Mouse model of ischemic acute kidney injury: technical notes and tricks

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Acute Kidney Injury (AKI) is a major kidney disease associated with high mortality in human patients. Recent basic science and epidemiologic studies have further suggested a causal role of AKI in the development and progression of chronic kidney disease. Clinically, ischemia is a leading cause of AKI, which may result from a variety of conditions, such as decreased cardiac output, renal vascular occlusion or obstruction, and kidney transplantation. In vitro models, including renal cell cultures, isolated renal tubules, and isolated perfused kidneys, are valuable for the research of the pathophysiological mechanisms of ischemic AKI. Nevertheless, in vivo whole animal models are indispensable, because of the limitation of the in vitro models to mimic the complexity of human body (45). Since the 1960s, various animal models of ischemic AKI have been developed and tested, and currently, two kinds of warm renal ischemia-reperfusion (IR) models are mainly used: 1) bilateral renal ischemic reperfusion (IR) (2, 6, 8, 13, 14, 17, 19, 22, 23, 25–27, 30, 31, 35, 39, 41–43, 46, 51, 53–57, 59, 61, 63, 67, 68, 75–80) and 2) unilateral renal IR (1, 9, 15, 18, 20, 21, 24, 29, 33, 34, 37, 38, 40, 44, 50, 58, 60, 62, 64, 66). Depending on whether the contralateral kidney is removed, the unilateral model can be further divided into two subtypes: unilateral IR with contralateral nephrectomy (15, 18, 20, 29, 38, 40, 44, 50) or without contralateral nephrectomy (1, 9, 21, 24, 45, 60). The bilateral ischemic AKI model is commonly used, because it is considered more relevant to human pathological conditions where blood supply is normally affected in both kidneys (2, 10, 11, 16, 30, 32, 37, 47, 49, 52, 70–74, 79). In the bilateral model, some studies performed decapsulation prior to renal ischemia (42, 51) that may have renoprotective effects, as reported earlier (69). However, decapsulation was not conducted in the majority of published studies.

The initial models of ischemic AKI were developed with experimental animals of relatively large size, such as dogs and rabbits (7, 28, 39). Rat models then became the most popular animal model, as among the ~1,300 publications of ischemic AKI animal studies since 1960s, half of them were conducted in rats. In 1990, the mouse was first introduced into the research field of ischemic AKI (65). The studies with mice were markedly promoted by the availability of various transgenic mice. In the past decade, there have been more studies using mice than those using rats. In addition, the size of a mouse is about 1/10 that of a rat, which means less drug consumption for experimental testing. Despite these notable advantages, the mouse model is known to have bigger variations, causing inconsistency in results. In recent years, we have optimized the mouse model of bilateral renal ischemic AKI. In this review, we share the lessons and experiences that we have learned and have gained in our laboratory. Specifically, we present a detailed experimental protocol and discuss the technical issues that we think may benefit our fellow researchers in this field to establish more reliable, consistent mouse models for ischemic AKI research.

Experimental Procedures for Bilateral Ischemic AKI in Mice

Preparation for experiment. The equipment, surgical tools and other materials (number needed in parentheses) are a homeothermic monitor system (1), animal hair clipper (1), tissue forceps with blunt points (2), tweezers with ultra-sharp points (1), dissecting and operating scissors with sharp points (2), micro-aneurysm clips (2), micro-aneurysm clip applying forceps (1), 4–0 Vicryl suture with 1/2-circle needle of 17-mm length; needle holder (1), Michel wound clips, Michel wound...
The right kidney is gently pushed out of body cavity with cotton swabs to expose the pedicle. A micro-aneurysm is used to clamp the pedicle to block the blood flow to the kidney to induce renal ischemia. The duration of right kidney ischemia starts from the time of clamping. Complete ischemia is indicated by color change of the kidney from red to dark purple in a few seconds. After verification of the kidney color changes, the kidney is returned to the abdomen cavity. The mouse is then laid on its right side for the left renal pedicle clamping and ischemia. There is around 1–1.5 min time latency between the right and left kidney clamping. However, the ischemic time of each side is recorded separately to ensure both kidneys receive the same durations of ischemia.

After the ischemia, the micro-aneurysm clips are released at desired times for each kidney to start the reperfusion, which is indicated by the change of kidney color to red. A Vicryl suture is used to close the muscle layer of the incision followed by the closure of the skin wound with Michel wound clips. Immediately after the wound closure, 0.5 ml warm sterile saline is given intraperitoneally to each mouse. The animal is then kept on a heating pad until it gains full consciousness before being returned to its housing cage.

Monitoring the Success of Renal Ischemia-Reperfusion

The success of renal IR is monitored at several levels. First, after clamping, the kidney color should change from red to dark purple, indicative of a successful renal ischemia. The immediate color change at the very beginning requires careful observation to notice. However, the kidney will be in deep dark purple color several minutes later. After removing the clips, kidney color should change back to red to indicate the reperfusion. Usually blood flow is restored immediately after removing the clips, thus special anti-coagulation procedure is not applied here in our experiments.

Second, depending on the severity of kidney injury, there may be a decline of renal function that can be detected by the increases in blood urea nitrogen (BUN) and serum creatinine. A few microliters of blood samples are sufficient for the BUN measurement using the urea nitrogen test kit from StanBio, while 20 μl are needed for the serum creatinine measurement using a kit (based on Jaffe Method) from StanBio. Therefore, we routinely measure the BUN to monitor the renal function before the renal ischemia and at different reperfusion time points. The BUN value in normal control C57BL/6 mice is around 20–40 mg/dl. After 30 min of bilateral renal ischemia, there are slight yet detectable increases in BUN between 6 and 12 h of reperfusion and marked increases between 24 and 48 h (Fig. 2). The serum creatinine is usually determined at the endpoint of the experiment, when larger volumes of blood can be collected when the kidney is removed. As expected, the BUN and serum creatinine increases depend on the severity of kidney injury. For example, in one of our previous tests, after 22 min of renal ischemia, the BUN value increased to 120–150 mg/dl at 48 h of reperfusion, after which BUN decreased toward basal levels as a result of kidney repair and functional recovery. After 25 min of ischemia, BUN reached ~300 mg/dl at 72 h of reperfusion. In C57BL/6 mice, 22 min and 25 min of ischemia will induce mild to moderate injury with the recovery of renal function in ~1 wk. Thirty minutes of ischemia induced very severe kidney injury, and a significant proportion of the

Fig. 1. Sites of flank incision of bilateral mouse model of ischemic acute kidney injury (AKI). The incision sites on the left (A) and right (B) sides are labeled with red line.
severely injured mice died at 72 h of reperfusion. The serum creatinine level usually increases to 1–1.5, 2, and 2.5 mg/dl after 48 h of reperfusion following 22, 25, and 30 min of ischemia, respectively.

Finally, the histological examination of kidney tissues by methods such as hematoxylin-and-eosin (H&E) staining, PAS staining, and TUNEL assay is the direct way to verify and localize the kidney injury. Fig. 3 shows representative images of H&E staining of kidney tissues with or without ischemic AKI. The typical renal tubular damage includes severe tubular lysis, loss of brush border, and sloughed debris in tubular lumen space. In ischemic AKI, the most severely injured site is the S3 segment of proximal tubules located at the outer stripe of outer medulla. We routinely conduct H&E staining to grade tubular damage (0, no damage; 1, 0–25% damaged tubules; 2, 25–50% damaged tubules; 3, 50–75% damaged tubules; 4, >75% damaged tubules) (10, 72). We also analyze apoptosis by TUNEL staining and immunofluorescence of active caspase 3; apoptosis can be quantified by counting positively stained cells (72–74).

Key Factors for a Consistent Mouse Model of Ischemic AKI

Mouse surgeon. A well-trained, skillful surgeon is the key to the establishment of a consistent, reliable mouse model of ischemic AKI. A good mouse surgeon not only can reduce surgical trauma but also can complete the whole procedure within the anesthesia time in a smooth, organized manner. As discussed below, 50 mg/kg pentobarbital sodium is normally used in our study for mouse anesthesia. This anesthesia only provides a little more than 1 h of time for the whole experimental procedure, which includes the surgery to expose renal pedicles, the waiting period for body temperature stabilization, 20–30 min of ischemic duration, and finally the closure of the wound. In our experience, a higher dosage of pentobarbital sodium (e.g., >60 mg/kg) prolongs anesthesia, but it significantly increases animal loss during surgery. Less experienced surgeons need longer operation time and, thus, may need to supplement anesthetics for the surgery to be completed, which can affect the final result and lead to animal loss. We emphasize that the surgery has to be conducted in a well-prepared and organized manner by a skillful surgeon. To this end, new surgeons have to fully understand each of the steps, watch the whole procedure, and practice until the whole experiment can be completed within the anesthesia time to yield comparable kidney injury results. Notable variations are introduced during high turnover or rotation of mouse surgeons. Thus, it is advised, unless unavoidable, not to change the mouse surgeon in a study.

Fig. 2. Blood urea nitrogen (BUN) level of C57BL/6 mice after different ischemia-reperfusion periods. Male mice of 8 wk were subjected to 22, 25, and 30 min of bilateral renal ischemia or sham operation. Serum samples were collected at indicated reperfusion times for BUN assay.

Fig. 3. Renal histology after ischemic AKI. Top: kidney tissues from C57BL/6 mice with 30 min of bilateral renal ischemia and 48 h of reperfusion or sham operation were stained by hematoxylin and eosin. IM, inner medulla. CT, cortex. Bottom: enlarged images of boxed area in the upper panels.
Animals. There are marked differences in the susceptibility to ischemic AKI among different mouse strains and even different colonies of the same strain. National Institutes of Health Swiss mice were shown to be resistant or less sensitive to ischemic AKI than C57BL/6 and BALB/c mice (12). A recent study further showed that 129/Sv mice are also less susceptible to ischemic AKI (48). The mouse strain or colony-related differences in injury susceptibility is particularly relevant in studies using transgenic and gene knockout mouse models. Although most of the transgenic mouse models are described to have comparable genetic background with wild-type strains (e.g., C57BL/6) after more than five generations of backcross, the wild-type mice from the same transgenic models may be significantly different in their ischemic injury sensitivity than the regular C57BL/6 mice. For example, our recent study established a Dicer-knockout mouse model in which Dicer was specifically deleted from the kidney proximal tubules (PT-Dicer-KO). This model had a C57BL/6 background, but the wild-type mice from this model were significantly more resistant to ischemic kidney injury than the regular C57BL/6 mice. As a result, longer (32 vs. 30 min for regular C57BL/6) ischemic time was needed to induce AKI with BUN of ~200 mg/dl and serum creatinine of ~2 mg/dl after 48 h of reperfusion (72). We routinely conduct pilot tests to determine the appropriate ischemic duration for a new mouse line to be studied.

Even within the same strain, different mouse colonies may show different susceptibility to ischemia AKI. Our laboratory maintains an in-house C57BL/6J colony established with breeders from The Jackson Laboratory. Mice from this colony are significantly more resistant to ischemic kidney injury than the aged matched male mice directly purchased from The Jackson Laboratory (Fig. 4). The cause of the difference is unclear, but it may be related to colony maintenance, which includes feeding, health, and stress of the animal. In this regard, if mice are shipped from a vendor or other outside sources, they need to have at least 1 wk of rest/stabilization before the experiment. To alleviate the strain and colony differences, we strongly recommend that littermate mice are tested in the same experiment. This is particularly important for Omics studies that analyze hundreds to thousands of genes, proteins, or metabolites and thus require very stringent controls to reduce false positives to narrow down the targets for further in-depth investigation. In studies using a transgenic model, the wild-type littermates from the model, rather than the mice from a matching strain, should be used as the controls. Such controls were included in recent studies to generate convincing evidence for the involvement of specific genes in the pathogenesis of ischemic AKI (5, 27, 51, 72, 80).

Ischemic AKI is also affected by the animal age. For example, Kusaka et al. (36) recently showed that aged rats (60–65 wk old) are more susceptible to ischemic AKI than young rats of 6–7 wk. In young adult mice of ~8–12 wk that are commonly used for ischemic AKI study, age differences of over 1 wk may cause detectable differences in kidney injury. Our suggestion is to use animals of the same or very similar (<1 wk difference) age in each experiment.

In addition, ischemic AKI is known to be affected by sex. Interestingly, while the female mice are generally more resistant to ischemic AKI than males, they are more sensitive to cisplatin-induced nephrotoxic AKI (57, 74). Male mice are commonly used for ischemic AKI research due to their better consistency and sensitivity, which is caused mainly by testosterone (57). The health condition of the animals is another factor that should be considered. Some transgenic or gene knockout models may develop disease conditions that affect AKI.

Key Equipment

In addition to the general surgical tools, the key equipment, especially the microaneurysm clips that are used to induce renal ischemia and the thermostatic system for the body temperature control, are important to the success of the experiment. Appropriate tools and equipment should be purchased and designated for mouse experiment. The microaneurysm clips for mouse renal pedicle clamping are quite fragile and should be handled carefully with specially designed applying forceps to avoid any damage. We use the clips from George Tiemann (item no. 160–863), and similar microaneurysm clips are available from Biomedical Research Instruments and Roboz Surgical Instrument Company. Those clips can be reused multiple times if they are handled properly and with care. Normally, we replace the clips after ~100 operations or if the surgeon detects damages to the clip, which can cause uneven kidney color changes with nonischemic spots after clamping. The thermostatic station with a rectal probe to monitor and control the mouse body temperature in the accuracy of 0.1°C is necessary because, as discussed below, the variation in body temperature is probably the single most critical factor affecting the severity of ischemic kidney injury. Our thermostatic stations were purchased from Harvard Apparatus (item no. 507222F), and similar equipment is available from other manufacturers.

Surgery

Dehydration status and surgery time. Kidney injury is greatly affected by the dehydration status of the body. Normally, experimental mice are kept in animal facilities with 12:12-h light-dark cycle. Mice are nocturnal, meaning they are more active in the night time. Thus, the mice housed in dark are more active and drink more water, whereas they sleep more and drink less during the light period. In general, the mice housed under 12:12-h light-dark cycle are more dehydrated in the afternoon than in the morning. Thus, it is highly recommended to conduct the ischemia experiment at a certain time of the day.
for all experiments in one study. We normally induce renal ischemia at 2:00 PM–5:00 PM.

Anesthesia

We choose to use pentobarbital sodium for anesthesia after we tried a few other anesthetics. Ketamine/xylazine was initially recommended by the veterinarian of our animal facility. We had to give it up because the dosage of ketamine/xylazine to ensure 1 h of anesthesia for operation frequently caused animal death. Isoflurane is another drug that we tested for anesthesia that can be switched on and off easily with the gas anesthesia machine, and it is not a controlled substance. However, isoflurane itself has renoprotective effects during renal ischemia-reperfusion injury (79) and consistently, obvious kidney injury could not be induced in mice even after 30 min of bilateral renal ischemia in our pilot tests. In our hands, pentobarbital works best. There may be occasional animal loss following pentobarbital anesthesia, but this is rare if the dosage is well controlled (start from 50 mg/kg and supplement with 5 mg/kg during surgery when necessary). Combined with buprenorphine, this dosage of pentobarbital provides a sufficient level of anesthesia and sedation for the completion of the surgery.

Body Temperature Maintenance

The body temperature of the experimental animal during ischemia is one of the most important factors that affect the severity of AKI. We used to do surgery with only a heating pad to keep mice warm during surgery. However, when our laboratory moved to a new space, there was a notably bigger variation of kidney function after ischemic injury (Fig. 5). Over a period of time troubleshooting, we finally figured out that the fluctuation of room temperature (and thus the mouse body temperature during surgery) was the cause of the variation. To solve this problem, we purchased a homeothermic monitor system, which has a rectal probe to accurately control the body temperature of the mouse under surgery and renal ischemia, which greatly reduced the variation of the outcome (Fig. 5).

Operation Time

To minimize variation, both total operation time and the ischemic time latency between right and left kidneys need to be consistent between the animals and experiments. The timeline of our typical experiment is depicted in Table 1.

1. Anesthesia and preparation for surgery (30 min) involves injection of pentobarbital sodium and buprenorphine, skin preparation, and stabilization of the body temperature on the heating pad of the homeothermic monitor system.

2. Surgery and clamping (10 min) involve the preparation of the renal pedicles, body temperature recovery, and clamping the renal pedicle. In this step, the time latency between the clamping of the right pedicle and the left pedicle is controlled within 1 to 1.5 min. Although it may not matter which side to start, we suggest to clamp the right side first because the right pedicle is relatively short and more difficult to clamp. After the right clamping, it is easy to clamp the left pedicle in 1–1.5 min.

3. The duration of renal ischemia (20–30 min) is determined by the experimental design.

4. Wound closure (5 min) involves suturing of muscle layer and clipping closure of the wound.

Minimization of Surgical Trauma

The purpose of the surgery is to induce ischemic AKI. It is important to minimize other trauma associated with the surgery. Currently, both midline incision (laparotomy) and flank incision are being used by different investigators to expose renal pedicle for clamping. We recommend flank incision, which is much less traumatic. It is also important to limit the incision size. In addition, we recommend the use of Vicryl suture to close the muscle layer of the wound as it is absorbable. Closure of the wound with clips without suturing the muscle layer may lead to incomplete wound healing and infection.

Table 1. Timeline of surgical procedure for bilateral ischemic AKI in mice

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Injection of pentobarbital and buprenorphine, skin preparation</td>
</tr>
<tr>
<td>25–30</td>
<td>Body temperature stabilization</td>
</tr>
<tr>
<td>2</td>
<td>Renal pedicle preparation</td>
</tr>
<tr>
<td>5</td>
<td>Body temperature recovery</td>
</tr>
<tr>
<td>2–3</td>
<td>Renal pedicle clamping</td>
</tr>
<tr>
<td>20–30</td>
<td>Renal ischemia</td>
</tr>
<tr>
<td>5</td>
<td>Wound closure</td>
</tr>
<tr>
<td>60–75</td>
<td>Total</td>
</tr>
</tbody>
</table>

AKI, acute kidney injury.
Post surgery Care

Good post surgery care is not only humane, but it is also a way to reduce experimental variation and prevent the loss of animals. Regularly, we give saline supplementation immediately after the surgery to prevent dehydration of the mouse. After returning the mouse to its cage, easily accessible water and food supplies are also necessary. The surgery may cause mobility difficulty of the animal. If the water and food are at high positions, it is important to keep some gel food on the floor of the cage. In general, pain reliever is not needed since the mouse behavior does not show significant signs of distress after the initial dose of buprenorphine.

Although the variation of the mouse model of ischemic AKI is generally larger than other (rat) models, relatively consistent results can be achieved with attention to the above-described factors. As shown in Fig. 5, in one of our experiments, after 30 min of ischemia and 48 h of reperfusion, BUN increased to an average of 230 mg/dl with a standard deviation of 31, while serum creatinine was 2.32 ± 0.35. In such severe AKI conditions, we usually detect a <50 mg/dl variation in BUN and <0.5 mg/dl in serum creatinine. For statistical analysis, 5 or 6 pairs of mice are necessary to show the difference between the two groups. Animals are excluded from a study if any of the following occurs: 1) there is abnormal kidney morphology and kidney function before surgery; 2) there are noticeable abnormalities in other organs; 3) surgery is not well performed or accidents occur during surgery; or 4) test substance delivery fails.

Conclusions

Bilateral renal I/R in mice is a commonly used model for AKI research. Nevertheless, the mouse model is less stable than others (e.g., the rat model). However, a well-trained, skillful surgeon who follows the afore-described procedure and pays particular attention to the technical issues, should be able to establish a relatively consistent mouse model for the investigation of the pathogenic mechanism and identification of therapeutic approaches.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: Q.W. and Z.D. conception and design of research; Q.W. performed experiments; Q.W. and Z.D. analyzed data; Q.W. and Z.D. interpreted results of experiments; Q.W. prepared figures; Q.W. drafted manuscript; Q.W. and Z.D. edited and revised manuscript; Q.W. and Z.D. approved final version of manuscript.

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