Ureteral obstruction as a model of renal interstitial fibrosis and obstructive nephropathy

Robert L. Chevalier[1], Michael S. Forbes[1] and Barbara A. Thornhill[1]

[1]*Department of Pediatrics, University of Virginia, Charlottesville, Virginia, USA*

Renal fibrosis is the hallmark of progressive renal disease of virtually any etiology. The model of unilateral ureteral obstruction (UUO) in the rodent generates progressive renal fibrosis. Surgically created UUO can be experimentally manipulated with respect to timing, severity, and duration, while reversal of the obstruction permits the study of recovery. The use of genetically engineered mice has greatly expanded the utility of the model in studying molecular mechanisms underlying the renal response to UUO. Ureteral obstruction results in marked renal hemodynamic and metabolic changes, followed by tubular injury and cell death by apoptosis or necrosis, with interstitial macrophage infiltration. Proliferation of interstitial fibroblasts with myofibroblast transformation leads to excess deposition of the extracellular matrix and renal fibrosis. Phenotypic transition of resident renal tubular cells, endothelial cells, and pericytes has also been implicated in this process. Technical aspects of the UUO model are discussed in this review, including the importance of rodent species or strain, the age of the animal, surgical procedures, and histological methods. The UUO model is likely to reveal useful biomarkers of progression of renal disease, as well as new therapies, which are desperately needed to allow intervention before the establishment of irreversible renal injury.

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Correspondence: *Robert L. Chevalier, Department of Pediatrics Box 800386, University of Virginia Health System, Charlottesville, Virginia 22908, USA. E-mail: rlc2m@Virginia.Edu*

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Renal fibrosis is regarded as the final common pathway for most forms of progressive renal disease, and involves glomerular sclerosis and/or interstitial fibrosis. Because most renal disorders (whether glomerular or interstitial, congenital or acquired) lead to renal fibrosis, there is great interest in identifying underlying factors to prevent or reverse the changes. For over 50 years, surgical renal ablation in a variety of animal species has been used to model progressive renal disorders. A seminal paper published in 1981 emphasized the role of glomerular hyperfiltration in the initiation of lesions in remnant glomeruli in rats.[1] Subsequent studies showed that while renal ablation leads to glomerulosclerosis in rats, there is little apparent glomerular injury in C57BL/6 mice (the most commonly used 'wild-type' experimental mice).[2] However, other mouse strains (129/Sv and Swiss-Webster mice) develop significant glomerular sclerosis,[2] underscoring the effect of genetic background on the renal response to renal ablation. While the focus of investigation remained centered on the glomerulus for many years, attention turned also to tubular and interstitial injury, with models of ischemia/reperfusion, immune injury, and nephrotoxins.[3] Clinical studies have also demonstrated that progression of renal insufficiency is much better correlated with renal interstitial fibrosis than with glomerular pathology.[4]

In the 1970s, unilateral ureteral obstruction (UUO) in the rabbit was shown to result in proliferation of renal interstitial fibroblasts and their transformation into myofibroblasts.[5] Subsequent studies in the rabbit showed increased interstitial collagens I, III and IV, fibronectin, and heparin sulfate proteoglycan.[6] Since then, animal models of UUO have been expanded and refined to elucidate the pathogenesis of obstructive nephropathy as well as mechanisms responsible for progressive renal fibrosis.[7,8] In contrast to adults, in whom diabetes and hypertension are the major etiologies of renal failure, congenital urinary tract obstruction is the most important identifiable cause of renal failure in infants and children.[9]

The development of animal models of renal fibrosis presumes an understanding of the disorder under investigation. Unfortunately, there is no general agreement regarding a strict definition of renal fibrosis, which underscores the need for ongoing investigation of a process central to most progressive renal disease. A number of terms have been

applied to the pathological process of renal accumulation of collagen or extracellular matrix: fibrosis, sclerosis, and scarring.[10] This process appears to represent a maladaptive response to injury, which optimally should result in healing of the wound.[3,11] If the insult is prolonged (as with chronic UUO), the outcome is irreversible renal injury. If the insult is removed (by relief of obstruction), or if the fibrotic response is blocked (by inhibition of gene expression, protein production, or receptor response), renal injury may be prevented or reversed.

Variables in the development of models of UUO

As early studies of the renal consequences of UUO were performed in the rabbit and dog, the preponderance of current studies are based on the rat and mouse. Since 1950, there have been over 10,000 publications involving ureteral obstruction, nearly 1000 of which address fibrosis (Medline). The advantages of using UUO as a model of renal fibrosis include the absence of an exogenous toxin, the lack of a 'uremic' environment, and the availability of the contralateral kidney as a control. Using the contralateral kidney as a control, however, does not take into account its cellular, metabolic, and functional renal compensatory changes in response to UUO.[12] For this reason, comparison of renal changes resulting from UUO should be compared with those in sham-operated animals (unless comparing a therapeutic intervention on the obstructed kidney). Contralateral nephrectomy in animals subjected to partial UUO reveals the effects of reduced renal mass superimposed on the obstructive injury. Surgical models of UUO have the added advantage of allowing variation in the severity, timing, and duration of obstruction, as well as the opportunity to study recovery following relief of the obstruction.

Complete UUO initiates a rapid sequence of events in the obstructed kidney, leading within 24 h to reduced renal blood flow and glomerular filtration rate.[8] This is followed within several days by hydronephrosis, interstitial inflammatory infiltration (macrophages), and tubular cell death attributable to apoptosis and necrosis. Tubular epithelial cell death is caused by a number of stressors resulting from UUO, including ischemia, hypoxia, oxidant injury, and axial strain caused by tubular dilatation. There appears to be a close association between progressive fibrosis and tubular cell death.[13] Following complete UUO in the rat or mouse, the progression to a severely hydronephrotic kidney with marked loss of renal parenchyma takes place over 1–2 weeks, with more severe fibrosis in the neonate than the adult (JJ Minor, KA Gordon, MSF, BAT, RLC, unpublished data).

Because most cases of clinical congenital obstructive nephropathy involve partial, rather than complete obstruction, models of partial UUO have been developed in the neonatal rat and mouse.[14,15] These models are also useful in the study of the pathogenesis of renal fibrosis, because the lesions develop more slowly and may be better suited to the study of therapeutic manipulations.[16]

Technical considerations

The surgical procedure for creating an animal model of UUO is relatively straightforward, if performed as a single operation in an adult rat. Morbidity and mortality will be reduced by using a temperature-controlled operating table heated to body temperature, with the animal anesthetized with isoflurane/oxygen and with the use of a high-quality binocular microscope to visualize the operating field. This approach is particularly important in mice (especially in the neonatal period), or if the obstruction is to be relieved or reversed by a subsequent operation. If this be the case, meticulous technique is essential to avoid adhesions and tissue damage, and establishment of ureteral patency must be documented at the time of study.[14,15] Ligation of the ureter is the technique used most frequently. However, fistulae can form around the ligature, allowing urine to bypass the obstruction (unpublished observations). Adhesions forming around the ligature can increase the difficulty in removing the ligature if recovery is to be examined: this is a greater problem with adult than neonatal animals. Small vascular clips can be placed around the ureter, but this approach can injure the ureter, or allow urine to pass if not closed correctly. A piece of silastic tubing can be folded perpendicularly across the ureter to create an obstruction, or the tubing can be slit and fitted around the ureter longitudinally, forming a sleeve and creating partial obstruction. Partial UUO can also be created by inserting the ureter in a surgically created slit in the underlying psoas muscle. However, this technique leads to variable (often very mild) degrees of partial obstruction.[17] A more reproducible method of creating variable, reversible partial UUO has been developed in the neonatal mouse.[15] This involves the placement of a fine ligature around the ureter and a piece of stainless steel wire of known diameter, which has been placed parallel to the ureter. After ligation, the wire is slipped out, leaving a partial obstruction with the desired luminal diameter. This ligature can be removed at various intervals, allowing the study of recovery of the lesions.

Glomerular filtrate rate can be measured following relief of UUO in the rat or mouse, using standard clearance techniques.[18,19] A novel model of UUO has been described in the adult mouse subjected to contralateral nephrectomy at the time of ureteral reimplantation following 10 days of complete UUO.[20] While this model allows serial measurement of blood urea nitrogen as a reflection of glomerular filitrate rate in the postobstructed kidney, the development of significant proteinuria suggests that secondary glomerular injury contributes to the lesions. Tubular function can be measured in rats following the release of UUO or bilateral ureteral obstruction, and can be correlated with the expression of renal epithelial sodium channel or aquaporins.[21,22] As described below, the UUO model is well suited to the discovery of new biomarkers which predate irreversible injury and functional changes.

The development of reproducible animal models of UUO, particularly in mice, requires an experienced animal surgeon.

The use of mutant strains of mice increases the possibility of greater susceptibility to intraoperative or postoperative mortality compared with wild-type controls. Operation on neonatal animals requires that the pups be accepted by the mother, and nursed successfully. It is important to monitor the daily weight gain of each animal, to assure that overall growth is adequate; kidneys should also be weighed at the time of harvest, to document parenchymal growth.

Renal fibrosis can be quantitated by digital morphometry of renal collagen distribution using tissue sections stained with Mallory trichrome (Figure 1a) or picrosirius red (Figure 1b). Types I, III, and IV collagen fibrils are identified by these methods,[23] with Type IV constituting the majority of tubular basement membrane. The aniline blue of Mallory trichrome may also stain tubular casts, however (Figure 1a), and picrosirius red is better discriminated by image analysis software programs (Figure 1b). Moreover, the correlation with tissue hydroxyproline content of collagen identified by picrosirius is superior to collagen stained with trichrome.[23] It is important to use an unbiased sampling approach: image analysis software can be used to measure randomly selected fields on the tissue section. In addition, total renal collagen content can be quantitated, although this does not directly address its distribution among renal compartments.

Because of the importance of the number of glomeruli per kidney as a determinant of progression of renal disease, an unbiased rigorous approach has been developed: the disector technique.[24] Comparison of this time-consuming approach with simply counting all glomeruli in a single mid-polar planar section of a whole kidney showed excellent correlation in both rats and mice.[25,26] The latter technique is therefore more practical for most rodent models of UUO.

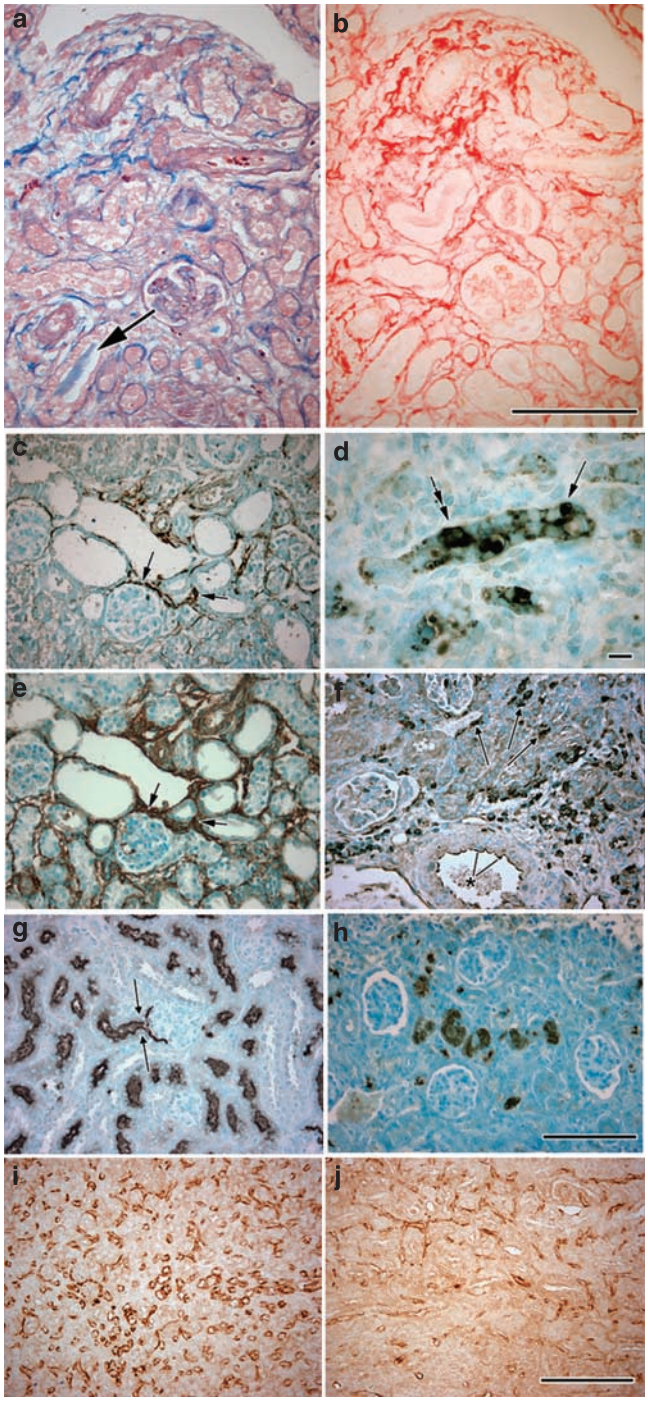

Figure 1 | Representative histologic sections of kidneys from mice subjected to unilateral ureteral obstruction (UUO). (**a, b**) Comparison of serial 3-μm paraffin sections of adult mouse kidney after 7 days of UUO, showing histochemical stains for collagen. ((**a**), Mallory trichrome; (**b**), picrosirius red). Although there is general agreement between the aniline blue staining of the trichrome and the red of the picrosirius stain, the trichrome may also stain structures such as cast material (arrow); furthermore, the distinct color of the picrosirius stain, is better recognized by image-analysis software. Scale bar = 100 μm for (**a**) and (**b**). (**c**) Adult mouse kidney after 7 days of UUO, showing macrophages identified by F4/80 immunostaining, which is restricted to cells in the interstitium. (**d**) Adult mouse kidney after 7 days of UUO, showing TUNEL staining. A degenerating proximal tubule is undergoing both apoptosis (single arrow) and necrosis (double-headed arrow); both categories of cell death are detected by this procedure. Apoptosis is characterized by cell contraction, blebs, and condensed nuclear material, while necrotic cells are swollen with diffuse TUNEL-positive staining. (**e**) α-Smooth muscle actin (α-SMA) staining in a section serial to the field shown in panel (**c**). Immunostaining is extensive in interstitial cells, and only in a few instances (arrows) is there evidence of colocalization of macrophage- and myofibroblast-specific staining. (**f**) Six-week-old mouse kidney with partial UUO applied at birth; section stained for fibroblast-specific protein-1 (FSP-1, also known as S100A4, a protein associated with intermediate filaments). Although numerous interstitial cells stain positively (arrows), vascular profiles within glomeruli and in arterioles (asterisk) are also stained. (**g**) Three-week-old mouse kidney with partial UUO applied at birth. Staining with *Lotus tetragonolobus*-derived lectin shows proximal tubules. At this stage of obstruction many tubules are normal, but some are beginning to attenuate at their junction with the glomerulus (between arrows). (**h**) Six-week-old mouse kidney with partial UUO applied at birth. Crowded atubular glomeruli surround *Lotus* lectin-staining fragmented remnants of the original proximal tubules, which have become detached from their glomeruli. Scale bar = 100 μm for panels **c**, **e**, **f**, **g**, and **h**. (**i**, **j**) Neonatal mouse kidneys after 14 days of UUO (**i**, contralateral kidney; **j**, obstructed kidney). Selective staining of the vascular endothelium with PECAM-1 antibody shows diminished microvessel density in the obstructed kidney. Scale bar = 100 μm.

As discussed below, animal models have revealed an extraordinary complexity in the renal cellular response to UUO, and there is significant disagreement regarding the pathogenic implications of fibrosis. On one hand, renal interstitial matrix accumulation is regarded as a primary cause of peritubular capillary obliteration, tubular atrophy, and progressive renal insufficiency.[27] The alternate view is that renal inflammation leads to glomerular and tubular damage of some nephrons, which in turn leads to injury to remaining nephrons, with interstitial fibrosis representing a secondary manifestation of disease.[10]

Recent studies have revealed major pathways leading to the development of renal interstitial fibrosis following UUO (Figure 2): (1) interstitial infiltration by macrophages (Figure 1c), which produce cytokines responsible for tubular apoptosis and fibroblast proliferation and activation; (2) tubular cell death by apoptosis and necrosis (Figure 1d), leading to the formation of atubular glomeruli and tubular atrophy; (3) phenotypic transition of resident renal cells. Chronic UUO activates the renin–angiotensin system, with production of reactive oxygen species and nuclear factor-κ B, which promotes macrophage infiltration[28] and renal tubular apoptosis and interstitial fibrosis in rats.[29] Classically activated macrophages can generate tumor necrosis factor-α, which mediates proapoptotic signaling and renal tubular cell apoptosis following UUO.[30] By contrast, alternatively activated macrophages generate anti-inflammatory cytokines, and induce cell survival and proliferation.[31]

In addition to the differentiation of infiltrating hematopoietic stem cells and proliferation of resident interstitial fibroblasts, tubular cells can undergo epithelial–mesenchymal transition,[32] by which process epithelial cells acquire mesenchymal characteristics and invade the interstitium to contribute to the deposition of extracellular matrix (Figure 2).

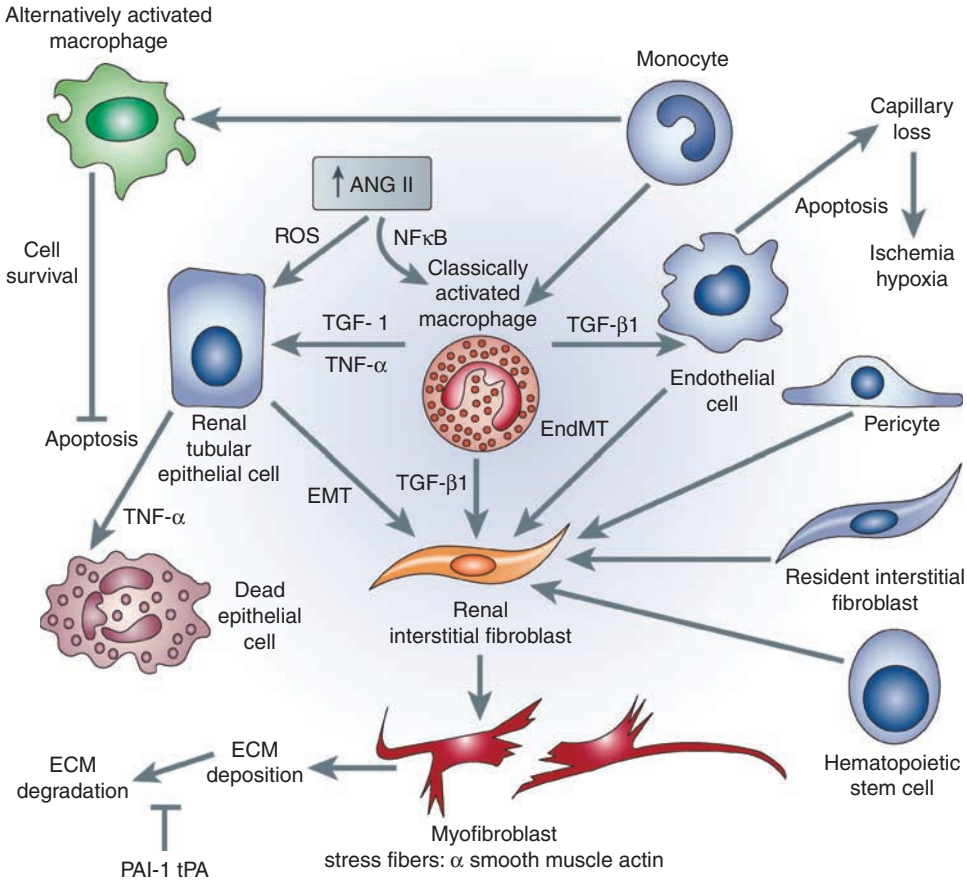

Figure 2 | Renal cellular interactions in the rodent kidney subjected to unilateral ureteral obstruction (UUO). The interstitium is infiltrated by monocytes, which are 'classically' activated to macrophages that release cytokines such as TGF-β1 and tumor necrosis factor-α (TNF-α). In turn, TGFβ1 promotes a phenotypic response of tubular epithelial cells either to undergo apoptosis (leading to tubular atrophy) or to undergo epithelial–mesenchymal transition (EMT), becoming fibroblasts that migrate to the interstitium. Angiotensin II (ANG II), produced by the activation of monocytes, stimulates the production of nuclear factor-κ B (NF-κB), which leads to the recruitment of more macrophages, as well as to the production of reactive oxygen species (ROS), which aggravates renal tubular injury. In contrast, alternatively activated macrophages can enhance tubular cell survival and proliferation. Endothelial cells can undergo endothelial–mesenchymal transition (EndMT) or apoptosis, which leads to capillary loss and secondary renal ischemia and hypoxia. Resident pericytes and infiltrating hematopoietic stem cells can also differentiate into fibroblasts. Under the stimulus of cytokines, such as TGF-β1 produced by macrophages or other cells, fibroblasts synthesize stress fibers and undergo further differentiation to become myofibroblasts. The myofibroblasts are contractile and augment the deposition of the extracellular matrix (ECM), leading to progressive interstitial fibrosis. This process is augmented by a decrease in ECM degradation, mediated by plasminogen-activator inhibitor-1 (PAI-1) and tissue-type plasminogen activator (tPA).

Similarly, endothelial cells can undergo endothelial–mesenchymal transition,[33] or can undergo apoptosis, leading to capillary loss and consequent renal ischemia and hypoxia.[34] In addition to tubular cells and endothelial cells, pericytes can also differentiate into myofibroblasts,[35] which express α-smooth muscle actin (Figure 1e), and are major contributors to interstitial extracellular matrix. The importance of endothelial cells and pericytes as a major source of renal collagen-producing cells following UUO has only recently been recognized, which shifts attention from the tubular epithelial cell to the renal vasculature as a focus for renal fibrotic injury.[33,35] Significant advances in understanding the process of epithelial–mesenchymal transition followed the development of an antibody to fibroblast-specific protein-1 (Figure 1f), which permitted the tracking of the lineage of cells undergoing phenotypic transition.[36] However, it should be recognized that this antibody binds also to a number of cell types, including endothelial cells (Figure 1f) and macrophages.[35] This problem can be addressed with the application of molecular lineage tracing techniques.[33,35] The use of immunohistochemistry to localize individual cell types can be combined with digital morphometry to quantitate the parameters and to make statistical comparisons. In addition, lectins (or other segment-specific markers) can be used to identify tubular segments, permitting further refinement of localization of cell types. Certain lectins, such as derived from *Lotus tetragonolobus* (which labels mouse proximal tubules) (Figure 1g), permit identification of tubular cell remnants after significant tubular injury and fragmentation resulting from UUO (Figure 1h).[15] However, *Lotus* lectin can also bind to intercalated cells of collecting ducts and elastic tissue of arterioles.[37] Thus, as with the tissue identification of collagen, apoptosis, and fibroblasts, the accurate identification of tubular segments requires an experienced microscopist to interpret the localization of the marker in the context of renal morphology. Peritubular capillaries can be identified by immunohistochemistry using antibody to platelet endothelial cell adhesion molecule-1. As shown in Figures 1i and j, UUO in the neonatal mouse leads to decreased peritubular capillary density in the renal medulla.

Use of genetically engineered mice subjected to UUO

In the past decade, many important pathways responsible for renal fibrosis have been elucidated, using mice subjected to gene deletion or increased expression (either transgenic or delivery of the gene). These have been reviewed recently, summarizing the data leading to the elucidation of molecular pathways.[38,39] A number of studies point to endogenous renal angiotensin II as a central mediator of the renal response to UUO, including inflammation, apoptosis, and interstitial fibrosis.[40] In neonatal mice with 0, 1, 2, or 4 functional copies of the angiotensinogen gene subjected to UUO, a linear relationship existed between the number of gene copies and the severity of renal fibrosis.[41] This effect is independent of systemic hemodynamic changes. At the present time, angiotensin inhibition represents the principal clinical therapeutic approach to slowing or preventing the progression of most forms of renal disorders.

For each of these pathways, factors contributing to progressive renal fibrosis are balanced by counteracting factors. Thus, heme oxygenase-1 suppresses monocyte chemoattractant protein-1,[42] a stimulator of interstitial macrophage accumulation; the oncoprotein bcl-2 counters caspase-induced tubular apoptosis;[43] and bone morphogenetic protein-7 opposes epithelial–mesenchymal transition stimulated by transforming growth factor-β1.[39,44] Similarly, factors promoting collagen degradation offset those promoting its synthesis and deposition (Figure 2).[45] Improved understanding of these interrelationships should lead to new methods of limiting or reversing fibrotic injury,[13] as well as to the identification of urinary biomarkers that result from renal injury or response to therapy.[39] Several important lessons have been learned from these studies, underscoring the complexity of the interactions. While as described above, angiotensin II plays a central role in the increase of fibrosis due to UUO, these effects are driven by stimulation of the renal angiotensin Type 1 receptors: stimulation of Type 2 receptors actually suppresses fibrosis.[46] Also of note, while most macrophages infiltrating the obstructed kidney appear to be injurious ('classical activation'), other macrophage populations may attenuate inflammation ('alternative activation').[31,47] Furthermore, factors having a salutary effect on one renal compartment can also have an injurious effect on another.[48] While the glycoprotein osteopontin stimulates the accumulation of macrophages in the obstructed kidney and contributes to renal fibrosis, osteopontin also suppresses renal tubular apoptosis, which reduces tubular injury.[49,50] In contrast, death-associated protein kinase contributes to tubular apoptosis in mice with UUO, but attenuates the progression of fibrotic injury.[51] These findings highlight the need for caution in the interpretation of experimental results.

In addition to the known counteracting factors described above, unidentified counteracting factors can confound interpretation in studies of gene knockout mice that have been subjected to UUO. An effect of deletion of a gene seen at an early time point following UUO may disappear later.[38] Thus, suppression of a specific molecule may impair or retard fibrosis early in the process, but overlapping pathways can erase the effects over time. As renal fibrosis may not develop until late in the disease process, these other pathways may actually be of greater therapeutic importance. For these reasons, both early and late observation points should be included in such studies. The selection of time points of study using models of UUO depends largely on the model and the question being addressed. Complete UUO leads to rapid destruction of the renal parenchyma, such that most of the renal cellular changes have taken place by 2–3 weeks. Partial UUO has the advantage of better reflecting clinical obstructive nephropathy, as renal cellular responses progress over a number of weeks. Relief of either complete or partial UUO, furthermore, allows the study of recovery at various intervals. In a report of temporary complete UUO in the

neonatal rat, renal functional recovery was complete after 1 month, but after 1 year 80% of function was lost, at which time fibrotic changes also appeared in the contralateral kidney.[52,53] The latter are presumably the result of gradual deterioration of the postobstructed kidney, and hyperfiltration by the contralateral kidney.

Effect of species

The use of animal models of human disease should take into account the possibility of species-specific signaling pathways. A number of studies have shown a reduction in the renal production of epidermal growth factor following UUO in the rat or mouse,[54–56] as well as a reduction in urinary epidermal growth factor in human obstructive nephropathy.[57,58] Administration of exogenous epidermal growth factor to neonatal rats with UUO reduces tubular apoptosis and interstitial fibrosis, and has a salutary effect 1 month following release of obstruction.[59] This effect is mediated in the rat by maintaining phosphorylation of BAD, a proapoptotic molecule.[60] However, administration of epidermal growth factor potentiates renal injury due to UUO in the neonatal mouse, a response mediated by elevated Src activity in mouse tubular cells, and not detected in rat or human tubular cells.[56,61]

Effect of age

As noted above, the study of neonatal models of UUO is important because obstructive nephropathy is a clinically important cause of renal failure in infants and children. In contrast to the consequences of UUO in the adult animal, obstruction to urine flow in the embryonic kidney can interfere with morphogenesis of kidneys and urinary tract, although obstruction later in fetal life or in the neonate can prevent renal growth and maturation. A number of animal models of congenital obstructive nephropathy have been developed, including the chick embryo, fetal rabbit, and opossum.[62] Use of the opossum represents a novel approach to fetal research, as the postnatal marsupial is essentially an extrauterine fetus, in which ureteral obstruction can be accomplished by withdrawing the animal from its mother's pouch.[63] Renal maturation in the fetal sheep is more similar to that of the human, in which nephrogenesis is complete before birth. A number of studies have been performed in this model, showing that recovery following relief of obstruction is directly proportional to the duration of intrauterine decompression, and inversely proportional to the duration of obstruction.[64] Notably, UUO early in gestation leads to compensatory growth of the contralateral kidney in the fetal lamb.[62] This indicates that compensatory growth is not necessarily dependent on functional demand, as excretory function in the fetus is accomplished by the placenta. Experimental UUO in the fetal monkey reveals glomerular as well as tubulointerstitial changes, similar to those found in human fetal obstruction.[65,66]

In contrast to the primate, sheep, and guinea pig, in which nephrogenesis is complete before birth, only 10% of nephrons are functional at birth in the rat or mouse, with the remainder maturing postnatally.[67] The early postnatal period in these species parallels that of the midtrimester human fetus, and allows the study of the effects of UUO during the period of most rapid nephrogenesis. Chronic UUO in the neonatal rat increases renal interstitial accumulation of collagen types I, III, and V, with increased type IV in tubular basement membrane.[68] Although surgical models of UUO in the neonatal mouse reveal the effects of obstruction without intrinsic defects in renal development, the use of genetically altered strains reveals molecular mechanisms involved in the response of the developing kidney to surgical obstructive injury.[38]

In human studies and animal models, fetal and neonatal UUO results in reduced nephron number, with the magnitude of reduction being dependent on the severity and duration of obstruction.[14,25] The mechanisms of nephron loss include glomerulosclerosis, phenotypic transition of glomerular cells with disappearance of glomeruli, and glomerulotubular disconnection leading to the formation of atubular glomeruli.[14,15,53] Chronic UUO in the neonatal rat markedly increases renal renin production, with persistence of the fetal renal pattern of renin distribution along afferent arterioles.[55] This is reversed by relief of obstruction.[52] Similarly, markers of renal tubular and interstitial maturation retain a fetal pattern in neonatal rats subjected to chronic UUO.[55] The use of the neonatal UUO model can shed light on the obstructive lesion in the adult: neonatal renal pericytes express NG2 and α-smooth muscle actin (pericyte markers), which disappear with normal maturation, but persist in response to neonatal UUO.[35]

In investigating mechanisms of obstructive injury in the immature kidney, it is important to recognize the role(s), in normal renal development, of factors deemed injurious to the obstructed adult kidney. Thus, while angiotensin inhibition has been shown to ameliorate renal lesions due to UUO in adult animals,[69] the administration of angiotensin-converting enzyme inhibitors to neonatal rats with UUO can actually exacerbate the renal lesions and augment renal fibrosis.[16] Similar considerations arise in the examination of other factors, such as transforming growth factor-β1 or endothelial nitric oxide synthase.[70,71]

In conclusion, the use of animal models of UUO has provided many new insights into the pathogenesis of obstructive nephropathy, and of progressive renal fibrosis in general. The promising biomarkers of disease severity, progression, and response to therapy have already been revealed by the model,[39] and it is likely that application of emerging technologies will ensure its usefulness well into the future.

DISCLOSURE

All the authors declared no competing interests.

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