ESTABLISHING A RAT MODEL OF CHRONIC KIDNEY DISEASE VIA FIVE-SIXTHS NEPHRECTOMY

Nguyen Thi Dieu Linh[1], Le Thi Thanh Nhan[1], Nguyen Pham Ngoc Mai[2], Pham Thuy Phuong[3]

[1] *Hanoi University of Business and Technology*

[2] *University of Science and Technology of Ha Noi*

[3] *Vietnam University of Traditional Medicine*

ABSTRACT

Objective: *To develop a rat model of chronic kidney disease through surgical removal of five-sixths of total renal mass, serving as a foundation for future pharmacological and therapeutic studies.*

Subjects and methods*: Experimental study on white rats, randomly divided into 10 rats/group, including control group with surgery without kidney removal and given distilled water, model group with surgery to remove 5/6 of the rats' kidneys and given distilled water.*

Results: *After 2 weeks of surgery to remove 5/6 of the rats's kidneys, in the model group, the concentrations of urea, creatinine, 24-hour urine volume and 24-hour proteinuria of the rats increased compared to before surgery. Histological images of the rats kidneys showed interstitial expansion, glomerular hypertrophy, and tubular dilation. The survival rate of rats was 100%.*

Conclusion: *The 5/6 nephrectomy model successfully reproduced key clinical and pathological features of CKD with a high survival rate. This stable and reproducible model provides a valuable experimental basis for studying the pathophysiological mechanisms of CKD and for evaluating potential pharmacological or therapeutic interventions in future research.*

Keywords*: Chronic kidney disease, White rats.*

INTRODUCTION

Chronic kidney disease is a serious and common disease in kidney and urinary diseases and is also a complication of some internal diseases such as diabetes, hypertension, gout [1]. This is a global health problem, with a rapidly increasing frequency and requiring huge treatment costs [2]. According to the publication of the Global Burden of Disease Study in 2010, chronic kidney disease ranked 27th in the list of causes of total deaths worldwide in 1990 and increased to 18th in 2010 [3]. In the face of the constantly increasing incidence of the disease, research on the pathogenesis as well as measures to prevent and treat chronic kidney disease has become a hot topic in the medical community at home and abroad. Currently, many authors have developed animal models of chronic kidney disease for research purposes. Models have been successfully created in many types of animals such as rats, rabbits, and the methods used mainly include physical, chemical, and biological methods. In which, the 5/6 nephrectomy method and the adenine method are often used in experiments. The 5/6 nephrectomy method is also known as the Platt method, because Platt successfully established a model of chronic kidney disease by surgically cutting 5/6 of the rats kidney for the first time in 1952. This model is basically suitable for research conditions in Vietnam, the basic mechanism of the model is similar to the pathogenesis of chronic kidney disease in humans. Therefore, this study was conducted with the goal of building a model of chronic kidney disease in white rats by the 5/6 nephrectomy method.

SUBJECTS AND METHODS

Subjects

White Wistar rats , male, adult, healthy, weight from 200 - 250g. Experimental rats were provided by the Animal Department - Vietnam Military Medical Academy.

Study time and location

- Time: From April 2024 to October 2024.

- Location: Department of Pharmacology, Institute of Pharmacy Training, Military Medical Academy.

Department of Pathology and Forensic Medicine, 103 Military Hospital.

Machines and tools for research

- Biochemical Systems International Srl, Italy, model 3000 Evolution.

- Humancout 30TS hematology analyzer, Human, Germany.

- Universal 320 refrigerated centrifuge (Hettich - Germany).

- Analytical balance 10-4, model CP224S (Sartorius - Germany).

Corresponding author: Pham Thuy Phuong
Phone number: (+84) 983654033
E-mail: thuyphuongydhctvn@gmail.com

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- Electronic balance 2200g with accuracy of 0.01g (HL-Japan).

- Powerlab system (ADInstruments - Australia) with LabChart v8.0.0 software and peripheral devices used to measure mouse blood pressure by tail-cuff method (pressure cuff and blood pressure probe; ML 125 NIBP amplifier).

- Specialized blunt-tip curved needle used to give mice medicine.

- Small animal surgical instruments.

Methods

White rats of 20 divided into 2 group, each group of 10 rats:

- Group 1 (OP): Surgery without kidney removal + drinking distilled water.

- Group 2 (model): Surgery to remove 5/6 kidneys + drink distilled water.

The model of chronic kidney disease was performed in white rats according to the method described by Lu, J. R et al. (2014), with modifications [4]. The rats were operated on under sterile conditions. The surgery was carried out in 2 stages:

Stage 1: Remove 2/3 of the left kidney.

The rats were anesthetized with 50 mg/kg body weight of sodium pentobarbital injected intraperitoneally. After anesthesia, the rats were placed in a lateral position on an operating table lined with a warm pad. The hair was shaved and the surgical area was disinfected with 10% betadine solution. Next, the skin was incised diagonally parallel to the lower left rib. The fascia and connective tissue were dissected, and the left kidney was exposed from the renal capsule using a fork and a wet cotton swab. The dissection and exposure were carried out carefully, avoiding touching the adrenal gland. Separate and place a 4.0 thread below the renal artery using a homemade tool. Place the catheter (400 µm) parallel to and close to the renal artery. Temporarily ligate the renal artery and catheter with the pre-placed thread. After the renal artery was temporarily occluded, use an electric knife to cut off the upper 1/3 and lower 1/3 of the left kidney, respectively. Slowly loosen the renal artery knot combined with controlling bleeding by compressing a gelatin sponge onto the incision surface. The surgical area is cleaned with saline and gently patted dry with sterile gauze. Next, gently place the kidney in the abdominal cavity and suture the connective tissue, abdominal wall muscles, and back in layers; suture the skin and disinfect the site with 10% betadine solution. After surgery, the rats are placed in a warm cage and monitored postoperatively until the animal regains consciousness.

Stage 2: Removal of the entire right kidney

Fifteen days after the surgery to remove 2/3 of the left kidney, the rats had completely recovered, and surgery was performed to remove the entire right kidney. The rats were anesthetized, disinfected, and the skin was cut diagonally parallel to the lower right rib and the right kidney was exposed from the renal capsule similar to the surgery in stage 1. Then, a 4.0 thread was exposed and threaded through the renal pedicle using a homemade tool. The renal pedicle was tightened, including the renal artery, vein, and ureter. Finally, the right kidney was removed by cutting across the blood vessels and ureter right next to the kidney, far from the place where the thread was tied. The connective tissue, the abdominal and back muscles were sutured in layers; the skin was sutured and disinfected with 10% betadine solution. After surgery, the rats were placed in a warm cage and monitored postoperatively until the animal regained consciousness. After 7 days of surgery, the rats could be returned to the group.

The surgical control rats underwent the same surgical steps as the model surgery, however, the surgical process stopped at the step of separating the fascia and connective tissue to expose the kidney, without exposing the kidney from the renal fibrous capsule and without causing damage to the renal parenchyma. Then, the connective tissue and fascia of the abdominal and back walls were sutured in layers; the skin was sutured and the site was disinfected with 10% betadine solution. As an adaptive response to the removal of the kidney, the remaining kidney would hypertrophy, increase glomerular filtration, progress to proteinuria, increased urea, blood creatinine, hypertension, renal fibrosis with glomerular sclerosis and tubulointerstitial scarring over time.

The criteria for determining whether the rats model successfully induced chronic kidney disease were evaluated 15 days after the second surgery: The rats were alive, the surgical wound was dry and healed well, and one of the signs of renal dysfunction was blood urea, blood creatinine, and 24-hour urinary protein increased by 1.5 times or more compared to before surgery.

Blood sampling technique

The mice were immobilized by grabbing the nape of the neck and clamping the tail, then briefly anesthetized with Ketamine (90 mg/kg, intraperitoneal injection). A glass capillary tube was used to puncture the subocular plexus at a 30° angle to collect blood. After obtaining a sufficient amount, the tube was withdrawn, hemostasis was applied, and the injection site was disinfected.

Urine collection method

Urine volume was assessed at T_0 (immediately before drug administration) and T_c (last day after drug

administration) at 7:30 am. On the day of evaluation, each mouse was placed in a separate cage with a tray and urine was collected in a cup.

Data processing

Data are expressed as Mean ± SD. Statistical analyses were performed using SPSS 20.0 software. Differences between groups were evaluated using the Student's *t*-test for independent samples. A *p*-value < 0,05 was considered statistically significant.

The sample size (n = 10 rats per group) was determined based on previous experimental studies on chronic kidney disease models using the 5/6 nephrectomy method, which demonstrated that this number provides sufficient statistical power to detect biologically meaningful differences in biochemical and histopathological parameters between groups. This sample size also ensures ethical use of laboratory animals while maintaining reliability and reproducibility of the results.

Errors and error correction measures

Methods are applied to minimize possible errors during data collection, analysis and processing:

- Research animals were selected relatively uniformly, healthy, without deformities or unusual signs.

- The time to perform the experimental steps between the rats batches is uniform at the same time.

- Data is measured carefully and accurately using standardized and highly accurate laboratory equipment and machinery.

- Process data using specialized software on the computer. Store data and information using notebooks and photos.

Ethics in research

The study was approved by the Vietnam University of Traditional Medicine's Thesis Defense Council.

The research was carried out in accordance with the approved outline.

The study was conducted on white rats, the number of animals used in the experimental models was limited to a minimum, sufficient to obtain results that ensure reliability and sufficient statistical processing. Honesty in data processing.

Rats that died during the experiment (if any) and rats after the experiment was completed were all handled according to regulations.

The selection of experimental animals, the conditions for raising, caring for and using animals all strictly follow the "Guidelines for basic content of appraisal of preclinical research results of modern drugs, traditional medicines, vaccines and medical biological products" of the Ministry of Health.

RESULTS

Results of general condition assessment of rats

The results of the general condition assessment showed that the rats in the model group showed ruffled fur, darker fur, and were less active and ate less. Decreased compared with the surgical control group, with the manifestation becoming more pronounced over time.

Rats weight assessment results

Table 1. Weight of rats in the study groups

Research group	Rats weight (g)		p_{b-a}
	Immediately after the 2nd surgery (a)	15 days after 2nd surgery (b)	
0P (1)	198,08 ± 18,92	208,21 ± 20,69	< 0,05
Model (2)	196,36 ± 19,05	195,62 ± 19,55	> 0,05
p_{2-1}	> 0,05	< 0,05	-

Table 1 shows that at the initial time, there was no significant difference in body weight between groups (p>0,05). Although rats in the model group tended to weigh less than those in the control group after kidney removal, the difference was not statistically significant due to the small weight of the removed kidney (~1g, p>0,05). However, 15 days after the second surgery, rats in the model group did not gain weight, while those in the control group did, resulting in a statistically significant difference (p<0,05).

Results of serum urea and creatinine concentrations in rats

Table 2. Serum urea concentration of rats in the study plots

Research groups	Serum urea concentration (mmol/l)		p_{b-a}
	Before surgery (a)	15 days after 2nd surgery (b)	
0P (1)	5,72 ± 0,71	5,88 ± 0,84	> 0,05
Model (2)	5,68 ± 0,74	8,96 ± 0,98	> 0,05
p_{2-1}	> 0,05	< 0,01	-

Table 2 shows that preoperatively, serum urea levels were similar between groups (p>0,05).

However, 15 days after the second surgery, the model group showed a significant increase in serum urea compared to both baseline and the surgical control group (p<0,01).

Table 3. Serum creatinine concentration of rats in the study groups

Research groups	Serum creatinine concentration (µmol/l)		p_{b-a}
	Before surgery (a)	15 days after 2nd surgery (b)	
0P (1)	89,20±9,85	90,10 ± 9,92	> 0,05
Model (2)	88,90± 9,54	139,60±14,26	> 0,05
p_{2-1}	> 0,05	< 0,01	-

Table 3 shows that, at the preoperative time, the serum creatinine concentration of the rats in the batches were the same (p>0,05). At time 15 days after the second surgery, serum creatinine concentration of rats in the model group increased (p<0,01) compared to before PT as well as compared to the control group.

Results of 24-hour urine volume and 24-hour proteinuria evaluation of rats

Table 4. 24-hour urine output of rats in the study groups

Research groups	24-hour urine output (ml)		p_{b-a}
	Before surgery (a)	15 days after 2nd surgery (b)	
0P (1)	14,93±3.12	13,65±2,97	> 0,05
Model (2)	14,77±3.63	21,15±2,63	< 0,05
p_{2-1}	> 0,05	< 0,05	-

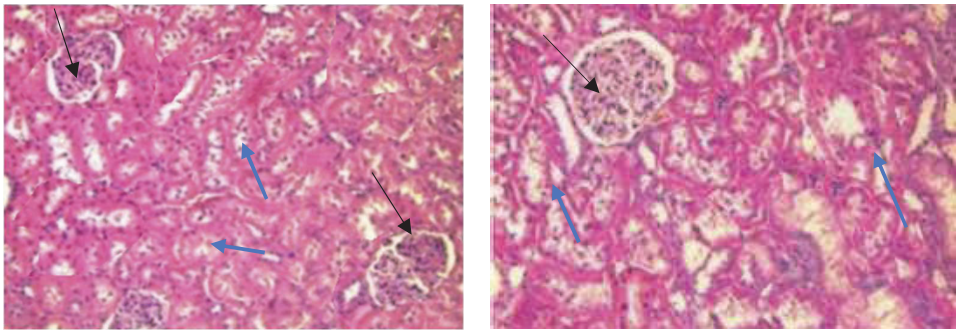
Table 4 shows that, at the time before surgery, the 24-hour urine volume of rats in the groups was the same (p>0,05). At 15 days after the second surgery, the 24-hour urine volume of rats In the model group, the increase was statistically significant compared to before PT as well as compared to the control group with p<0,05.

Table 5. 24-hour urinary protein of rats in the study groups

Research groups	24-hour urinary protein of rats (mg/24h)		p_{b-a}
	Before surgery (a)	15 days after 2nd surgery (b)	
0P (1)	235,68±42,32	243,27±37,42	> 0,05
Model (2)	219,85±39,75	358,31±52,45	< 0,05
p_{2-1}	> 0,05	< 0,05	-

Table 5 shows that, at the time before surgery, the 24-hour urinary protein of rats in the groups was the same (p>0,05). At 15 days after the second surgery, the 24-hour urinary protein of rats in the increased model there was statistical significance compared to before surgery as well as compared to the control group with p<0,05.

Results of histological evaluation of rats kidney



Control group **Model group**

Figure 1. Microscopic images of rats kidneys in the study groups (HE x 200)
(1.Glomerulus (black arrow); 2. Renal tubule (green arrow))

The histological structure image of the rats kidney in the control group shows normal renal parenchyma, renal cortex with glomeruli, renal tubules and blood vessels between renal tubules with normal structure. The histological structure image of the rats kidney in the model group shows interstitial dilatation, glomerular hypertrophy, and renal tubular dilatation.

DISCUSSION

Our research results show that after 2 weeks of surgical removal of 5/6 of the rats kidneys, the concentration of urea, creatinine in the rats's blood increased, the 24-hour urine volume and 24-hour protein of the rats increased. These are important indicators to assess kidney function damage. Our results of evaluating these indicators when creating the model are completely consistent with those of other authors [4],[5]. In the authors' research, we have not found a standard to determine a successful model before giving the rats the research drug, however, the research results on blood tests, urine, kidney histopathology... are all relatively complete and clearly show the manifestations of chronic kidney disease. Perhaps the removal of 5/6 of the kidneys, leaving only 1/6 of the kidneys, is a fairly large kidney injury, a stable injury in all rats and cannot be recovered, with the inevitable result being the progression of chronic kidney disease. The model of chronic kidney disease by cutting 5/6 kidneys of white rats has also been previously implemented at the Department of Pharmacology, Military Medical Academy, with the results of the blood urea, creatinine and 24-hour urinary protein indexes of rats increasing from 1.5 to 2 times compared to before surgery. An increase of 1.5 times or more can be considered a clear increase in the pathological condition. Therefore, to standardize the model, we chose the criteria for evaluating the successful implementation of the model in addition to the standard of living rats, dry surgical wound with good healing, with the additional criteria of having one of the manifestations of renal dysfunction: blood urea, blood creatinine, 24-hour urinary protein increased by 1.5 times or more compared to before surgery. The results of our study are consistent with the results of the study by Kohei Hayashi et al. (2020) [6], and also show the progression of kidney disease in the model.

Thus, with the model we applied in this study, the results of causing chronic kidney disease in rats are clear and consistent with the pathogenesis in humans. It is considered a suitable model in domestic research conditions as well as a model currently being used by many authors and published in international journals, with good updating.

CONCLUSION

Thus, the model of chronic kidney disease in white rats by cutting 5/6 of the rats kidney resulted in the manifestation of progressive adrenal damage to become chronic. The results of the blood urea, creatinine, 24-hour urine volume and 24-hour proteinuria of the rats increased in the model group along with the change in the histological structure of the rats kidney, which is clear evidence of chronic kidney disease in the research rats.

This study was limited by a relatively small sample size and a short follow-up period. Further studies with larger groups and longer observation times are needed to evaluate the long-term stability of the model and its applicability in pharmacological or therapeutic research.

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