonylation modification of multiple proteins (**Figure 4c**) and c-Src activation (**Figure 4d**) in LV homogenates. PNx surgery did not cause significant loss of body weight at four weeks post-surgery. However, the PNx model by pole ligation method did not induce hypertension. There was no significant difference between sham and PNx mice in systolic BP, diastolic BP and mean BP (data not shown) measured at both one day before the step-one surgery and one day before sacrifice, which is consistent with the notion that the C57BL/6 mouse strain is not an established hypertensive model[20,21]. The data also suggest that this PNx model is unlikely a model of acute renal failure. Moreover, the PNx-mediated cardiac changes, but not impaired renal function, were attenuated by blockage of c-Src activation or induction of heme oxygenase-1 (HO-1)[18].

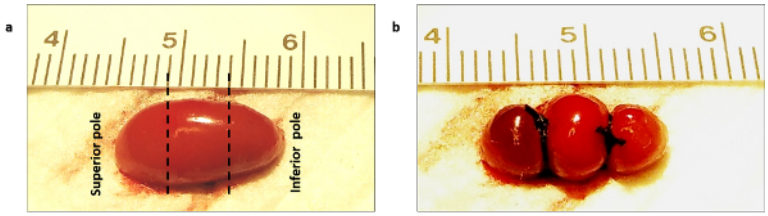

Figure 1: Pole ligation of left kidney. The pictures showed left kidney before ligation (left) and after ligation (right). The doted lines in the left picture showed the estimated ligation lines. Please click here to view a larger version of this figure.

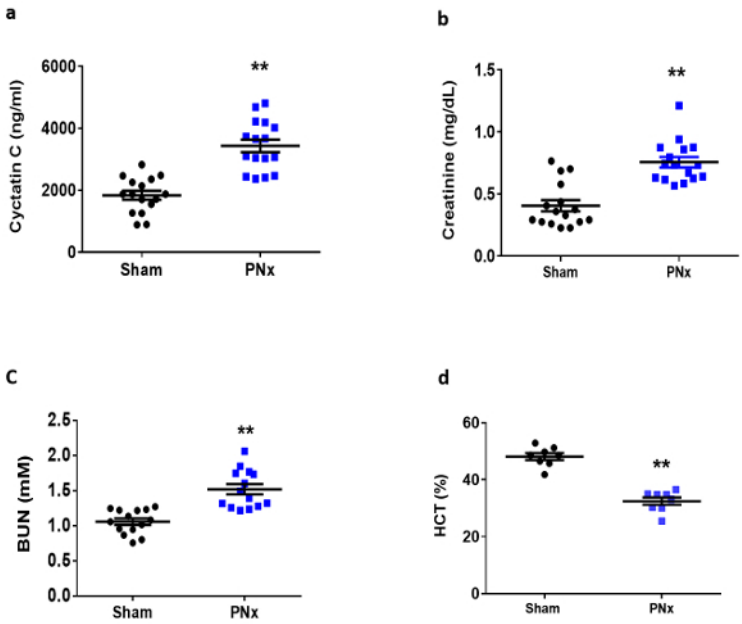

Figure 2: PNx with pole ligation caused impaired renal function and anemia. At four weeks post-surgery, the PNx surgery significantly increases plasma levels of (**a**) Cystatin C (n = 16), (**b**) creatinine (n = 16), (**c**) BUN (n = 16), compared to the sham surgery. PNx also significantly reduced (**d**) hematocrit (HCT) in PNx mice (n = 8). Data were expressed as Mean ± SEM. **, p <0.01, sham-operated mice vs. PNx-operated mice. Please click here to view a larger version of this figure.

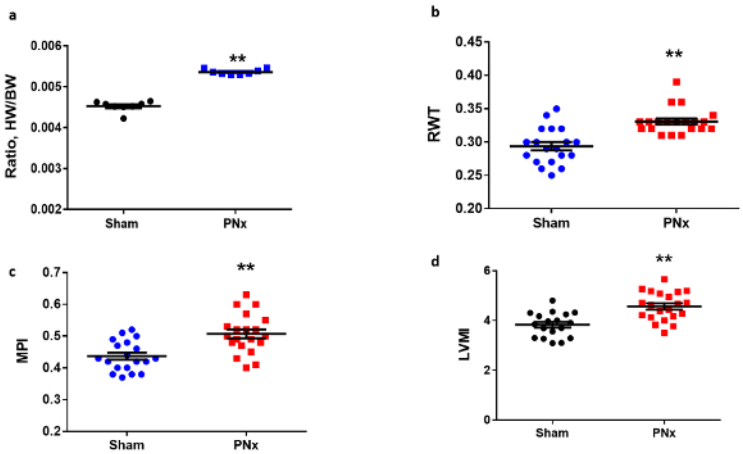

Figure 3: PNx with pole ligation stimulated cardiac hypertrophy. At four weeks post-surgery, the PNx surgery significantly increases (**a**) heart weight/body weight ratio (**a**, n = 8), (**b**) relative wall thickness (RWT, n =1 9-21), (**c**) myocardial performance index (MPI, n = 19-21), and (**d**) left ventricle mass index (LVMI, n = 19-21). Data were expressed as Mean ± SEM. **, p <0.01, sham-operated mice vs. PNx-operated mice. Please click here to view a larger version of this figure.

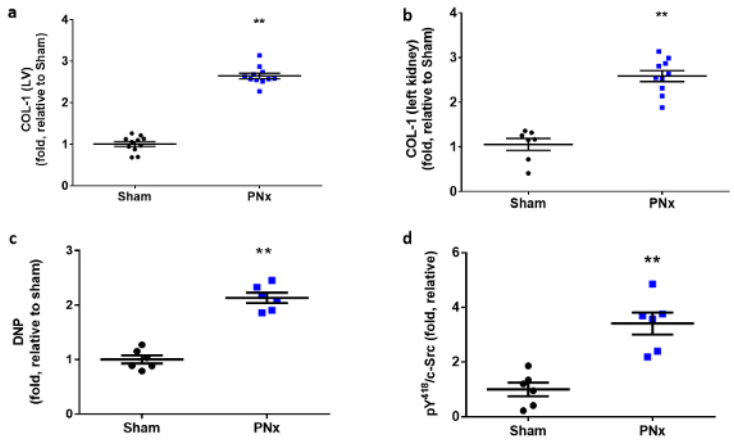

Figure 4: PNx with pole ligation stimulated type I collagen expression, protein carbonylation and c-Src activation. At four weeks post-surgery, the PNx stimulated type I collagen expression in the left ventricle (LV) homogenates (**a**, n=11) and left remnant kidney homogenates (**b**, n = 7-10). Also in LV homogenates, the PNx stimulated direct protein carbonylation (**c**, n = 6) and c-Src activation (**d**, n = 6). Data were expressed as Mean ± SEM. **, p <0.01, sham-operated mice vs. PNx-operated mice. Please click here to view a larger version of this figure.

Discussion

The rat 5/6th PNx model has been widely used to study CKD. Because of the much smaller kidney size in mouse, the classical artery ligation and pole resection are very challenging in mouse models with possible high mortality rates and unexpected bleeding/blood loss.

We adopted a mouse PNx model with pole ligation to overcome the bleeding/blood loss. This PNx model takes less time with improved survival rate and high reproducibility. This pole ligation model develops the phenotypic changes of human uremic cardiomyopathy at the time of four weeks post-surgery, and thus provides a model to study its mechanism(s) and therapeutic target(s).

In this PNx model, the 5/6th PNx is an approximate estimation of renal mass reduction since, and up until now, there is no method to exactly calculate the remnant renal mass of the two kidneys that have different sizes/masses. However, the ligation should be performed in a very similar way to reduce operation variation. We estimate that keeping about 40% of the left kidney (or about 20% of total renal mass) can significantly decrease the mortality rate (by about 20%) without sacrificing the development of the uremic cardiomyopathy phenotype. Another issue is the force of ligation which requires practice.

In comparison with the 5/6th PNx models by pole resection or renal artery ligation[15,20], the pole ligation method is easy, quick, and less instrumentally demanding. The possible advantages of this PNx model include the following: 1) avoidance of possible blood bleeding as seen in the PNx model by pole resection or artery ligation, 2) impaired renal function with increase in oxidative stress caused by the inflammation response to the pole ligation, 3) lower mortality rate (about 20%), and 4) quick development of uremic cardiomyopathy phenotype. A successful pole ligation shows a progressive irreversible damage and degradation of the ligated poles. The ligated poles are visible at two weeks post-surgery (**Figure 4**), and mostly disappear at four weeks post-surgery (**Figure 5**). Comparison of the ligated poles at two weeks and four weeks post-surgery, indicates the inflammation reaction responding to the pole ligation.

This PNx model may be useful to study the mechanism(s) of drug action and therapeutic drug screening. We recently use this PNx model to investigate the role of the Na/K-ATPase signaling and oxidative stress in the development of uremic cardiomyopathy. Administration of pNaKtide, a specific antagonist of the Na/K-ATPase signaling, not only prevents the development of uremic cardiomyopathy but also reverses the onset of uremic cardiomyopathy[18].

The critical steps within the protocol are as follows:1) A more aggressive ligation (remnant left kidney mass is less than 30%) will significantly increase the mortality rate (about 50%). 2) The force of ligation requires practice to reach the point that the ligation restricts the blood supply to the poles without breaking the capsule and kidney tissue. Breaking the kidney tissue leads to intra-renal bleeding that can cause death after removal of the right kidney. A successful ligated pole is discolored within 1 - 2 min after ligation, due to the limit of blood flow to the pole. 3) Special attention is required to avoid ligating the ureter of the left kidney or damaging the ureter when ligating the poles. A mouse with ligated or damaged ureter will not survive after removal of the right kidney. This may be the most noticeable disadvantage of the pole ligation method. 4) Maintenance of body temperature during surgery process and post-surgery care. 5) Control of possible infection using antibiotics.

Disclosures

The authors have nothing to disclose.

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