Review Article

Animal Remnant Kidney Model of Chronic Renal Failure Revisited

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Animal models have been the mainstay of experimental means to study chronic renal failure. An ideal experimental animal model provides a stable uremic milieu to allow experimental manipulation. The model should be technically simple to produce and have a reproducible degree of glomerular filtration rate reduction. There should also be close mimicry of human chronic renal failure without additional (unwanted) physiologic changes. This review article outlines the various choices of animal models in relation to the aforementioned criteria, with particular emphasis on their advantages and shortcomings. In principle, reduction of renal mass is undertaken in these models, after which adaptive changes take place in the remaining/remnant kidney. The glomerular changes, in proportion to the number of nephrons resected or damaged, are characterized by growth as well as hyperfunction. To date, the five-sixths nephrectomy model has remained the state-of-the-art prototype, although it must be acknowledged that no single animal model can ever duplicate the original condition of human kidney disease. It is in this context that a thorough understanding of each animal model allows the most effective modeling strategy in biomedical research. Insofar as the ideal animal model does not exist, researchers should use the biologic and biochemical diversity among the models to experimental advantage. It is expected, with good reason, that the differences can tell us as much as the similarities. Attention to selection of appropriate animal models is important to further advance the nephrology research frontier. [Hong Kong J Nephrol 2003; 5(2):57–64]

Key words: animal model, five-sixths nephrectomy model, remnant kidney model

動物模型的應用，一直是慢性腎衰竭實驗的主要方式。一個理想的動物模型，在技術上應易於製作，在腎小球過濾速率降幅上具有可再現性，以提供穩定的腎衰竭環境供實驗操作之用。同時，該模型應可準確模擬人類慢性腎衰竭的狀況，且不會產生額外 (不必要) 的生理變異。根據這些準則，本文對多個可供選擇的動物模型作一回顧，並集中討論各者的利與弊、原則上，這些模型均採用腎組織的減量，令餘下／殘餘的腎臟逐漸出現適應性的變化。其中的腎小球變化 — 肥大及功能過度 — 與被切除或受損的腎元數目呈正比。雖然目前「六分之五腎切除模型 (five-sixths nephrectomy model)」被認為是動物模型的典範，然而須注意的是，在現階段尚未有一個動物模型，能完全模擬人類腎病的真正狀況，因此，研究人員必須對每個動物模型作充分瞭解，並善用各模型間的生物及生化多樣性於生物醫學研究中。我們有理由預期，各模型間的異異性與相似性對我們有同等的意義，注重動物模型的適當選取，對腎臟科學研究的進一步發展相當重要。

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INTRODUCTION

Our understanding of chronic renal failure and its complications have been made possible by means of experimental animal models, mostly in the rat. A wide range of experimental (dietary and pharmacologic) therapies explored in such models have also proven to be clinically efficacious. Numerous methods, albeit not well standardized, are available for inducing the model of chronic renal failure and each has been strictly defined in a given animal species. None of them can ever duplicate the original condition in a human kidney. Rather, it is the purpose of this review article to outline the characteristics of each model, with special emphasis on their individual advantages and drawbacks.

CHARACTERIZING AN ANIMAL MODEL

The term “animal model” in the sense of biomedical research refers to a simplified representation of a more complex system, with the hope that the information thus obtained can be transferred to a more complex system. The ideal experimental uremic model should constitute a reproducible research environment that is similar to human chronic renal failure, thus bridging the gap between the artificial environment of an in vitro system on the one hand and the complicated in vivo milieu of the human on the other. Preferably, the animals should be maintained in stable chronic renal failure for relatively long periods of time for experiments of long-term design. To illustrate these criteria in animal model selection, a total nephrectomy model (an anephric model) would not qualify as an ideal long-term model because the animals would survive for less than 72 hours without dialysis support. Remnant kidney models, induced in one way or another, provide an acceptable animal survival rate but with less reproducibility (in terms of the degree of uremia). Furthermore, the model should, theoretically, be free of additional pathophysiologic processes; ligation of the ureter as a means of inducing experimental uremia would be confounded by the elements of acute renal failure and immune response [1,2], for example.

Three main ways of inducing experimental uremia are categorized in the Figure. Among these models, reduction of the renal mass by surgery is the most common technique chosen.

SURGICAL FIVE-SIXTHS NEPHRECTOMY

Czerny performed the first human partial nephrectomy in 1887 [3], in which the renal capsule was peeled back for the area to be resected. Two years later, the first animal partial nephrectomy was performed by Tuffier [4], who removed one kidney and a portion of the contralateral one thereafter. No changes in the elimination of water or urea were demonstrated. In 1899, removing three-quarters of the canine renal mass was attempted by Bradford, resulting in polyuria and wasting [5]. In 1905, Heinecke carried out unilateral nephrectomy and resection of one-half of the other kidney [6]. Up to six additional resections had to be performed in order to reduce the amount of hypertrophied renal mass. Over the subsequent years,

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Figure. Classification of various animal models for chronic renal failure.
One problem with the contemporary mammalian five-sixths nephrectomy model has been the variable degree of glomerular/renal injury. In general, removal of the renal mass is expected to cause a significant decrease in the glomerular filtration rate of rats, and achieve a steady level around 2 weeks after unilateral nephrectomy. The degree of uremia thus achieved, nevertheless, was reported to be highly variable. The possibility of inducing standardized and stable uremia at predetermined levels would make the five-sixths nephrectomy model more attractive. Our experience and data from others [30] invariably demonstrated the critical importance of minimizing the error margin in assessing the amount of residual renal tissue or remnant mass. Simply put, the main reason for the variable glomerular filtration rates attained has been the failure to appreciate the extent of the compensatory renal

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response by the remaining renal tissue. Meticulous resection of the kidney poles (under direct vision) in order to standardize the amount of remnant mass left behind is the key to model reproducibility.

Alternatively, renal mass ablation by specially designed diathermy or electrocoagulatory probe has been employed [31–34], although at a higher cost. Compared to surgical resection, better hemostasis was achieved this way. Electrocautery, on the other hand, damages the residual tissue underlying the cortical burn to a certain extent. Histopathology after the ablative procedure might not, therefore, represent the progressive injury that is seen in a diminished pool of initially normal nephrons, as is the case with the intact nephron hypothesis. Reproducibility of this model is less satisfactory compared to surgical resection [35], which is easy to understand with the reasoning outlined before. Only the area of destroyed cortex can be defined by means of electrocautery (despite standardization of the current intensity and length of probe contact), whereas the number of nephrons left behind is still variable. A corollary of the electrocautery method is the application of cryosurgery to reduce renal mass [36], which might be less expensive. Again, the method itself entails the additional mechanism of (potentially undesirable) immunologic renal injury. In particular, circulating antibodies against renal extracts and immunoglobulin G deposits along the glomerular basement membrane and in the mesangium were demonstrated in animals after renal cryoablation [37].

A cautionary note, however, on the design of the almost century-old five-sixths nephrectomy model is that the original idea was to simulate progressive kidney disease (such as that seen in chronic hypertension, diabetes or other sclerosing processes) in the absence of immunologic injury. Nevertheless, the current belief is that any chronic progressive renal failure has an immunologic/inflammatory component. This is supported by the recent body of in vivo experimental evidence regarding the efficacy of mycophenolate mofetil on the progression of five-sixths nephrectomy-related progressive renal failure [38–40]. Recruitment of inflammatory cells is being recognized irrespective of the initial renal insult, and even in experimental models of a “non-immunologic” nature. Macrophage infiltration, for example, was demonstrated shortly after renal mass reduction in rats subjected to five-sixths nephrectomy [40].

There is also legitimate concern that most renal diseases in humans are not characterized by a sudden decrease in the functioning renal mass, as mandated by the current surgical model. Stated another way, more gradual loss of renal mass might, intuitively, mimic more closely the natural history of progressive renal disease. Recently, a modification of the original remnant kidney model has been proposed, with respect to gradual renal ablation. Briefly, it involves an initial left heminephrectomy (removal of the upper and lower poles of the left kidney), followed by removing the remainder of the left kidney 3 weeks later, and subsequent right heminephrectomy another 3 weeks later [41]. Higher albuminuria and increased focal segmental glomerulosclerosis were demonstrated in this gradual renal ablation model, compared to the conventional five-sixths nephrectomy method. This novel remnant kidney model merits further evaluation, inasmuch as it extends the potential of the contemporary five-sixths nephrectomy model.

**Vascular Ligation Method**

Selective ligation of the renal arteries provides an alternative method of inducing experimental uremia. Briefly, unilateral (right) nephrectomy is combined with ligation of most of the primary and secondary divisions of the main left renal artery. The degree of renal infarction is assessed by discoloration (for example, 75% of the kidney) and, hence, is interpreted as ischemia that progresses to infarction. Segmental infarction of the contralateral kidney is, thus, achieved [42–45]. According to our experience in small-sized animals [46–49], the prolonged operative time with microsurgery and variation in the degree of uremia achieved are disadvantageous.

Our previous evaluation of surgical resection compared to vascular infarction methods, for example, showed a consistent degree of glomerular filtration rate reduction in the former, resulting in 20% to 30% of normal creatinine clearance in Wistar rats. This compared favorably with the wide-ranging creatinine clearance (0.3% to 74% of normal) from the infarction model. In particular, the standard deviation of blood urea nitrogen in the surgical resection model was substantially lower than that derived from the infarction model [46]. The feasibility of achieving a reproducible chronic renal failure model by vascular ligation, even in the case of larger animals, is also compromised by the formation of collateral vessels that bypass the ligated branches and the inconsistent ramification pattern of the renal artery [50]. In accord with our experience in rat models, the ligation technique induced a much less consistent degree of azotemia than did the surgical resection technique of five-sixths nephrectomy in canine models [16,50].

The following caveats should be understood in light of the uncertainty induced by vascular ligation. Renal ablation by compromising the vascular supply might induce an element of renovascular hypertension, as occurs in the classical Goldblatt model. Propensity for hypertension in such models that leave infarcted renal tissue in situ has often been a concern because the
presence of systemic hypertension greatly influences the progression of the experimental model. In the presence of severe systemic hypertension, for example, glomerulosclerosis was documented in rats after limited (40%) nephrectomy only [51]. More recently, additional injury mechanisms induced by the vascular ligation method, as opposed to conventional surgical renal ablation, have been reaffirmed. Such evidence comes from the studies comparing surgical renal mass reduction with models of vascular ligation [52–54]. With an equivalent reduction in renal mass, the uremic animals in the vascular ligation model demonstrated significant proteinuria and glomerulosclerosis that were not seen in surgical mass reduction models. Glomerular capillary hypertension was also considerably higher in the case of renal infarction [53]. In addition to the mixture of systemic hypertension in the remnant kidney model, increased deposition of immunoglobulins was demonstrated in the remaining kidney, as derived from experimental infarction [55], thus implying an additional element of immunologically mediated injury. Of note, the contributory role of immunologic factors in the vascular ligation renal infarction model was suggested [55] when the disease process was prevented by the administration of cortisone or 6-mercaptopurine.

In view of the burgeoning interest in uremic models of various animals, it is worth emphasizing that studies of vascular ligation have not always demonstrated the same degree, or even type of abnormalities, as evidenced in most studies using rats [56,57]. The pathology of the rabbit vascular ligation model, for example, is predominantly tubulo-interstitial nephropathy as characterized by hypercalcemia, hypercalciuria and urinary stones, but minimal glomerular scarring [58]. Likewise, proteinuria is modest and arterial hypertension is inconsistent despite the presence of glomerular sclerosis in the dog model of uremia induced by vascular ligation [59–61]. Despite the proteinuria and histologic glomerular alterations, dogs might have preserved renal function for up to 4 years after renal infarction [61]. The vascular ligation model applied to cats yields all of the features seen in the rat model, including hypertension, proteinuria and glomerular sclerosis [62,63]. Certain strains of mice demonstrate none of the characteristic pathophysiology of the rat model [57]. In a similar manner, genetic factors of susceptibility to glomerulosclerosis and disease progression operate in the rat, depending on the strain being studied [26,64,65]. As a general rule, Wistar, Sprague-Dawley and Lewis rats are commonly chosen for the uremic animal models, based on their propensity to develop progressive, age-related glomerular lesions. In addition, within the rat model itself, the nature and extent of compensatory renal growth depends, to a great extent, on their age at the time of renal ablation [26,66,67]. Hypertrophy predominates in adults, whereas less compensatory growth occurs in old rats. This highlights the potential pitfalls in extrapolating information gathered from one particular animal model to another, which could be as erroneous as directly transferring lessons from animal to human conditions.

**Chemical Nephrectomy**

Another way to induce experimental renal failure is via the administration of nephrotoxic chemical agents. Among them, parenteral use of uranyl nitrate, adriamycin or adenine ingestion has been evaluated for inducing renal damage [7,68–70]. The disadvantage is clear. Biologic or pathologic effects of these chemical agents on the lymphoid and other organ systems would be difficult to monitor and control. Demonstration of anemia in laboratory animals, for example, gives rise to a diagnostic conundrum. Does it indicate the sequelae of uremia or the effects of the agent on hematopoiesis? Likewise, most of the immunologic models of kidney failure [71,72] lead to nephrotic syndrome or specific glomerular disease which, in many ways, alters nutrition, amino acid and protein metabolism.

Moreover, immunologic and toxic experimental models seldom represent stable and far advanced renal failure. Animal susceptibility to nephrotoxins also varies. For example, the nephrotoxic threshold of the human kidney to aminoglycoside antibiotics is significantly lower than that of laboratory animals, and this threshold even varies among different strains (and ages) of rats [73,74]. Given the substantial inter-individual variation in the dose response curve for most of these chemical agents, their effects in individual animals are difficult to predict. The diverse actions of these agents are also recognized, as illustrated by the classic example of adriamycin, which induces proteinuria (resembling minimal change nephropathy in humans) by glomerular damage in rats after a single intravenous injection [75], whereas serial injections lead to chronic progressive renal failure [69].

**From Animal to Human: Bench to Bedside**

It becomes obvious from this review that the variety of animal models behave in different ways. The question therefore arises: how good are they at shedding light on our understanding of human chronic renal failure? In particular, previous therapeutic successes in rats failed to demonstrate the expected response in humans with progressive renal disease [76].

Notwithstanding the practical advantages of using animal models for experimental purposes, there is little doubt that numerous differences exist between them.
and humans. Several of these differences deserve attention. First and foremost, direct comparisons of animal remnant kidney models with human kidneys with similar nephron mass losses are lacking. If any, the degree of reduction in renal mass in humans (such as with unilateral nephrectomy or transplant donation) is relatively modest compared with the ablation or five-sixths nephrectomy model in animals [56]. Second, animal experimentation typically involves a small number of rodents of a given gender and age, all of them being subjected to a uniform procedure such as standardized five-sixths nephrectomy. This represents a distinct homogenous cohort of animals with comparable renal injury, in contradiction to clinical trials enrolling subjects with wide-ranging age, blood pressure, nephropathy etiology and rate of disease progression. End points are more tangible in rodents because they progress to end-stage renal failure over a relatively short period of time, i.e. in terms of several months as opposed to a decade or more in humans. Secondary end points such as serial renal histology are, again, never practical in the clinical scenario. A third obvious problem is the timing of intervention. The experimental animal uremic model allows timely introduction of dietary or pharmacologic intervention shortly after renal insult, a convenience not often encountered in our practical daily care of chronic kidney disease patients.

To learn the most from animal models, it is noteworthy to emphasize that there is, as yet, no experimental equivalent of human chronic renal failure disease. With these perspectives in mind, the differences can, perhaps, tell us as much as the similarities.

**CONCLUSIONS**

Most established animal models of chronic renal failure bear some similarities to clinical chronic renal failure, but none is fully representative and ideal (Table). Regardless of the animal model and animal species, the criteria for an acceptable experimental uremic model are that it: (i) is appropriate as an analog of human kidney disease in the broad sense and, preferably, has a standardized degree of uremia; (ii) provides the ease and amenability to experimental manipulation; (iii) is specific to chronic renal failure without other concomitant disease processes; and (iv) has an excellent track record with established biologic properties including morphology (renal pathology) and functional (laboratory) data.

By and large, renal mass reduction is thought to closely resemble human kidney disease in which an initial insult causes loss of a selected population of functioning nephrons, leading to functional and metabolic alterations in the remnant “intact” nephrons. While there has been little dispute about the time-honored five-sixths nephrectomy as the prototype model for renal failure, the essential question always arises as to whether or not, or to what extent, we can extrapolate the chosen model to the human kidney and disease process being studied.

To summarize, the odysseys of experimental animal models are epitomized by the remarks of Dr. Peter Kennedy [77], “No one should expect to find a perfect animal model but ... there is still much to learn from very close corollaries.”

**REFERENCES**

9. Chanutin A, Ferris EB. Experimental renal insufficiency produced

**Table.** Comparison of currently available animal models of chronic renal failure.

<table>
<thead>
<tr>
<th>Model</th>
<th>Ease of application</th>
<th>Reproducibility</th>
<th>Devoid of additional effects</th>
<th>Similarity to human chronic renal failure</th>
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<td>Surgical 5/6 nephrectomy</td>
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<td>Nephrectomy plus electrocautery</td>
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<td>Vascular ligation method</td>
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Desirability of individual model denoted by * (least desirable) to *** (most desirable), varies with animal species being applied.

Animal uremic models


