Chapter 11. Experimental models for renal disease – reduction in renal mass

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Rationale for model development

Chronic kidney disease (CKD) is a public health problem worldwide. With constantly increasing incidence, high rates of mortality and morbidity, and frequent inexorable progression to end-stage renal disease (ESRD), the understanding of the pathogenic mechanisms of CKD has received significant interest over the past decades. Almost irrespective of the original insult to the kidney, and even when this original disease is not manifest anymore, CKD progresses continually. This led to the concept that after the loss of a critical mass of nephrons, the pathogenic mechanisms for progression become manifest and lead to progressive loss of remnant kidneys. Based on experimental micropuncture studies, allowing the direct measurement of single nephron function, it was hypothesized that the increased work of the remnant normal glomeruli, a compensatory response for the initial loss of function, was a maladaptive process, eventually leading to further loss of nephrons due to advancing glomerulosclerosis.

This concept developed by Barry M. Brenner in the eighties required an adequate experimental model for testing. The laboratory rat was the animal model of choice and techniques
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to reproduce the initial loss of nephron mass were developed and refined. These models are intensively used today to gain a deeper understanding of the pathogenic mechanisms involved in disease progression, and most importantly to test different therapeutical approaches designed to slow down or even to prevent development of ESRD.

Model implementation

Based on initial clinical studies, it became apparent that an initial loss of approximately 75-80% of the total renal mass would adequately reproduce the insult leading to CKD, that progresses to ESRD. This model is known as the 5/6 nephrectomy (5/6NX) and can be achieved using two different approaches.

1. 5/6 nephrectomy by infarction
This model involves surgical removal of one kidney and obstruction of blood flow to 2/3 of the remnant kidney.

Infarction of 2/3 of the left kidney
The left kidney is preferred for this surgical approach due to the better exposure in the abdominal approach. In the rat, the left renal artery is longer and directed downwards. Considering that the surgical procedure requires a relatively short time, while survival and long-term maintenance of the laboratory rat is a must (in order to follow CKD progression), the choice of anesthetic has to be made based on the rapidity of the recovery.

Therefore, inhalation anesthetics are preferred, such as Isoflurane (5% in oxygen). Notwithstanding the advantages of
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inhalation anesthesia (direct and rapid control of depth), its cost and complexity may direct the investigator towards the use of parenteral anesthetics, such as Ketamine/Xylazin, which also provide an adequately short time of recovery.

Once the animal is anesthetized, the abdominal area is shaved from the sternum to the pelvic area, preferably using electric clippers, designed for veterinary use. The area is sterilized with Povidone – Iodine, using the two-step method, with a scrub solution first (7.5%, containing surfactants) followed by the 10% solution. It is important to note that, once the animal is ready to be returned to the cage, the dried povidone solution must be removed using alcohol, due to its local irritant effect and the ease of access to the area by the rat, who will try to alleviate itching by chewing on the final sutures. The animal is then placed on a heating table or heating pad throughout the procedure, while its core temperature is maintained at 37°C, as monitored by a rectal thermometer (Fig.1).

![Fig.1. Animal prepared for surgery](image-url)
Access to the abdominal cavity is obtained by a vertical midline incision, starting about 1 cm below the sternum and extending downward by about 2 cm (according to the size of the rat, numbers above for a 250 g rat). The incision is made in two steps: first the skin is raised using a toothed forceps and cut using blunt scissors; then the abdominal muscles are cut over the linea alba, by carefully lifting the abdominal muscle wall using toothed forceps to prevent accidental pinching of the organs below. It is advised to start with a small transversal incision at the lower end (2-3 mm) and then extend it vertically. Any bleeding from the wound edges can be resolved by applying pressure with cotton gauze or by using cautery. The incision edges can be retracted using small animal adjustable retractors or simply by passing a thicker suture thread (2-0 USP) through each of the wound sides and attaching the ends to the surgery table. The wound edges should be maintained moist at all times by covering them with gauze drenched in normal saline. This will allow for shorter and more efficient postoperative wound healing.

Using cotton swabs, the intestines and mesentery are gently moved to the right side of the abdomen and loosely secured in place by using folded 4x4 gauze. This will expose the left side of the abdomen, and the spleen will be used as a reference to find the left kidney. Using cotton swabs, the left kidney is freed from the surrounding tissue and detached from the retroperitoneal fat. Care must be taken so the capsule of the kidney is not damaged during manipulation of the kidney. The left kidney is placed on a piece of gauze and elevated from the surrounding organs (Fig. 2).
Using fine cotton tipped swabs, the pedicle is cleaned from the capsule, with gentle, repetitive, clockwise movements. It is essential that this step is performed carefully, in order not to damage the ureter or the very fine renal nerves that run not only within the pedicle but also through the surrounding connective tissue (intact renal innervation is necessary in order to preserve the functionality of the normal mechanisms involved in regulating renal function during the follow-up). A breech of no more than 3-4 mm in diameter is sufficient to get access to the renal pedicle. Within the renal pedicle, the renal artery is usually situated over the vein and easily identifiable as a thin, pink vessel, as opposed to the vein, which is about 3-4 times larger and has a dark-blue color. In order to distinguish the artery from any other resembling tissue such as fibrous bridges or nerves, it can be gently compressed using fine microsurgical blunt forceps: flow of blood will be obvious
going towards the kidney. If the artery is attached to the vein (which is rarely the case at this level) it must be separated using fine blunt curved end microsurgical forceps inserted between the artery and the vein and opened progressively – the vein is very fragile so manipulation with very sharp end microsurgical forceps is not advisable.

In close proximity to the pedicle, the renal artery sends out three branches, usually visible in the uppermost layer of the surgical field (Fig. 3).

![Fig. 3](image)

**Fig. 3.** Exposure of the renal branches (left); ligation of the inferior polar pedicle with infarction of the lower pole of the kidney

Two of the branches are then cleaned of surrounding connective tissue (either the upper and lower or the two upper branches). At this step, a microforceps must be used with great care, in order not to puncture the renal vein, which is immediately below the arterial branches. Also, any extrarenal branches of the renal artery must be identified, so that blood flow to other organs is not interrupted. This is especially the case when the artery for the adrenal gland emerges from the renal artery in close proximity to the pedicle.
Each of the branches are then lifted up using curved end, blunt forceps, and another forceps is used to pass two 4-0 silk threads underneath. The branches are then tied off with two knots without sectioning. The left kidney is then placed back in the abdominal cavity.

After the kidney is replaced, it should be checked for adequacy of the procedure, namely reduction of blood flow to 2/3 of its mass (Fig. 4). The non-perfused regions will quickly become whitish, while the normally perfused regions will have the same purple color as before the procedure.

**Right nephrectomy**

The right kidney is identified by gently lifting the liver using cotton swabs, with care not to damage the liver, or to pull on the round ligament which could cause rupture of the liver lobe and difficult to stop bleeding. The right renal artery emerges perpendicular to the aorta and is much shorter than the left.
The right kidney is decapsulated using a sheet of dry cotton gauze and very gentle “squeezing” of the kidney out of the capsule, starting from the outer surface of the kidney. The renal pedicle is cleaned from connective tissue using middle-out lateral movements of two cotton swabs. At this point, it is best to identify the ureter emerging from the renal pelvis and running downwards and tying it off with a 3-0 silk thread. Alternatively, the ureter can be tied off together with the vascular pedicle, but it is possible that it can be left out of this suture due to anatomical variations. A hemostat is then placed over the vascular pedicle and two knots (silk, 2-0) are tied on either side of the hemostat. The pedicle is then cut between the two knots. Finally, the vascular stump is replaced in the abdominal cavity.

After all organs are rearranged in the abdominal cavity, the incision is sutured in two layers. The muscle layer is closed using non-reabsorbable polypropylene sutures. This suture has to be discontinuous, with individual sutures every 3-4 mm. Then the skin incision is closed preferably using metal clips so the animal cannot remove them while in the conscious condition. If clips are not available, polypropylene sutures can also be used, but they will have to be removed after the skin wound is healed.

After removal of gas anesthesia, the animal is monitored until it moves spontaneously. It is then returned to its home cage and further monitored until normal behavior is observed. It is preferable that the animal is kept in the cage alone until the abdominal wound is completely healed. Once the animal is awake, antibiotics and analgesics must be administered.
Issues and troubleshooting

• Obstruction of blood flow to precisely 2/3 of the infarcted kidney is often difficult to achieve. Perfusion (or lack thereof) has to be monitored after the ligation of the two renal artery branches. If infarction area is too small, another branch has to be identified and tied off. If the infarction area is too big, one or more branches have to be released.

• Excessive manipulation of the main renal artery can lead to reflex vasoconstriction that blocks blood flow to the whole kidney. Warm saline can be applied to the area before verifying the extent of infarction. In addition, one or two drops of a topical anesthetic (such as lidocain) can be applied due to its direct vasodilator properties upon the vascular smooth muscle.

• Performing the infarction on one kidney in the same procedure as the nephrectomy is generally well tolerated. However, when the procedure is applied to animals of different strains, which may display concomitant pathologies, it may be necessary to first perform the nephrectomy and the infarction delayed by at least a week. In this case, paramedian ventral incisions can be done for each of the kidney. Some investigators may prefer a dorsal, retroperitoneal approach, with incisions on each side of the spine. Notwithstanding the advantage of smaller incisions and avoidance of the open abdominal procedure, this approach is technically more challenging as the surgical planes are reversed and for the infarction procedure, the left renal vein sits on top of the artery. The retroperitoneal approach can however easily be used for the right nephrectomy, when the two-step procedure is preferred.
2. 5/6 nephrectomy by removal of renal mass
Given the above concerns related to the quantitative precision of the infarction and also mechanistic differences between the two models, as will be discussed below, surgical removal of the upper and lower pole of one kidney, together with contralateral nephrectomy, may be preferred. Access to the left kidney is obtained in the same manner as described above. In contrast to the infarction procedure, the integrity of the kidney capsule is not maintained and the kidney must be first decapsulated (using a dry piece of cotton gauze). A small flat piece of plastic is placed underneath the kidney. With the kidney held in place with a pair of cotton swabs, placed on either side of the renal pedicle, the upper and lower poles of the kidney are removed using a fine sharp scalpel blade. The cut has to be done in one movement, starting medially and extending laterally, perpendicular to the longitudinal axis of the kidney. Care must be taken in identifying and isolating extra-renal branches of the renal artery (adrenal artery) and the ureter. The profuse bleeding on the remaining surfaces of the kidney is stopped by covering it with a 1x1 cm piece of hemostatic, gelatin based compressed sponge such as Gelfoam ®. Gentle pressure is applied on both cranial and caudal ends of the kidney until the sponge forms a relatively secure bond to the kidney surface (usually ~2-3 min). If no further bleeding occurs, the kidney can be returned to the abdominal cavity, and the incision is closed as described above (Fig. 5).
Issues and troubleshooting

- Sometimes the bleeding of the remnant kidney is difficult to stop. Addition of another piece of hemostatic sponge can solve this issue. Some investigators may prefer to place a temporary suture on the renal pedicle to restrain the bleeding. While this is acceptable for very short periods of time (up to 30 seconds), prolonged ischemia followed by reperfusion of the renal stump may have effects beyond the direct reduction in renal mass, thereby confounding the experimental results.

- For long term experiments, peritoneal adherences and also adherence of other abdominal organs to the remnant kidney may become an issue. While this is true also for the infarction model, adherences are more commonly found when surgical removal is used.
**General experimental and mechanistic considerations**

While both the infarction and surgical models of reduced renal mass closely reproduce the reduction in nephron number (5/6 of the total renal mass), there are considerable differences between the two approaches in terms of the physiological variables affected, and the choice of either procedure to mimic human disease has to be carefully considered.

Several experimental studies indicated that the overall kidney and glomerular hypertrophy, reduction in glomerular filtration rate, renal blood flow, as well as serum creatinine levels are similar between animals in which equivalent reduction in renal mass was obtained by infarction or surgical removal. However, the typical glomerulosclerosis and increased protein excretion as found in progressive CKD patients were only found in renal infarcted animals. This was accompanied by development of hypertension exclusively in the infarcted group. In addition, the degree of glomerulosclerosis was directly proportional with the level of hypertension in these animals, irrespective of whether only one pole of the kidney or both were infarcted. These data indicate that the progression of CKD in these models depends on the concomitant development of hypertension and give support to the contention that systemic and glomerular hypertension due to increased glomerular transmission of high blood pressure is the culprit for the progression of glomerular damage of the remnant nephrons. Moreover, this suggests that hypertension that develops following infarction of the kidney may be caused by local factors related to the infarction zone, such as activation of the renal angiotensin system or inflammatory factors released locally.

Therefore, for a close reproducibility of the clinical CKD situation, we suggest the use of the surgical model of reduced
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renal mass, followed by the addition of a high salt diet (8%). This model will mimic the progressive development of hypertension and glomerular injury as seen in patients with CKD, without the confounding effects of factors released from the infarcted area.

Experimental results from this model are presented below:

Fig. 6. Evolution of mean arterial pressure as measured directly every 10 minutes by telemetry in rats subject to 5/6 reduction of renal mass (RRM) by surgical excision, as compared to sham-operated rats. Note that hypertension develops only when a high salt diet is administered, starting on the third week.
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Fig. 7. Renal histological aspect of kidneys as stained with Mason’s Trichrome. Left panel: sham operated rats displaying normal renal histology. Right panel: RRM by surgical excision + 6 weeks of high salt diet. Note extensive glomerular sclerosis and tubulointerstitial fibrosis (blue by Mason’s Trichrome staining)

References:

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with reduced renal mass. - The FASEB Journal 2006 (20): A1192
