Animal models of chronic kidney disease: useful but not perfect

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ABSTRACT

Animal models of chronic kidney disease (CKD) approximate
the human condition and are keys to understanding its
pathogenesis and to developing rational treatment strategies.
The ethical use of animals requires a detailed understanding
of the strengths and limitations of each species and the
disease model, and the way in which findings can be trans-
lated from animals to humans. While not perfect, the careful
use of animal experiments offers the opportunity to examine
individual mechanisms in an accelerated time frame.

INTRODUCTION

Research involving animals is a critical step in understanding
physiology, pathophysiology and the development of therapies
in both humans and animals. Animal experiments have pro-
vided us with fundamental concepts of kidney biology, ranging
from hypertension and diabetes, to renal manifestations of sys-
temic disease such as lupus and diseases primarily affecting the
kidney such as glomerulonephritis and tubular necrosis.

Importantly, as in human research, strict ethical standards
to maximize the likelihood of obtaining the knowledge
sought balanced against minimal animal discomfort are con-
sidered in experimental design. To address these concerns,
the 'Three R's' have become the cornerstone of animal-based
science [1]. These principles emphasize the 'Replacement'
of animal experimentation by other methods where possible, the
'Refinement' of animal experiments to maximize the evidence
obtained and the 'Reduction' in the number of animals used
to the minimum required [1]. In this Journal, we require all
authors to indicate whether the institutional and national
guideline for the care and use of laboratory animals was
followed. However, while this approach is admirable, the
guidelines are relatively abstract in form and need to be
applied to the actual conduct of animal research. In this
review, we therefore hope to point out the value of well-con-
ducted animal research, at the same time as the limitations
which in turn might reduce unnecessary animal experiments
in the future.

CHOICE OF SPECIES

The first major decision when contemplating the use of animal
models is to choose the species involved. Rodent models remain
the most popular species to approximate human disease. There
has been a progressive increase in studies using mouse models
of renal disease, with a corresponding decrease in the use of rats
(Figure 1).

There are many reasons for this exponential rise in murine models of kidney disease. Because of their small size,
many more mice can be housed in a small space, and they
are therefore cheaper to maintain. Extensive inbreeding gener-
ates genetically defined strains, metabolic or immunological
variants may lead to spontaneous disease models and variants
can have abnormalities in specific disease pathways, all of
which increase the utility of mice [2]. The availability
of mouse-specific reagents has often made it the species of
choice for immune disease. However, it is the relative ease
with which single genes can be targeted in the mouse that
has most contributed to the recent explosion in their use.
The greatest increase in mouse use has occurred in the last
three decades since mouse models of gene deletion or over
expression began appearing, and which now abound. In some
cases, the consequent phenotypes are very predictable and
precise. For instance, the hormone relaxin is an endogenous
inhibitor of collagen synthesis. The relaxin null mouse
develops more severe renal fibrosis than its wild-type counterpart, and this is completely abrogated by the infusion of recombinant relaxin [3]. Unfortunately, the outcome is often not so clear-cut, when the deletion of the gene does not result in the predicted phenotype. If the gene is essential to development, the knockout may be embryonically lethal. Redundancy of the gene product, where other pathways compensate for a missing gene or protein, is very common. Recently, conditional deletion or promotion has gone some way to bypassing these problems. Accordingly, insertion of a substrate or cell-specific promoters has allowed researchers to either switch off or induce gene expression at a desired point in time in the development of the animal or disease, or to target gene deletion to specific cell populations [4]. An assumption of such gene-based mouse research is that the gene and protein product are expressed and regulated in the same way in the human as in the mouse; an inherently fragile assumption [2]. Although a protein function may be the same in both humans and rodents, species-specific differences in the molecular regulation of a gene often make it difficult to extrapolate an analysis of the animal gene to physiological conditions in the human body.

Despite having much to recommend them, mouse models of kidney disease have a number of inherent disadvantages and limitations. Surgical manipulations are widely used to model human disease. These may reproduce the injury that causes renal disease (e.g. ureteric obstruction), reproduce the consequences of renal injury (e.g. renal ischaemia, reduction in renal mass) or involve collection of specimens from micro-environments. Surgical methods of inducing disease are technically easier to perform in larger animals such as rats and rabbits, as are micropuncture studies [5]. Blood pressure measurement is problematic in the mouse [6]. The reduction in housing costs associated with the small size of mice may be counterpoised against smaller amounts of experimental material; for instance, a mouse kidney weighs ~10 times less than a rat kidney. This provides less opportunity to concurrently examine multiple variables such as RNA, protein expression, biochemistry and metabolic processes and to perform extensive simultaneous or sequential observations from individual animals.

In designing experiments, we often underestimate important differences within rodents. The predominance of inbred mouse strains means that murine animal models are inherently different from rat models which are generally based on outbred strains. For decades, studies in immunology, and more recently genomics, have utilized the genetic purity of mouse strains. Inbred strains are more stable, uniform, repeatable and better defined than outbred stocks and are indeed the underlying basis of both gene knock-out and gene knock-in studies. Conversely, outbred stocks are undefined in genetic terms, as it is unknown what genes each animal carries. Nomenclature such as Sprague–Dawley or Wistar can be misleading because animals sourced from different breeders will have different characteristics, with no standard Wistar or Sprague–Dawley stock to compare against [7].

For many reasons, the pathophysiology in rat models is often not replicated in the mouse. For instance, many of the mouse strains used in the generation of genetically modified animals are particularly resistant to glomerulosclerosis and metabolic and immune-mediated mechanisms. The specific underlying genetic basis of this variability is, however, not clear [8]. While humans and rats have only one copy of the renin gene, mice have two alternate genotypes, either a single gene or two copies at the renin locus. The result is that mice with two renin genes have plasma renin activities 10-fold higher than their single-gene counterparts [8]. Given the central role of the renin–angiotensin system in renal disease, this alone may be an important confounding variable.

A number of non-rodent species are used, albeit less frequently. Theoretically, the closest approximation to human biology should be in non-human primates, though even then the heterogeneity is enormous [9]. There are pressing reasons for the relative paucity of the use of non-human primates in experimental renal disease. A great debate has surrounded the ethics of experiments in non-human primates. On the one hand, the genetic, structural and functional similarities of monkeys and chimpanzees to humans make them ideal experimental models. However, the strong possibility that these animals sense pain, suffering, anxiety and social relationships in the same way as humans makes them deserving of specific ethical consideration. Non-human primates are also expensive to obtain and house, can share infection with humans and with their intelligence and strength can be difficult to handle.

Nevertheless, non-human primates have been used in a number of different circumstances including infection [10], pre-eclampsia [11], stroke [12] and cognition [13] research and vaccine development [14]. Transgenic models exist [15, 16], although we know of no renal-specific examples. These have posed further ethical dilemmas; the genetic modifications may result in direct harm and the potential for social exclusion [15]. As a consequence, the use of higher-order non-human primates is banned in some countries [15]. Experiments in non-human primates are by necessity limited to very small numbers of animals, particularly when
compared with rodent research; accordingly, the statistical value of such studies is often questionable. The development of human-mouse chimeras, so-called humanized mice, may offer a partial solution [17], though likely will be the subject of great ethical controversy. Dogs have featured prominently in the development of nephrological care, particularly in dialysis and transplantation where this animal species was the subject of most initial successes. Abel et al. [18] published a seminal paper on the removal of diffusible substances from the circulating blood of living animals (mainly dogs) by dialysis. Vascular anastomosis and organ transplantation owe enormously to early work using dogs [19]. The dog was the animal in which azathioprine was shown to be effective in immunosuppression to allow allotransplantation of kidneys, spearheading the development of human renal allografts in the early 1960s [19]. Unilateral ureteric obstruction (UUO), one of the most commonly used models of chronic kidney disease (CKD) in mice and rats, is well described in the dog [20]. Canine models of pre-eclampsia exist [11]. Many developmental pharmaceutical companies still use dogs to test the efficacy and safety of new agents. However, ethical considerations have limited the use of companion animals, and certainly the community has strong feelings about using dogs for research purposes, emphasizing the need for strict ethical controls on their use.

Animal experiments require the existence (or establishment) of reliable breeding and holding facilities. Farm animals inherently fit these requirements.

Pigs have reasonable similarities to humans in respect of size, metabolism and renal anatomy. They have thus been favoured as a potential source of kidneys for xenotransplantation into man, and therefore, the subject of extensive research into immunological barriers [21]. The large size of fully grown farm pigs (up to 250 kg) limits routine laboratory use; hence, several types of miniature pigs (minipigs) have been often used [22]. The multipapillary sheep kidney has been the subject of research into renal physiology [23], and to generate antisera. Increasing evidence of disease transmission, particularly of viruses and prions from animals to man, possibly will reduce the use of farm animals, particularly as sources of xenografts.

There are important anatomical and functional distinctions between animals and humans. The severity of glomerulosclerosis in animal models varies considerably within species, suggesting that the genetic background independently affects the disease process. Structural issues include the unilobular-unipapillary structure of rodent, rabbit and dog kidneys compared with the multipapillary kidney of humans, pigs and sheep [22] and the gender-related renal anatomical dimorphism found in mice [24]. An inescapable conclusion from the above discussion is that animal disease phenotypes are greatly influenced by species and strain.

**CHOICE OF A DISEASE MODEL**

There are few if any animal models of renal disease that faithfully reproduce all aspects of the human pathophysiology and clinical syndromes. There are, however, many good models of particular aspects of disease [25]. We will only outline a few of the more commonly employed strategies.

Ischaemia is likely the most common contributor to the cause of acute kidney injury (AKI) in the human population. The importance of research into ischaemic AKI is underscored by the fact that morbidity and mortality associated with AKI have not appreciably declined in recent years [26]. Bilateral or unilateral clamping of the renal pedicle has been used by many groups to simulate ischaemic AKI [27]. Although it was long thought that the long-term consequences of AKI were relatively benign in patients who survive and recover renal function, it is increasingly recognized that AKI greatly increases the risk of developing subsequent CKD [26, 28]. In a rat model of ischaemic acute renal failure, we found that despite a short-term return to normal renal function, long-term histological outcomes were not benign [29]. The consequences are proportional to the period of ischaemia; even small differences in ischaemia time significantly change the characteristics of the model. These differences can make comparative studies of therapeutic interventions problematic. Additionally in the human, AKI is often the consequence of many other factors including sepsis, tissue injury or multi-organ failure, reducing the direct application of this model to human AKI.

Diabetic nephropathy is regarded as the single largest cause of CKD. Animal models only approximate its pathogenesis. The mouse models of diabetic complications consortium were specifically established to develop innovative mouse models to more closely mimic the human complications of diabetes [30, 31]. Type 1 diabetes has been most commonly modelled by injection of streptozotocin (STZ). Structural similarity between glucose and STZ results in STZ being transported into pancreatic β-cells, with subsequent destruction. However, STZ sensitivity is dependent on a number of factors including glucose transporter expression by pancreatic β-cells, with significant variation in both inter- and intra-species sensitivity to the drug [32]. Widespread glucose transporter expression means that the cytotoxicity of STZ is not restricted to islet cells but also affects kidney and liver cells, hence, the need for carefully controlled experimental design. Mice are much more resistant than rats to STZ, and are usually injected with a high dose on consecutive days [33]. Inter-animal variation is considerable, even with inbred strains; hence, many investigators are forced to discard animals which do not become sufficiently hyperglycaemic.

Akita mice have type 1 diabetes mellitus caused by a spontaneous point mutation in the Ins2 gene which leads to misfolding of insulin, resulting in pancreatic β-cell failure. Akita mice develop pronounced and sustained hyperglycaemia with high levels of albuminuria, and consistent renal histopathological changes, suggesting that these mice may be suitable as an experimental platform for modelling diabetic nephropathy. One key feature of diabetic kidney disease in Akita mice is that the severity of renal injury is significantly influenced by genetic background when back-crossed [34].

Type 2 diabetes accounts for the vast majority of patients with diabetic nephropathy. Much about type 2 diabetes has been learnt from models with single-gene mutations [35]. Mutations of the leptin receptor have resulted in the db/db
mouse. The db/db mouse develops hyperglycaemia, glomerular hypertrophy and sclerosis with age [36]. Mutations of the leptin receptor are, however, a rare cause of type 2 diabetes in humans, and the direct relevance to the human condition may thus be argued. Obese Zucker rats are an inbred strain characterized by mild glucose intolerance and peripheral insulin resistance, similar to that found in humans with type 2 diabetes. These abnormalities precede the development of albuminuria and glomerular injury [37].

Experimental models of diabetes only reproduce some of the pathophysiology and histology of human diabetic nephropathy [38]. Histological changes (glomerulosclerosis and Kimmelsteil–Wilson nodules) are generally much less severe than that seen in the human condition. Declining glomerular filtration rate is often not seen. Given that both can take decades to develop in the human, it seems likely that their absence is a function of the relatively brief life of the mice or rats used in such experiments. A number of accelerated models have been developed in attempts to more closely model the human condition, including uninephrectomized or hypertensive animals [39].

Interest in obstructive renal disease has been rekindled by our need to study the mechanisms of tubulointerstitial disease. UUO is one of the most popular models of fibrosing kidney diseases. The model has the advantages of reproducibility with little inter-animal variation, a short time-course of obstruction after a few days [40]. The latter has proved a particularly useful model to study regeneration [40].

There are, however, a number of inherent disadvantages with the UUO model. One major deficiency is the inability to accurately measure changes in renal function. The remaining unobstructed kidney compensates for much of the loss of function in the obstructed kidney, and accordingly only modest changes occur in serum creatinine or urea concentrations. Progressive fibrosis is so rapid, severe and irreversible that it limits the testing of possible ameliorating interventions unless they are expected to have profound effects. Notwithstanding the above, many putative anti-fibrotics have shown therapeutic potential in this model, at least when introduced prior to injury [41–43].

The reduction in renal mass that accompanies CKD manifests itself as progressive glomerulosclerosis and tubulointerstitial fibrosis. This can be reproduced experimentally by subtotal nephrectomy. Two different experimental approaches are used; uninephrectomy followed by either surgical excision of approximately half of the remaining tissue, or ligation of polar branches of the renal artery [25]. The latter model is associated with severe hypertension. It is most commonly performed in the rat [44]. The rate of progression to renal failure is very closely related to the amount of tissue infarcted or excised, which is technically difficult to reproduce consistently in every animal. Accordingly, it may be necessary to pair for renal function at a certain post-operative point to allow for the technical variation in the extent of nephrectomy. Rabbit and mouse sub-total nephrectomy models have been less extensively studied, and are less reliable. In the rabbit hypercalcaemia and nephrolithiasis rather than glomerulosclerosis occurs. Great strain variation in the severity of glomerulosclerosis seen in the mouse [45].

Experimental animal models have been pivotal to our understanding of antibody-mediated glomerular disease, and likely will continue to be critical in translating developments in molecular biology and medicine into therapies in man. The range of models is now vast, and expanding, using injection of preformed antibodies to glomerular antigens, active immunization against glomerular antigens and immunization against non-renal circulating antigens.

One of the earliest animal models of human glomerulonephritis (Masugi nephritis) was based on the injection of anti-kidney antisera, raised in a second animal, into the animal species from which the kidney was obtained, resulting in an injury due to antibodies to the glomerular basement membrane (anti-GBM antibodies) [46]. A huge repertoire of anti-GBM antibody-mediated animal models have since been described, including passive (injection of pre-formed antibodies to GBM) and active (induction of an immune response to those antibodies) phases or models. The similarity to human anti-GBM disease with Goodpasture’s syndrome was demonstrated by induction of anti-GBM renal disease by injection of lung tissue [47]. The passive phase is valuable for investigations into glomerular permeselectivity. The autologous disease is important in understanding acute crescentic glomerulonephritis. Disappointingly, much of the data addressing abrogation of injury in the autologous phase have demonstrated that therapies introduced after induction (as in the human condition) are much less efficacious than those preceding the injury. Nevertheless, understanding the pathophysiology has resulted in the current plasma exchange and immunosuppression regime employed in this disease.

Mesangial cell proliferation and expansion of the extracellular matrix are important components of the pathophysiological changes in various glomerular diseases. Injection of an antibody to Thy-1, an antigen found on glomerular mesangial cells, results in mesangiolyis due to necrosis and fibrin deposition with monocyte/macrophage infiltration [48]. This is followed by mesangial cell proliferation and mesangial matrix expansion, similar to that seen in human IgA nephropathy. However, Thy-1 nephritis only partially models this since there is no evidence of immunoglobulin A deposits. Others have injected pre-formed IgA immune complexes, or IgA producing myeloma cells into mice to induce IgA deposits, but these lesions do not fully mimic the wide range of lesions seen in human IgA nephropathy.

The ddY mouse has elevated levels of circulating IgA and mouse IgA mesangial deposits [49]. Nevertheless, these mice do not have increased proteinuria, or haematuria. This model, as with other mouse models of IgAN, demonstrate that mouse IgA complexes can be deposited in the mesangium, but none of them completely reproduce the human disease. This may be due to differences in the IgA system between humans and mice. Mice only have one type of IgA with a low (and different) glycosylation pattern. Moreover, mice do not express the IgA Fc receptor CD89 homologue.
Berthelot et al. [50] have described a very pertinent model in which mice expressing both human IgA1 and human CD89 developed signs of IgAN with increased leukocyte infiltration, haematuria, proteinuria and reduced renal function. This model is important since mice expressing only human IgA1 displayed only endocapillary IgA1 deposits, with no signs of mesangial injury or kidney dysfunction. In contrast, expression of two human molecules in the mice resulted in typical mesangial injury observed in patients. 

Much of our understanding of the pathophysiology of membranous nephropathy resulted from immunization of rats with tubular brush border antigens (Heymann nephritis) [51]. The recent discovery that the target antigen in the glomerulus is not the same as in Heymann nephritis, but in ~80% of cases is the phospholipase A2 receptor (PLA2R) no doubt will lead to development of a whole new range of animal models and hopefully to targeted therapies [52]. However, neither rodents nor rabbits express PLA2R on their podocyte surface. Transgenic models based on this antigen are reportedly under development [53]. Other potential podocyte antigens reported to be involved in human membranous nephropathy that may prove instructive in animal models include aldose reductase and manganese superoxide dismutase (SOD2) [53, 54].

TRANSLATION FROM ANIMALS TO HUMANS

Putting aside the many obvious differences between animals and humans, and experimental and spontaneous diseases, the use of animal models to validate therapies for human use raises other questions. In order to minimize variance in animal experiments, it is usual to select a single species (preferably an inbred strain) of animal, of a single gender and at a specific age. Often, as in STZ diabetes or sub-total nephrectomy, subsequent selection then occurs to restrict the range of the severity of injury. This could not be further from the heterogeneity of humans, whether seen on the street or in the clinic. The reality is that animal models do not reflect the broadness of our patient population.

Similarly there is often a mismatch between the detailed outcomes measured in animal research but not in human trials. For instance, in the search for agents to reduce fibrosis and progression of CKD, in the animal multiple histological, histochemical and biochemical parameters are often measured and followed, whereas in man the more restrictive end points of mortality, glomerular filtration or proteinuria are commonly used. Whether the multiple surrogate markers measured in the animal can be readily extrapolated to the human condition is questionable.

Already mentioned has been the need for demonstrating the efficacy in established disease. While prevention and very early treatment strategies are important proof-of-principle experiments for any new therapy, the clinical reality is that most patients present with established disease.

Finally and perhaps most importantly, animal experiments have a limited role in the modern evaluation of safety in clinical practice. While an enormous effort is given to evaluating toxicity in animals, including short- and long-term effects and safety during reproduction, embryogenesis and development, distressingly there are still unexpected adverse events being reported, not only in the human early clinical studies but also with long-term post-marketing surveillance.

CONCLUSIONS

Animal models have been, and for the foreseeable future, will remain pivotal to bridge the gap between the test tube and the human condition—the bench to bedside translation. Despite our best efforts, these animal models are fraught with imperfections, approximations and open to misinterpretation. The ongoing technical advances, particularly with new and efficient gene transfer techniques [55] and the use of human-mouse transgenic animals [17], may help close the gap but many difficulties are inherent. Translational research involving animals requires careful experimental design. It is our moral obligation as scientists to minimize the use of animals in research, and to use them in a manner that maximizes the likelihood of obtaining good quality information, which is then interpreted carefully.

CONFLICT OF INTEREST STATEMENT

None declared.

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Pathophysiology and treatment options of chronic renal allograft damage

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ABSTRACT

Chronic rejection is a poorly understood entity albeit a frequent cause of graft failure. Despite the advent of new immunosuppressive agents, neither the slope of graft destruction nor the frequency is ameliorated. There are a number of hypothesis which try to explain the conundrum of chronic graft destruction: ongoing rejection, antibody-mediated rejection, poor choice of organs, hyperfiltration, calcineurin inhibitors (CNI) nephrotoxicity and non-compliance among them. None of these hypotheses can explain all features of the process, thus, it is likely that they act in combination. What seems to be clear is a beneficial effect of early angiotensin-converting enzyme (ACE)/AT1 blocker treatment. It is less clear, however, whether a reduction or a switch from CNIs to other immunosuppressants prolongs graft survival. This review highlights the pathophysiological aspects that are important for the development of chronic allograft damage in the context of possible treatment options.

PATHOPHYSIOLOGY OF CHRONIC ALLOGRAFT DAMAGE

Despite major improvements in the care of patients after kidney transplantation, one of the major problems during the long-term follow-up of these patients is chronic allograft damage. However, this is a difficult term as it is often mixed with terms such as chronic rejection that comprise only some parts of the complex pathogenesis of this entity. In the complex pathogenesis of chronic allograft damage, which is only partly understood today, alloantigen-dependent and independent factors act together to initiate inflammatory reactions that eventually lead to tissue damage with a loss of nephrons in the graft followed by fibrosis and tubular atrophy (TA). This leads to graft dysfunction and eventually graft failure.

When successful kidney transplantation programmes started in the mid-1950s of the last century, the major problem was graft loss within the first days after transplantation. The focus