Uraemic Cardiomyopathy in Different Mouse Models

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Uraemic cardiomyopathy (UCM) is one of the most common complications in chronic kidney disease (CKD). Our aim was to compare characteristics of various UCM mouse models. Mice were assigned to the following groups: the pole ligation group, 5/6 nephrectomy group (5/6Nx), uninephrectomy plus contralateral ischemia followed by reperfusion group (IR), adenine group, and sham group. Mice were sacrificed at 4, 8, and 16 weeks after surgery in the pole ligation, 5/6Nx, and IR groups, respectively. In the adenine group, mice were sacrificed at 16 weeks after the adenine diet. The structure and function of the heart and the expression of fibroblast growth factor 23 (FGF-23) and growth differentiation factor 15 (GDF-15) in hearts were assessed. The mortality in the 5/6Nx group was significantly higher than that in the pole ligation, 5/6Nx, and IR groups. Echocardiogram and histological examination showed cardiac hypertrophy in the adenine, 5/6Nx, ligation group, and IR group. In addition, cardiac fibrosis occurred in all CKD modeling groups. Interestingly, cardiac fibrosis was more serious in the IR and adenine groups. FGF-23 expression in sham mice was similar to that in modeling groups; however, the GDF-15 level was decreased in modeling groups. Our results suggest that the four models of UCM show different phenotypical features, molding time and mortality. GDF-15 expression in the hearts of UCM mice was downregulated compared with sham group mice.

Keywords: uraemic cardiomyopathy, mouse models, surgical method, adenine, GDF-15, FGF-23

INTRODUCTION

Cardiovascular disease is the most common complication of chronic kidney disease (CKD) and the main cause of death among dialysis patients (1–3). A large number of patients with kidney disease die within 3 years after the diagnosis of uraemic cardiomyopathy (UCM) (4). Among the many cardiovascular complications, UCM is characterized by left ventricular hypertrophy (LVH) and diastolic dysfunction (5). To better understand its pathogenesis and explore prevention and treatment methods, it is essential to establish an effective UCM animal model.

Although the rat is an extremely useful model for studying cardiomyopathy of renal failure (6, 7), there are currently many genetic manipulations in mice, and it is easier to perform additional genetic manipulations in the mouse system. The 5/6 nephrectomy (5/6Nx) (8–10), uninephrectomy plus contralateral ischemia followed by reperfusion (IR) (11), pole ligation (12) and adenine diet models (13) of uraemic cardiomyopathy in mice have been reported in recent years. However, mice are small, and surgery is more difficult than surgery in rats. At present, there is no research comparing the advantages and disadvantages of various mouse UCM models.
Therefore, we performed this study to test the survival rate, convenience of the surgery, and degree and characteristics of heart damage in different mouse models.

METHODS

Experimental Mouse Models of Uraemic Cardiomyopathy

All animal surgical protocols and procedures were approved by the Animal Ethical and Welfare Committee of Nanjing Medical University. Male C57BL/6J mice were purchased from the Experimental Animal Center of Nanjing Medical University. All animals were maintained at 19–21°C on a 12-h/12-h light-dark cycle, fed a standard rodent diet and allowed free access to drinking water. Eight-week-old mice were randomly assigned to the following experimental groups: sham group, 5/6 nephrectomy group (5/6Nx) (Figure 1A.a), pole ligation group (Figure 1A.b), uninephrectomy plus contralateral ischemia followed by reperfusion group (IR) (Figure 1A.c), and adenine group. Under anesthesia (pentobarbital), 5/6Nx mice (n = 45) were produced by removing the two poles of the left kidney; haemostasis was achieved by electric cautery and pressure, and the right kidney was removed 1 week later. In the pole ligation group mice (n = 34), the two poles of the left kidney were ligated using a suture string and with appropriate force. After 1 week of recovery, the right kidney was removed. In the IR group (n = 20), the left kidney was subjected to 30-min of ischaemia followed by reperfusion on a warming platform. Subsequently, the right kidney was removed. The addition of adenine to the diet is a chemical method to induce CKD in mice. To investigate the effect of adenine on cardiac damage induced by CKD, mice (n = 15) were fed standard pellet chow containing 0.20% adenine (Sigma-Aldrich, St. Louis, MO) for 16 weeks. For sham animals (n = 14), a sham operation was performed whereby both kidneys were decapsulated and replaced intact. Mice in the pole ligation group and 5/6Nx group underwent non-invasive transthoracic echocardiography tests at 4 and 8 weeks after surgery, respectively, and the IR, adenine, and sham groups underwent tests at 16 weeks after surgery or adenine treatment. Then, mice were anesthetized with 50 mg/kg pentobarbital for sacrifice, with exsanguination performed by removing the eyeball. The heart and kidney were harvested from each animal and dissected for tissue analysis (Figure 1B).

Serum Biochemistry

Blood urea nitrogen (BUN) and serum creatinine (Cr) levels were measured using a Hitachi 7180 auto analyser (Hitachi High Technologies Co., Tokyo, Japan). BUN and Cr concentrations were evaluated using urease-glutamate dehydrogenase and enzymatic methods, respectively.

Haematoxylin-Eosin and Masson Staining

The hearts and kidneys of mice were perfused with 4% paraformaldehyde and fixed in 10% paraformaldehyde for 2 days before being sectioned. After staining heart tissue with haematoxylin-eosin (HE) and Masson and kidney tissue with HE, histological characteristics were observed under a light microscope.

Echocardiography and Doppler Analysis

Transthoracic echocardiography was performed non-invasively with a high-resolution ultrasound imaging system (Vevo 2100; VisualSonics, Inc, Canada) equipped with a 30-MHz mechanical transducer. The mice were lightly anesthetized with 2% isoflurane/100% oxygen and placed on a warming platform (set to 37°C) for the duration of the recordings. Two-dimensional guided M-mode echocardiography was performed in the parasternal long-axis view. Left ventricular ejection fraction (LVEF), fractional shortening (FS), the ratio of left ventricular transmirtal early peak flow velocity to left ventricular transmirtal late peak flow velocity (the E/A ratio), left ventricular end-diastolic anterior wall depth (LVAWd), left ventricular end-systolic anterior wall depth (LVAsWs), left ventricular end-diastolic posterior wall depth (LVPWd), left ventricular end-systolic posterior wall depth (LVPWs), and relative wall thickness (RWT) were measured. M-mode and Doppler measurement data represent 3–6 averaged cardiac cycles from at least two scans per mouse.

Fibroblast Growth Factor 23 (FGF-23) and Growth Differentiation Factor 15 (GDF-15) Immunohistochemistry

Immunohistochemistry was performed on the hearts to analyse the protein expression of FGF-23 and GDF-15. Hearts were fixed in 10% paraformaldehyde and embedded in 80% methyl methacrylate (MMA) (BDH Chemicals, Poole, England) with 20% dibutlyphthalate (Merck, Darmstadt, Germany) after dehydration in an increasing alcohol series. Three-micrometer-thick tissue sections were cut using a Polycut 2500 S microtome (Reichert-Jung, Nussloch, Germany). To remove MMA, tissue sections were incubated in 50% xylene and 50% chloroform. Subsequently, sections were rehydrated in a series of decreasing alcohol concentrations. Next, tissue sections were incubated with Tris-EDTA for 20 min and washed again three times with PBS buffer. Tissue sections were incubated with 5% bovine serum albumin to prevent non-specific binding of the secondary antibody. Subsequently, sections were incubated overnight at 4°C with polyclonal rabbit anti-GDF-15 (Proteintech, Wuhan, China, Catalog number: 27455-1-AP) and rabbit anti-FGF-23 (R&D, Minnesota, USA, Catalog numbers: 26291) at dilutions of 1:800 and 1:100, respectively. The next day, tissue sections were incubated with polyclonal 1:100 biotinylated goat-anti-rabbit (Zhongshan, Beijing, China) for 1 h and with 1:200 horseradish peroxidase labeled streptavidin (Invitrogen, Life Technologies, Bleiswijk, The Netherlands) for 1 h. Chromogenesis was performed by treatment of the sections with AEC reagent (Invitrogen, Life Technologies, Bleiswijk, The Netherlands) for 6 min and by counterstaining with haematoxylin. Finally, the sections were mounted with ClearMount Mounting Solution (Invitrogen, Life Technologies, Bleiswijk, The Netherlands) and covered with a coverslip. Finally, microcosmic images were observed under a light microscope.
STATISTICAL ANALYSES

For continuous variables, data are expressed as the mean ± SEM. Statistical analysis was performed using unpaired, two-tailed T-test and one-way ANOVA with Tukey’s multiple comparison test. For discrete variables, we used the chi-squared test. Statistical analysis was performed using SPSS 13.0 (SPSS, Inc., Chicago, IL, USA). A value of $P < 0.05$ was considered significant.

RESULTS

Comparisons of Survival Rate, Pathology, and Kidney Function in Different Models

Eight-week-old mice were randomly assigned to the following experimental groups: sham groups ($n = 14$), 5/6 nephrectomy group ($n = 45$) (Figure 1A.a), pole ligation group ($n = 34$) (Figure 1A.b), uninephrectomy plus contralateral ischemia followed by reperfusion group (IR) ($n = 20$, and) adenine group ($n = 15$). After modeling, the final study sample comprised 83 mice: 14 in the sham group (survival rate 100%), 15 in the adenine group (survival rate 100%), 17 in the 5/6Nx group (survival rate 37.7%), 22 in the pole ligation group (survival rate 64.7%), and 15 in the IR group (survival rate 75%) (Figure 2A). The mortality in the 5/6 Nx group was significantly higher than that in the pole ligation, IR, and adenine groups. Renal histology indicated glomerular hypertrophy, renal tubular casts, and renal tubular epithelial cell injury in the adenine group, 5/6Nx group, pole ligation group, and IR group (Figure 2B). BUN and Cr were significantly higher in the four modeling groups than in the sham group. In
addition, the BUN and Cr levels in the adenine group were also significantly higher than those in the 5/6Nx group, IR group, and ligation group. Interestingly, BUN and Cr levels in the pole ligation group were only slightly higher than those in the 5/6Nx group and IR group (Figure 2C).

**Comparisons of Body Weight (BW) and Heart Weight in Different Models**

After surgery or an adenine diet, body weight in the adenine group, 5/6Nx group, pole ligation group, and IR group decreased compared to that in the sham group, and weight loss in the adenine group was particularly significant (Figure 2D). There was a significantly greater heart weight and higher ratio of heart weight to body weight in the 5/6Nx group, pole ligation group, and IR group than in the sham group. Although the heart weight of the adenine group was slightly less than that of the sham group, the heart weight-to-body weight ratio was significantly higher (Figure 2D).

**Cardiac Histology Changes in Different Models**

A majority of CKD mice developed cardiac hypertrophy. We observed significant increases in ventricular wall thickness in the 5/6Nx groups, pole ligation model group, and IR group mice compared with the sham group via HE staining. Interestingly, the whole heart got bigger in the 5/6Nx group, pole ligation group, and IR group, but slightly reduced in the adenine group. We also observed significant increases in ventricular wall thickness relative to the heart volume in adenine group (Figure 3A). Because cardiac fibrosis plays an important role in the development of UCM, we performed Masson staining on myocardial tissue and found that compared with the sham group, the adenine group, 5/6Nx group, pole ligation group, and IR group all had obvious myocardial interstitial fibrosis. In addition, cardiac fibrosis was more severe in the IR and adenine groups (Figure 3B).

**Comparisons of Cardiac Echocardiography and Doppler in Different Models**

Echocardiography showed that LVSPs, LVDPw, and RWT in the 5/6Nx group, pole ligation group, and IR group were significantly thicker than those in the sham group, suggesting cardiac hypertrophy in the CKD mouse model (Figures 4A,B). The weight of mice in the adenine group was less than half of that in the sham group (sham group weight vs. adenine group weight: 32.378 ± 0.740 g vs. 12.413 ± 0.856 g, P = 0.000). We further evaluated the differences in the ratio of LVW/LVDPw, LVW/LVDPw, and RWT to body weight between the adenine group and sham group and found that the above parameters were much higher in the adenine group (Figure 4C). In addition, LVEF and FS in the adenine group were higher than those in the sham group; however, there were no significant differences in these parameters among the 5/6Nx groups, pole ligation group, IR group and sham group (Figure 4B). Analysis of diastolic function revealed no change in the E/A ratio between the sham group and the 5/6Nx group, pole ligation model group, and IR group. However, compared with the sham group, the adenine group showed a significant reduction in the E/A ratio (Figures 5A,B).

**Myocardial FGF-23 and GDF-15 Protein Expression in Heart Tissue**

Cardiac FGF-23 immunostaining was similar among the five groups (Figures 6A,B). However, immunohistochemistry of the heart tissue showed that the expression of GDF-15 decreased in the 5/6Nx group, adenine group, pole ligation group, and IR group compared to the sham group (Figures 6C,D).

**DISCUSSION**

Chronic kidney disease is a significant medical problem globally (14). Indeed, the majority of patients with CKD will die from cardiovascular disease (CVD) before progressing to end-stage renal disease (ESRD) (15). UCM is a widely prevalent cardiovascular disease in patients with CKD or ESRD, accounting for ∼50% of deaths due to CKD (11). UCM in patients with CKD or ESRD leads to LVH, left ventricular dilation, left ventricular systolic and diastolic dysfunction, and cardiac fibrosis. Severe UCM can lead to sudden cardiac death even in those without cardiac symptoms.

Although the rat is an extremely useful model for studying the cardiomyopathy of renal failure (4), mouse UCM models were developed due to the wide availability and ease of genetic manipulation in the mouse system. Because of the much smaller size of the kidney, however, classical surgical modeling is very challenging in mouse models with possible high mortality and unexpected bleeding or blood loss. Several UCM mouse models have been reported, but there has been no research comparing the advantages and disadvantages in different models. We used 5/6Nx, IR, pole ligation and an adenine diet to induce UCM and compared the merits and demerits of several models. Although surgery is difficult and the mortality is high, 5/6 nephrectomy is a classic method of inducing the cardiovascular complications of CKD such as UCM. The basic operation involved removing the upper and lower thirds of the left kidney, keeping the complete renal hilus, and removing the right kidney 1 week later. As a classic modeling method for CKD and its cardiovascular complications, 5/6 nephrectomy has been widely used in a large number of experiments. However, the difficulty of surgery and high mortality after surgery still plague researchers. When Kukida et al. studied CKD vascular calcification, they planned to use a 5/6Nx and high-phosphorus diet to induce CKD vascular calcification. Because of the high mortality of mice, they had to abandon the surgical modeling method and change to an adenine and high-phosphorus diet to induce vascular calcification (16). To successfully complete the surgery, researchers have to spend more time practicing surgical techniques and increasing the number of experimental animals, which not only prolongs the experimental time but also places pressure on scientific research funds. In our experiments, we found that excessive excision of the upper and lower poles of the kidney and a remaining left kidney mass <30% will significantly increase mortality. In addition, the haemostasis effect of the cut surface also has a significant impact.
on the survival rate of mice. For this reason, we used arterial clips to close the renal artery when removing the upper and lower poles of the kidney and used electric cautery to stop bleeding of the cut surface. After 8 weeks, cardiac echocardiography indicated that LVPWd, LVPWs, and RWT were greater in the surgery group than in the sham group, suggesting that 5/6 Nx mice developed LVH. Heart failure with preserved ejection fraction (HFpEF) characterized by LVH is a common cardiovascular complication of CKD (17). In our study, cardiac echocardiography showed no significant difference in LVEF and FS between the 5/6 Nx group and the sham group. In addition, HE staining showed that the heart was significantly enlarged and that the ventricular wall in 5/6 Nx mice was significantly thicker than that in the sham group, and Masson staining showed that myocardial fibrosis occurred in the 5/6 Nx group.

Recently, Wang et al. reported that the pole ligation method could induce UCM (12). The upper and lower poles of the left kidney were ligated gently with sutures, resulting in ischemia of the distal kidney, and the right kidney was removed 1 week later. In comparison with the 5/6 Nx models established by pole resection, the pole ligation method is easy, quick, and less instrumentally demanding. More importantly, we found that the mortality of the pole ligation method was significantly lower than that of the 5/6 Nx procedure. Echocardiography indicated

![FIGURE 2](image_url)
that the LVPWd, LVPWs, and RWT were greater in the surgical group than in the sham group, and there was no significant difference in LVEF and FS between the pole ligation group and the sham group. Furthermore, HE staining showed that the heart was significantly enlarged and that the ventricular wall of pole ligation mice was significantly thicker than that in the sham group. Masson staining showed that myocardial fibrosis occurred in the pole ligation model mice.
FIGURE 4 | Between-group comparisons of cardiac characteristics in echocardiography. (A) Representative M-mode echocardiograms from the sham, adenine group, 5/6th nephrectomy group (5/6Nx), pole ligation group, and uninephrectomy plus contralateral ischemia followed by reperfusion group (IR). (B) The mean LVAWs, LVPWs, LVPWd, RWT, LVEF, and LVFS in the five groups. Data are presented as the mean ± SEM (n = 14–22). (C) The mean LVAWs/BW, LVPWs/BW, LVPWd/BW, and LVPWd/BW in the sham and adenine groups. Data are presented as the mean ± SEM (n = 14–15). *P < 0.001, vs. Sham; **P < 0.01, vs. Sham; #P < 0.05, vs. Sham; ##P < 0.05, vs. Sham.
Hu et al. reported that ischaemia-reperfusion injury on one side plus nephrectomy on the contralateral side (IR model) also induced UCM (11). During the operation, the left renal artery was clamped for 30 min, causing renal ischaemia-reperfusion injury, and the right kidney was immediately removed. After 16 weeks, echocardiography demonstrated that LVPWd, LVPWs, and RWT were greater in the IR group than in the sham group, which suggested that the IR group mice developed LVH. However, there was no significant difference in LVEF and FS between the IR group and sham group. Cardiac tissue HE staining showed that the ventricular wall of the IR group was significantly thicker than that of the sham group, and Masson staining demonstrated that myocardial fibrosis also occurred. In addition, cardiac fibrosis was more serious than 5/6Nx and pole ligation groups.

In addition to the surgical method, another model of CKD is a chemical method involving the addition of adenine to the diet. A large number of studies have used 0.20% dietary adenine for 6 to 8 weeks to induce CKD in mice (18–20). However, this is not long enough for cardiovascular effects to develop. Thus, the diet regimen was extended to 16 weeks, causing the development of UCM. After 16 weeks, the body weight of the adenine group was significantly less than that of the sham group, while the ratio of heart weight to body weight was much higher than that of the sham group. HE staining showed that the heart was slightly reduced in the adenine group, but the ventricular wall of the adenine group was significantly thicker than that in the sham group. Masson staining demonstrated that myocardial fibrosis also occurred. Interestingly, it was more serious than 5/6Nx and pole ligation groups, but not as serious as IR group. Echocardiography suggested that the ratios of LVAWs, LVAWd, LVPWs, and LVPWd to body weight were all significantly higher than those in the sham group. In addition, LVEF and FS were significantly higher in the adenine group than in the sham group, which was different from the 5/6 Nx, pole ligation, and IR groups. Given that the hearts were slightly reduced but the thickness of the ventricular wall was increased significantly in the adenine group, the increase in LVEF and FS did not mean that systolic function improved; rather, it indicated that cardiac hypertrophy was serious. Doppler-derived mitral flow velocities revealed a reduction in the E/A ratio in adenine mice, and such an alteration is always accompanied by diastolic relaxation abnormalities (21). Interestingly, compared with that in the sham group, the E/A ratio did not change in the 5/6Nx, pole ligation, and IR groups.
Although the adenine model group is more convenient and heart damage is severe, the potential toxicity of adenine itself to the heart cannot be completely ruled out. More importantly, adenine caused significant weight loss in mice, which may cause metabolic and physiological dysfunction of various organs, resulting cardiac injury indirectly.

Although imaging and histological examination are the main diagnostic criteria of UCM, Atrial natriuretic peptide (ANP) and
β-myosin heavy chain (β-MHC) are well known as important biomarkers of heart failure and cardiac hypertrophy (22–24). In present study, we confirmed that four models of UCM mice showed cardiac hypertrophy and myocardial fibrosis by echocardiogram and histological examination. However, we did not detect expression of β-MHC and ANP, which is a limitation of this study.

The pathogenesis of UCM is complex, and the underlying mechanism is incompletely understood. It is thought that traditional cardiovascular risk factors, such as hypertension, volume overload, and anemia, are major causes of UCM; however, UCM may also develop independently of these above mentioned risk factors. The exact mechanism is still largely unknown. Growth differentiation factor-15 (GDF-15), also known as macrophage inhibitory cytokine-1 (MIC-1), is a novel member of the transforming growth factor β superfamily (25). It was identified in 1997 as a macrophage-derived inflammatory response cytokine (26). A large number of previous studies have reported that elevated levels of GDF-15 in circulation are closely related to cardiovascular death in CKD patients. Benes et al. reported that serum GDF-15 levels were closely related to poor prognosis in CKD patients with systolic dysfunction and heart failure (27). The mortality of patients with high serum GDF-15 was significantly higher than that of dialysis patients with low serum GDF-15 (28, 29). However, Liu et al. found that overexpression of GDF-15 could inhibit neuronal cell damage induced by oligomycin (25). Recently, Coll et al. reported that circulating elevated GDF-15 played an important role in metformin's benefit in terms of energy balance and weight (30). The exact biological function of GDF-15 is still poorly understood, and it exhibits a complex pattern of beneficial and harmful functions. Whether increased expression of GDF-15 can cause direct damage or may represent a protective response to biological stress is still an open question (31). Previous studies on GDF-15 have mainly focused on its expression in serum, and its expression in heart tissue in CKD has not yet been reported. More importantly, overexpression of GDF-15 in neural cells could protect cells from damage. Therefore, it is interesting and meaningful to explore the expression of GDF15 in hearts of UCM mice. We found that the expression of GDF-15 was significantly higher in the heart tissue of the sham group than in the same tissue of the adenine group, 5/6Nx group, ligation group, and IR group, indicating that GDF-15 expression downregulation in the heart tissue may be one of the mechanisms of UCM. Downregulation of GDF-15 in heart may be induced by traditional and non-traditional risk factors of cardiovascular complications of CKD. Mitochondrial dysfunction plays an important role in UCM (32), and GDF-15 improves mitochondrial function (22). The down-regulation of GDF-15 expression in the heart may be an important mechanism for the UCM.

A large number of studies have consistently confirmed that serum or plasma FGF-23 in CKD animals or patients is significantly increased (33–36). Cross-sectional studies have revealed that higher plasma or serum FGF-23 is associated with LVH in CKD, which can lead to congestive heart failure and subsequent death (37, 38). However, the results of studies on the expression of FGF-23 protein in hearts with heart failure or LVH are relatively few and inconsistent. Leifheit-Nestler et al. reported that cardiac FGF-23 was excessively high in patients with CKD and that enhanced myocardial expression of FGF-23 strongly correlated with LVH (39). However, a study carried out by Andersen et al. reported that FGF-23 protein expression in heart tissue of heart failure patients was similar to that in the control group (40). The increase in circulatory FGF-23 expression in CKD has been widely reported (33–38). However, there are few studies on the expression of FGF-23 in the heart, and the results are also inconsistent (39, 40). As it remains to be clarified whether the myocardium contributes to the release of FGF-23 in UCM, we aimed to assess FGF-23 protein expression in the cardiac of sham and UCM mice. In our present study, we found that FGF-23 protein expression in heart tissue in CKD mice was similar to that in sham mice, suggesting that the high circulatory FGF-23 in CKD does not cause accumulation of FGF-23 in the heart. FGF-23 activates promoting cardiac hypertrophy gene transcription by binding to FGF receptors (FGFRs) on the surface of cardiomyocytes membrane, instead of directly entering the cells (41). Therefore, the function and activity of the FGFRs may be more important. Considering that the pathogenesis of uremic cardiomyopathy is very complicated, the proteomics study of myocardial tissue may be very helpful for discovering new key pathogenic molecules of UCM.

**CONCLUSION**

The 5/6Nx model, pole ligation model, IR model, and adenine model can induce uraemic cardiomyopathy in mice. The mortality in the 5/6 Nx group is significantly higher than that in other three modeling groups. The ventricular wall thickness

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**TABLE 1 | Comparison of characteristics between different models.**

| Model   | The degree of kidney damage | Relative ventricular hypertrophy | Cardiac diastolic dysfunction | Heart size | Cardiac fibrosis | Mortality | Induction Time | Difficulty of operation |
|---------|----------------------------|---------------------------------|-----------------------------|------------|-----------------|----------|---------------|------------------------|
| 5/6Nx   | ++                         | +                               | -                           | Enlarged   | ++              | ++       | ++            | +                      |
| Pole ligation | + + +                   | +                               | -                           | Enlarged   | +               | +        | +             | +                      |
| IR      | +                         | +                               | -                           | Enlarged   | ++              | +        | ++            | +                      |
| Adenine | + + + +                    | ++                              | +                           | Reduced    | ++              | -        | + +           | -                      |
in the four modeling groups mice are significant increased. However, the heart size gets bigger in the 5/6Nx group, pole ligation group, and IR group, but slightly reduces in the adenine group. Four modeling groups all have obvious myocardial interstitial fibrosis. In addition, it is more severe in the IR and adenine groups. Echocardiography indicates that the mice in adenine group shows a significant decrease in cardiac diastolic function (Table 1).

In conclusion, researchers can choose the appropriate model according to the modeling time, surgical technique, characteristics of the heart damage, etc. In addition, we also found that the expression of GDF-15 in heart tissue was significantly downregulated in UCM mice compared with sham mice, suggesting that GDF-15 may be a novel molecular target worthy of further study.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article's supplementary material, further inquiries can be directed to the corresponding authors.

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**ETHICS STATEMENT**

The animal study was reviewed and approved by Animals Experiment Ethics Committee of Nanjing Medical University.

**AUTHOR CONTRIBUTIONS**

CC, CXie, HW, LW, and JZ performed the experiments and analyzed the data. HM and CXin edited and revised the manuscript. All authors approved the final version of the manuscript.

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