A Novel Standardized Method of Renal Biopsy in Mice

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Keywords

Renal biopsy · New standardized method

Abstract

Introduction: In animal models, it can be difficult to confirm therapeutic effects due to technical inconsistencies and other reasons. Although renal biopsy is widely used in clinical diagnosis, it is rarely used in animal experimental models, especially in mice, because the problems of surgery-induced renal injury and bleeding have not been solved. **Methods:** We developed an easy-to-use method of renal biopsy in mice and evaluated whether 3 consecutive renal biopsies can be performed in the same kidney in a standardized manner. This method was verified using 2 mouse models, a healthy mouse model and a unilateral ureteral obstruction (UUO) mouse model, and evaluated based on renal function and histological changes. **Results:** There were no perioperative complications in any of the model mice. There was no significant difference in serum Cr, 24-h urine protein, or kidney/body weight ratio between the biopsy and control groups. Each biopsy sample contained sufficient renal tissue.

The damage to the operated tissue was limited to the tissue near the biopsy site. Compared with renal tissues in the corresponding control group, the renal tissues obtained from the 3 biopsies (healthy model days 0, 4, and 7 and UUO model days 3, 7, and 10) and the remnant renal tissues after the biopsy showed no significant difference in the glomerular sclerosis index, degree of renal tubular damage, inflammatory response and renal fibrosis in these 2 models. **Conclusions:** Our new standardized method of renal biopsy in mice is a safe and cost-saving approach that allows repeated renal biopsies and ensures minimal trauma and sufficient sample size to quality in experimental disease models.

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Introduction

Renal biopsy can be used to identify the etiology and pathological changes in renal diseases and further determine the pathological classification and stage, which has become an important medical tool for the diagnosis, treatment, and prognosis of diffuse kidney diseases [1].

Although renal biopsy is widely used in clinical diagnosis, it is rarely used in animal experimental models, especially in experimental mice.

Mice have been widely used to establish various kidney disease models, such as ureteral obstruction and ischemia-reperfusion models. Technical inconsistency could directly affect the final results, especially in kidney ischemia-reperfusion injury models, which are complicated to produce and are easily affected by temperature and the tension and tightness of the clamps. It can be difficult to differentiate therapeutic effects from technical inconsistency. In addition, the market prices of some experimental mice requiring specialized treatment, especially transgenic transformation and recombination [2], such as gene-targeting CYP4F2 mice, Dicer1 mice, gene knockout flt mice, and Cre mice, are relatively expensive. Therefore, the application of renal biopsy in mouse experiments can not only reduce the number of experimental mice and save costs but also eliminate differences caused by various external factors, such as differences in manipulation among laboratory personnel or congenital differences between individual animals.

Previously, a team used an open surgery biopsy method to remove a quarter or a third of the kidney tissue of experimental rats, but this method may cause a series of side effects, such as a large surgical incision that may lead to serious bleeding complications and a low level of standardization that prevents a consistent size of the remnant kidney [3]. These conditions limit the possibility of repeat renal biopsy. Due to the small size of the mouse kidney and the size limitation of experimental equipment, standardized renal biopsy in mice is very rare, although mice are the most common animal models.

Therefore, we designed a specialized method of renal biopsy for experimental mice that is characterized by high operation standardization and a small trauma area. We verified that this new renal biopsy method can be used to complete at least 3 standard and effective renal puncture biopsies in the same kidney of the same mouse and does not cause secondary renal damage both in healthy model mice and in unilateral ureteral obstruction (UUO) model mice.

Materials and Methods

Animals

Male C57BL/6 mice (18–22.5 g) were purchased from the 2nd Affiliated Hospital Laboratories of Harbin Medical University (KY2018-171). The mice were housed in an air-conditioned room (22 ± 2°C; 40–70% relative humidity; 12:12 h light/dark cycle), fed commercial rodent chow, given water ad libitum, and acclimated for 1 week before use.

Fig. 1. Operational principle of the biopsy apparatus. **a** The cone hole of a 1-mL syringe is pressed firmly on the surface of the renal tissue to be biopsied, forming a seal. By applying pressure, kidney tissue with a diameter of 2 mm is pulled into the cone head hole. While maintaining the position of the pulled piston, an assistant places a polyester wire (8/0) wrapped around the syringe cone head around the tissue that is being aspirated; quickly pulling the polyester wire cuts the aspirated tissue. **b** Kidney biopsies of the same size were obtained by 3 identical pulls. **c** The full size of the renal biopsy specimen could be clearly observed by PASM staining. PASM, periodic acid-silver methenamine.

Experimental Design

Kidney Biopsy in Healthy Model Mice

Twelve healthy C57BL/6 mice were randomly divided into 2 groups (Biopsy [Bx] group and Control [Ctrl] group, $n = 6$ per group). On day 0, all C57BL/6 mice underwent a left nephrectomy. Then, the right kidney was exposed by a right back incision, a first-round biopsy with a diameter of 2 mm was performed at the upper pole of the kidney surface in the Bx group, and the weight of the biopsy tissue was recorded. On the 4th day, a second-round renal biopsy with a diameter of 2 mm was the Ctrl group underwent a sham operation. Performed on the inferior region of the other side of the kidney surface in the Bx group, and the weight of the biopsy tissue was recorded. On the 7th day, a third-round biopsy with a diameter of 2 mm was performed in the middle region of the right side of the kidney in the Bx group, and the weight of the biopsy tissue was recorded. Then, the animals were sacrificed in both groups on day 7, body weight, 24-h urine protein, and serum Cr were measured before the mice were sacrificed and the right kidneys were removed for histological examination.

Kidney Biopsy in Unilateral Ureteral Obstruction Model Mice

Twenty-four healthy C57BL/6 mice were randomly divided into 2 groups (Bx group, $n = 6$ and Ctrl group, $n = 18$). On day 0, both groups of mice underwent right ureteral ligation to establish the UUO model. On the 3rd day, the right kidney of the Bx group was exposed by a right back incision, and a first-round biopsy with

a diameter of 2 mm was performed at the upper pole of the right kidney surface to record the weight of the biopsy tissue. On the 7th day, a second-round kidney biopsy with a diameter of 2 mm was performed on the lower on the other side of the right kidney surface in the Bx group, and the weight of the biopsy tissue was recorded. On the 10th day, a third-round biopsy with a diameter of 2 mm was performed in the middle region of the right side of the kidney in the Bx group, and the weight of the biopsy tissue was recorded. Then, the mice in the Bx group were sacrificed. The mice in the Ctrl groups were sacrificed on the 3rd, 7th, and 10th days, respectively, and 6 mice were randomly sacrificed each time. Body weight, 24-h urine protein, and serum Cr of each group were measured before the mice were sacrificed. The right kidneys were removed for histological examination.

Operational Principle of the Biopsy Apparatus

A 1-mL disposable sterile syringe was used. The needle was removed, and the cone hole was pressed tightly to the surface of renal tissue to be biopsied, forming a seal. The pulled piston position can be controlled using the scale on the syringe. By applying pressure, kidney tissue with a diameter of 2 mm is pulled into the cone head hole. By maintaining the position of the pulled piston, an assistant places a polyester wire (8/0) wrapped around the syringe cone head around the tissue that is being aspirated; quickly pulling the polyester wire cuts the aspirated tissue (Fig. 1a). In this manner, equivalent-sized kidney biopsies can be repeatedly obtained by applying the same pulling force (Fig. 1b). The full size of the renal biopsy specimen could be clearly observed by periodic acid-silver methenamine (PASM) staining (Fig. 1c).

Histology and Immunohistochemistry

The renal tissues were fixed with 10% neutral formalin for 24 h and then embedded in paraffin. Histological morphology was observed by hematoxylin-eosin (HE) staining [4], the degree of glomerulosclerosis by PASM staining [5]. The degree of renal tubular injury and interstitial alteration was also evaluated by periodic acid-Schiff (PAS) staining and Kim-1 immunohistochemical staining [6]. F4/80 immunohistochemical staining as a marker of macrophages was used to evaluate the inflammatory response. Masson, M-Sirius red (Sirius) staining, and α -smooth muscle actin (α -SMA) immunohistochemical staining were used to observe the degree of fibrosis [7–9].

The degree of glomerulosclerosis was evaluated as described by Raij et al. [10]. Glomerulosclerosis can be divided into 0–4 grades according to the proportion of glomerulosclerotic foci in the glomerulus. Grade 0 indicates no glomerulosclerosis, grade 1 indicates no more than 25% damage to a single glomerulus, grade 2 indicates 26–50% damage, grade 3 indicates 51–75% damage, and grade 4 indicates 76–100% damage. The glomerular sclerosis index (GSI) was calculated based on this grading. For example, 5 of 40 glomeruli were +, 5 glomeruli were +++, and the other 30 glomeruli were normal; the GSI can be calculated as follows: $GSI = [(1 \times 5/40) + (3 \times 5/40)] \times 100 = 50$. The criteria for tubular injury and renal interstitial fibrosis were from the literature [11, 12]. The degree of renal tubular injury and renal interstitial fibrosis is expressed as the proportion of damaged renal tubules and the proportion of renal interstitial tissue and fibrosis, respectively. For statistical analysis, at least 10 visual fields of each specimen were randomly selected and photographed under the microscope (200 $\times$, Nikon DS Ri1), and the positive areas were statistically analyzed by Image ProPlus software.

Table 1. Analysis of serum Cr, 24-h urine protein, and KW/BW in healthy model mice

	Bx group	Ctrl group	<i>p</i> value
Scr, μ mol/L	20.83 $\pm$ 5.58	21.57 $\pm$ 5.31	0.8192
24-h UprV, g	0.60 $\pm$ 0.27	0.44 $\pm$ 0.18	0.5960
KW/BW, mg/g	7.43 $\pm$ 0.72	7.20 $\pm$ 0.99	0.6506

Serum Cr, 24-h urine protein, and KW/BW ratio on day 7 are expressed as the mean $\pm$ SEM. KW/BW, kidney weight/body weight; Bx, biopsy; Ctrl, control.

Statistical Analysis

All results are presented as the mean $\pm$ SEM for each group. Statistical differences were analyzed using one-way ANOVA, two-tailed *t* tests of independent samples, or nonparametric tests, and *p* values of <0.05 or 0.01 were considered significant.

Results

Morphology, Pathology, and Renal Function Did Not Differ Significantly between the Biopsy and Control Groups of Healthy Model Mice

Renal biopsies were performed in healthy model mice. There was no difference in body weight or serum Cr between the 2 groups before the operation (data not shown). We tested whether the new renal biopsy method affects the renal morphological parameters and renal function of the mice. On the 7th day, we measured the serum Cr, 24-h urine protein, kidney weight (KW), and body weight (BW) of all mice in the Bx and Ctrl groups. The data showed that there was no significant difference in serum Cr, 24-h urine protein, or KW/BW ratio between the Bx and Ctrl groups (Table 1). These results confirm that this new renal biopsy method does not cause changes in renal function or changes in gross renal morphology after repeated renal biopsies.

To further study the quality of the biopsy and the influence of the new biopsy method on renal histology, HE, PASM, PAS, Masson's trichrome, Sirius red and Kim-1, F4/80, α -SMA immunohistochemical staining were performed on the renal tissues obtained at days 0, 4, and 7 after biopsy, the remnant renal tissues (Rem) and the Ctrl group (Fig. 2a). There was no significant difference in the number of glomeruli in the 3 biopsies (Table 3), and the analysis showed that the average glomeruli in each cross section were 25.06 ± 9.87 ($n = 18$) and that the biopsy-related damage was limited to the renal tissue near the biopsy area. There were no significant differences in renal tissue morphology, GSI, tubule injury, inflammatory response, or fi-

Fig. 2. Histological changes and analysis of GSI score, tubular injury, inflammatory response, and kidney fibrosis in healthy model mice. **a** Representative images of HE, PASM, PAS, Masson's trichrome, Sirius red and Kim-1, F4/80, and α-SMA immunohistochemical staining among different groups in healthy model mice. **b** GSI score. **c** Proportion of renal tubular injury by PAS staining.

d The proportion of Kim-1 positive areas. **e** The proportion of F4/80 positive areas. **f** Proportion of kidney fibrosis by Masson staining. **g** The proportion of Sirius positive areas. **h** The proportion of α-SMA positive areas. Data are means ± SEM ($n = 6$). ns, no significance versus sham control; PASM, periodic acid-silver methenamine; PAS, periodic acid-Schiff.

Table 2. Analysis of serum Cr, 24-h urine protein, and KW/BW in UUO model mice

	Bx group	Ctrl group	<i>p</i> value
Scr, μmol/L	22.31±7.32	21.07±3.83	0.9766
24-h UprV, g	0.84±0.06	0.63±0.18	0.4038
KW/BW, mg/g	4.90±0.31	5.10±0.30	0.2967

Serum Cr, 24-h urine protein, and KW/BW ratio on day 10 are expressed as the mean ± SEM. UUO, unilateral ureteral obstruction; KW/BW, kidney weight/body weight; Bx, biopsy; Ctrl, control.

Table 3. The number of glomeruli in each renal biopsy sample in healthy and UUO model mice

	1st Bx	2nd Bx	3rd Bx
Healthy model	26.40±7.20	26.00±3.92	25.33±5.03
UUO model	32.67±5.69	30.40±6.58	29.67±9.29

The number of glomeruli in the three biopsies of the healthy mice group and the UUO mice group are expressed as the mean ± SEM. All p values >0.05. UUO, unilateral ureteral obstruction; Bx, biopsy.

brosis among the biopsy tissues, the Rem, and the Ctrl group (Fig. 2b–h). These results indicate that the new renal biopsy method not only provides sufficient renal tissue samples but also can reflect changes in renal histology.

Morphology, Pathology, and Kidney Function Did Not Differ Significantly between the Biopsy and Control Groups in Unilateral Ureteral Obstruction Model Mice

To verify the practicality of the new renal biopsy method in a disease mouse model, we selected 24 healthy C57BL/6 mice and established a UUO model. There was no difference in body weight or serum Cr between the 2 groups before the operation (data not shown). First, to explore the influence of the new renal biopsy method on renal function and general morphology in UUO model mice, the serum Cr, 24-h urine protein, KW, and BW of all mice were measured on the 10th day. The data showed that there were no significant differences in the serum Cr, 24-h urine protein, and KW/BW of the C57BL/6 mice between the Bx and Ctrl groups (Table 2). These results suggest that the new renal biopsy method does not cause damage or changes in renal function and general morphology in UUO model mice.

We further studied whether the new renal biopsy method provided sufficient renal biopsy tissue from both UUO model mice and healthy model mice and whether no histological changes occurred. Therefore, PASM, PAS, Masson, Sirius red and Kim-1, F4/80, and α -SMA immunohistochemical staining were performed on renal tissues obtained on days 3, 7, and 10 from the Bx, Rem, and Ctrl groups in UUO model mice (Fig. 3a, 4a). There was no significant difference in the number of glomeruli in the 3 biopsies (Table 3), and the analysis showed 34.47 ± 10.19 glomeruli ($n = 18$) in each cross section of the 3 biopsies. There were no significant differences in GSI, renal tubule injury, inflammatory response, or renal fibrosis between the Bx and Ctrl groups on days 3, 7, and 10, respectively, or between the Rem tissues and Ctrl group on day 10 (Fig. 3b–e, 4b–d). In conclusion, consistent with the results in the healthy model mice, the novel renal biopsy method had the same practicality in the UUO model, and the renal tissue effectively represented the physiological or pathological manifestations of the kidney.

Discussion

In animal models, it can be difficult to confirm therapeutic effects due to technical inconsistency and other reasons. Although renal biopsy is widely used in clinical

diagnosis, it is rarely used in animal experimental models, especially in mice, because the problems of surgery-induced renal injury and bleeding have not been solved. Therefore, we developed an easy-to-use method of renal biopsy in mice and evaluated whether 3 consecutive renal biopsies can be performed in the same kidney in a standardized manner. We verified our method in 2 mouse models: a healthy mouse model and a UUO mouse model. Morphology, pathology, and renal function did not differ significantly between the Bx and Ctrl groups in either the healthy model or the disease model. Therefore, our new standardized method of renal biopsy is safe, allows for repeated renal biopsies, and ensures sufficient sample size to quality in experimental disease models.

To our knowledge, no standardized mouse kidney biopsy has been introduced so far. The main reasons are that animal kidney biopsy must be carried out with direct renal exposure, and the volume of animal kidneys, especially mouse kidneys, is small; thus, the amount of kidney biopsy sampling is limited, and multiple biopsy sampling easily causes remnant renal function damage [13–15]. Holger Schirutschke's team developed a standardized renal biopsy device for experimental rats [16]. They modified the skin biopsy needle used in the clinic and performed standardized renal biopsy on rat kidneys using the cutting principle. However, at present, the use of standardized renal biopsy in mice is very rare. This method developed by us is more similar to clinical renal biopsy, and the experimental results have stronger medical relevance. This method of repeated renal biopsy on a single kidney can be used to evaluate any intervention treatment longitudinally and support the feasibility and effectiveness of long-term follow-up biopsy.

We developed a mouse renal biopsy technique and verified its practicality in 2 models: a healthy mouse model and a UUO disease mouse model. All C57BL/6 mice were alive without any perioperative complications, such as infection or bleeding. There was no significant difference in serum Cr between the 2 groups. A sufficient amount of kidney tissue for each biopsy was obtained from both models. After 3 biopsies, the remnant KW/BW in the 2 models was not significantly different from that of the control group. The damage to biopsy operation-related tissue was also limited to the tissue area near the biopsy site. Compared with tissues from the corresponding control group, the renal tissues obtained from the 3 biopsies and the remnant renal tissues after the biopsy had no significant difference in the GSI, the degree of renal tubular damage, the inflammatory response, or the degree of renal fibrosis. These findings indicate that the

Fig. 3. Histological changes and analysis of GSI score, tubular injury, and inflammatory response in UUO model mice. **a** Representative images of PASM, PAS staining and Kim-1, and F4/80 immunohistochemical staining among different groups in UUO model mice. **b** GSI score. **c** Proportion of renal tubular injury by PAS staining. **d** The proportion of Kim-1 positive areas. **e** The proportion of F4/80 positive areas. Data are means $\pm$ SEM ($n = 6$). ns, no significance versus control; UUO, unilateral ureteral obstruction; PASM, periodic acid-silver methenamine; PAS, periodic acid-Schiff.

Fig. 4. Analysis of kidney fibrosis in UVO model mice. **a** Representative images of Masson, Sirius red, and α -SMA immunohistochemical staining among different groups in UVO model mice. **b** Proportion of kidney fibrosis by Masson staining. **c** The proportion of Sirius positive areas. **d** The proportion of α -SMA positive areas. Data are means $\pm$ SEM ($n = 6$). ns, no significance versus control; UVO, unilateral ureteral obstruction; PAS, periodic acid-Schiff.

new renal biopsy method minimizes the possibility of renal injury and has a high degree of standardization. Through minimizing the complications related to renal biopsy, this new method provides standard-sized renal biopsy samples that effectively represent the physiological or pathological manifestations of the kidney and can be used for histological or molecular biological analysis.

This new biopsy method combines the working principle of pressure suction and cutting. A 1-mL disposable sterile syringe was selected for pressure suction. The diameter of the cone hole of the syringe is 2 mm, which en-

sures that the diameter of the biopsy tissue is 2 mm. This method effectively controls the uniformity of the size of the biopsy tissue by controlling the position of the pulled piston using the scale on the syringe. A high-strength polyester wire that is not easy to break and has a smooth surface and resistance to acids, alkalis, UV radiation, weather aging, and other characteristics was selected for cutting. As bleeding is one of the most common complications that should be considered in serial biopsies, it is also the main cause of death in operated mice. A high-strength polyester wire can quickly cut off the tissue, and

then the renal capsule will quickly wrap the wound. The contraction of the capsule can effectively stop bleeding, so there is no need for other hemostasis methods. The equipment needed for the biopsy method is disposable sterile clinical equipment that does not require additional disinfection, and the method of sampling and operation is simple; therefore, this method can be utilized in the vast majority of laboratories. The UUO model was selected as the disease model and is more suitable for the new biopsy method than the acute kidney injury model. Ureteral obstruction causes a series of renal morphological changes, such as hydronephrosis, which increases the difficulty of renal biopsy operation [17]. The possibility of performing a repeated biopsy in a single kidney allows for individualized, longitudinal evaluation of any therapeutic intervention by performing a zero biopsy (for baseline values) as well as a long-term follow-up biopsy. In our view, possible effects between the interventional and corresponding untreated groups could be accurately evaluated, avoiding any uncontrolled factors, such as technical inconsistency and individual differences.

However, there are still some limitations in this study. First, we chose only serum Cr as an indicator of renal function, which is not a more sensitive measurement of glomerular filtration rate than the inulin clearance method, so we cannot completely exclude slight changes in renal function [18]. Second, only one side of the kidney was biopsied in the 2 mouse models, and the kidney volume of the mice was small. To avoid extensive renal injury, minimal renal tissue was collected. Paraffin and frozen specimen preparation and molecular biological examinations cannot be performed at the same time. The renal biopsy method of mice in this experiment is the same as that of clinical renal biopsy. Fresh biopsy can be used for RNA detection. One method is to use a micro kit, the other is to perform 2 or more punctures at a time, but there is limitation for protein detection by this novel method. In the future, we can perform 2 or more punctures at a time, or puncture from bi-

lateral kidneys of mice at the same time, so as to ensure the sample size and meet the requirements of histology, biochemistry, molecular biology, and gene detection. Finally, this biopsy method is relatively simple but requires the help of an assistant. In the future, this method can be mechanized and automated. In conclusion, our new standardized method of renal biopsy in mice is safe and cost-saving approach, allows for repeated renal biopsies, and ensures minimal trauma and sufficient sample size, even in experimental disease models restricted to one single kidney.

Statement of Ethics

All experimental procedures and animal care protocols were approved by the Animal Committee of Harbin Medical University (KY2018-171). Animal experiments were performed in accordance with the Health Guidelines of the National Institutes for the Care and Use of Laboratory Animals.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This study was supported by research grants from the National Natural Science Foundation of China (Nos. 8187030729 and 81670616).

Author Contributions

Xinyue Mao and Bing Li developed the study concept and design, analyzed and interpreted the data, and drafted the manuscript. Xinyue Mao performed the experiments. Chang Wang, Zhihui Xu, Yixin He, and Yanpei Hou contributed reagents/materials/analysis tools.

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