Chronic unilateral ureteral obstruction in the neonatal mouse delays maturation of both kidneys and leads to late formation of atubular glomeruli

Michael S. Forbes, Barbara A. Thornhill, Carolina I. Galarreta, Jordan J. Minor, Katherine A. Gordon, and Robert L. Chevalier

Division of Pediatric Nephrology, Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, Virginia

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Chronic unilateral ureteral obstruction in the neonatal mouse delays maturation of both kidneys and leads to late formation of atubular glomeruli. Am J Physiol Renal Physiol 305: F1736–F1746, 2013. First published October 9, 2013; doi:10.1152/ajprenal.00152.2013.—Unilateral ureteral obstruction (UUO) in the adult mouse is the most widely used model of progressive renal disease: the proximal tubule is the nephron segment most severely affected and atubular glomeruli are formed after only 7 days of UUO. To determine the proximal tubular response to UUO in the maturing kidney, neonatal mice were examined 7 to 28 days following complete UUO under general anesthesia. Proximal tubular mass and maturation were determined by staining with Lotus tetragonolobus lectin. Superoxide was localized by nitroblue tetrazolium and collagen by Sirius red. Cell proliferation, cell death, PAX-2, megalin, α-smooth muscle actin (α-SMA), renin, and fibronectin were identified by immunohistochemistry. During the first 14 days of ipsilateral UUO, despite oxidative stress (4-hydroxynonenal staining), glomerulotubular continuity was maintained and mitochondrial superoxide production persisted. However, from 14 to 28 days, papillary growth was impaired and proximal tubules collapsed with increased apoptosis, autophagy, mitochondrial loss, and formation of atubular glomeruli. Fibronectin, α-SMA, and collagen increased in the obstructed kidney. Oxidative stress was present also in the contralateral kidney: renin was decreased, glomerulotubular maturation and papillary growth were delayed, followed by increased cortical and medullary growth. We conclude that neonatal UUO initially delays renal maturation and results in oxidative stress in both kidneys. In contrast to the adult, proximal tubular injury in the neonatal obstructed kidney is delayed at 14 days, followed only later by the formation of atubular glomeruli. Antioxidant therapies directed at proximal tubular mitochondria during early renal maturation may slow progression of congenital obstructive nephropathy.

apoptosis; atubular glomeruli; autophagy; mitochondria; proliferation

OBSTRUCTIVE NEPHROPATHY is a major cause of renal impairment in the infant, whereas diabetes and hypertension are the predominant causes in adults. The surgical model of complete unilateral ureteral obstruction (UUO) has been widely employed to study the pathogenesis of progressive renal disease, with most reports based on adult mice, and emphasis placed on renal interstitial fibrosis (1, 8). We recently described previously unrecognized cellular responses by the adult mouse kidney subjected to 14 days of UUO (16, 17). These include rapid widespread proximal tubular cell death, resulting in a 65% reduction in proximal tubular mass, collapse of the glomerulotubular junction, and formation of atubular glomeruli (16, 17). These phenomena are associated with proximal tubular oxidative injury and mitochondrial loss.

A review of the literature revealed a number of reports of glomerulotubular injury and/or atubular glomeruli in a wide range of pediatric renal disorders, including congenital nephrotic syndrome, cystinosis, and pyelonephritis (7). It is likely that this process has remained underappreciated in the study of clinical and experimental renal histopathology due to the challenges in demonstrating the lesions, requiring either serial sectioning of glomeruli or nephron microdissection. Microdissection of nephrons from patients with congenital obstructive nephropathy demonstrates focal narrowing of the glomerulotubular junction (“swan-neck”), and multiple diverticula of this tubular segment, which precede the formation of atubular glomeruli (2, 20). Our recent reports of renal cellular responses following complete UUO in the adult mouse (16, 17) demonstrate that injury to the proximal tubule leads to the formation of atubular glomeruli, a terminal event in this model of obstructive nephropathy. Although adaptations to renal injury depend on the nature and severity of the stimulus, the cellular environment, and maturational stage of the kidney, differences between the developing and adult kidney have rarely been taken into account when considering the effects of ureteral obstruction (6). We previously reported tubular cell responses in neonatal mice subjected to complete UUO, focusing on proliferation and apoptosis (3). In view of the recent evidence in adult mice for selective damage to the proximal tubule and the formation of atubular glomeruli, the present study was designed to determine the temporal cellular responses of proximal tubules and glomeruli in neonatal mice subjected to complete UUO before the completion of nephogenesis. Mice underwent UUO in the first 36 h of life, were examined through 28 days of age, and were compared with mice subjected to sham operation. The results reveal—in both obstructed and contralateral kidneys—substantial maturational effects on the response to UUO that are relevant to the interpretation of published and future studies that use this widely established model of renal disease.

MATERIALS AND METHODS

Experimental animals and surgical procedures. C57 Bl6 mice were subjected either to complete UUO or sham operation. Neonatal mice underwent UUO within 36 h of birth, a period during which nephrogenesis is ongoing (24). The time points selected for study represent key stages of renal maturation: completion of nephrogenesis (7 days), weaning (21 days), and achievement of an adult-type pattern (28 days) (38). The 14-day time point was included, as this was the end-point for the studies of adult mice with which the present results are compared (16, 17). All surgery was performed using sterile technique in accordance with an animal protocol approved by the University of

Address for reprint requests and other correspondence: R. L. Chevalier, Dept. of Pediatrics, Univ. of Virginia, Box 800386, Charlottesville, VA 22908 (e-mail: RLC2M@virginia.edu).

Virginia Animal Care and Use Committee. All animals were anesthetized with isoflurane plus oxygen, and the left ureter was exposed through a flank incision. In animals undergoing UUO, the ureter was ligated with 8–0 nylon, while in sham-operated mice the ureter was left undisturbed. Mice were treated with 0.1–0.2 mg/kg buprenorphine subcutaneously at the time of surgery and 12 h postoperatively. Eighty-five animals were used for this study. There were 6–8 animals in each UUO group and 3–6 in sham-operated groups. These sample sizes permit valid statistical comparison between groups, accounting for interanimal variation in renal morphometric parameters for mice subjected to complete UUO (16, 17).

**Tissue collection and processing.** All animals were injected with pentobarbital sodium-phenytoin sodium solution (Euthasol; Virbac, Ft. Worth, TX), and kidneys and ureters were exposed through an abdominal incision. Kidneys had urine drained from them at the time of removal and were weighed with renal capsules intact. The majority of kidneys were removed and fixed by immersion in 10% phosphate-buffered formalin. In some cases, kidneys were perfused with a solution of 1.5% glutaraldehyde in a solution of 3% dextrose, 3% dextran (43,500 avg. MW) and 50 mM CaCl2, or with 2.5% glutaraldehyde in HBSS, pH 7.4. Selected 14-, 21-, and 28-day animals were perfused with nitroblue tetrazolium to localize superoxide formation, as previously described (17). Formalin-fixed kidneys were washed in phosphate buffer, dehydrated through a graded series of ethanols and xylene, embedded in paraffin, and sagittally sectioned at 4 μm. Glutaraldehyde-perfused kidneys were cut into 50-μm coronal sections that were processed for plastic embedding as described previously (15); from these, plastic “semithin” sections (0.25 μm) of areas of interest were cut with glass knives on an ultramicrotome and stained with alkaline toluidine blue.

**Staining.** Fragmented DNA was detected using Apoptag (Chemicon, Temecula, CA) with DAB development (Biogenex, San Ramon, CA) and methylene blue counterstaining. This method is based on the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick-end labeling (TUNEL) reaction (21). For immunohistochemistry, sections were pretreated to quench endogenous peroxidase (H2O2 in methanol) and endogenous biotin (Avidin-Biotin blocking kit, Vector Laboratories, Burlingame, CA). Mitotic cells were detected with phospho-histone H3 (ser 10; Cell Signaling Technology #9701, Beverly, MA) at a 1:2,000 primary antibody dilution. Proximal tubules were selectively stained with either biontanized Lotus tetragonolobus agglutinin (Vector B1325) or with megalin antibody (ab76969, Abcam, Cambridge, MA; 1:500 dilution). Intertubular cell α-smooth muscle actin (α-SMA) content was localized by immunohistochemical staining using Sigma (St. Louis, MO) antibody A4-2547 at a dilution of 1:800, and fibronectin (Abcam #ab6328) at a dilution of 1:200. Picrosirius red staining was used to identify collagen deposition (Polysciences #09400, Warrington, PA). Oxidative damage was assessed with an antibody against 4-hydroxynonenal (ab48506, Abcam; 1:2,000 dilution). PAX-2 antibody (Zymed Laboratories 71–6000, Invitrogen, Grand Island, NY) was used at 1:500 dilution to demonstrate immature nephrons. Renin (goat-anti-rabbit) antibody was a gift from Dr. Tadashi Inagami of Vanderbilt University and was used at a dilution of 1:10,000.

**Quantitation.** Average parenchymal thickness was determined as previously described (17). Glomerular areas were measured in 50 glomeruli from three animals in each category (42). Morphometric measurements were performed in sagittal sections using a random selection of 10 microscopic fields at ×400 magnification distributed as described previously (17). TUNEL-stained specimens were scored for net apoptosis counts by manual counting of clearly identifiable apoptotic nuclei in 10 fields in a median sagittal section of each kidney. Quantitation of mitosis was carried out in similar fashion by manual counting of phospho-histone-positive mitotic nuclei. Since Lotus lectin affinity is a property of mouse proximal tubules that develops in utero (23), relative proximal tubule mass [VV(PT)] was determined in neonatal mice by image analysis (ImagePro Plus 5.1, Media Cybernetics, Silver Spring, MD) as described previously (17). For measurement of interstitial proteins, 10 fields, photographed at ×400 magnification and restricted to the subcapsular cortex, were used to quantitate α-SMA and fibronectin distribution, expressing cell staining with DAB reaction product as a percent area value.

In the case of UUO, seven animals were used for documentation and stereology of each group of specifically stained sections. In view of our finding that UUO induces altered kidney conformation not only in the obstructed but also in the contralateral kidneys of neonates after 14 days, measurements were also made for all parameters in age-matched, sham-operated animals (3–6 animals at 7, 14, 21, 28 days).

**Evaluation of glomerulotubular connection and relative populations of proximal tubules.** In the adult mouse model of UUO, we demonstrated that the presence of Lotus-lectin affinity in cells of the glomerular capsule can be used as an index of proximal tubule integrity (16). In younger mice, however, this criterion serves also as a measure of maturation, since the contribution to the capsule by cuboidal epithelial cells increases with the age of the animal up to adulthood (11). Because of the sexual dimorphism that develops in mice with respect to Bowman’s capsule morphology (males have a greater incidence of tall, proximal tubule epithelium-like cells than do females) (11), in older animals (21 and 28 days) measurements of percentage of Lotus-positive glomeruli were limited to male animals. The lower percentages of Lotus positivity in obstructed neonatal kidneys reflect the interaction of ongoing maturation and obstructive injury. To account for this, periodic acid Schiff (PAS)-hematoxylin-stained sections were also examined for basement membrane thickening. In addition, to determine the fraction of atubular glomeruli, 37 glomeruli from the obstructed kidneys of three 14-day-old mice were traced in serial consecutive sections stained with the Lotus-lectin technique, as described previously (16). Furthermore, 10 series of glomeruli from obstructed kidneys of 28-day-old mice were traced in PAS-hematoxylin-stained sections.

**Statistical analysis.** The SigmaStat program v. 3.0 (Aspire Software International, Ashburn, VA) was employed. Comparisons between groups were made using Kruskal-Wallis one-way ANOVA on ranks with pairwise multiple comparisons by Dunn’s method. Statistical significance was defined as P < 0.05.

**RESULTS**

**Growth and maturation of obstructed and contralateral kidneys.** Chronic UUO resulted in severe hydropnephrosis of the obstructed kidney with progressive decrease in weight from 7 to 28 days of life (Fig. 1, A and B). Weight of the obstructed kidney was greater than that of the contralateral kidney following 7 days of UUO, likely due to transient edema, as kidney DNA content and protein/DNA ratio are not altered by UUO in the neonatal rat (10). Parenchymal thickness of the obstructed kidney failed to increase during the first 14 days and continued to fall through 28 days (Fig. 1C). While the weight of the contralateral kidney exceeded that of the sham-operated animals by 21 days, parenchymal thickness lagged behind that of sham-operated mice at 14 and 28 days, reflecting the onset of hydropnephrosis and a delay in both medullary growth and papillary elongation in contralateral kidneys of 8 of 11 mice (Fig. 1, A and C).

**Maturation of glomeruli and tubules and their response to obstruction.** At 7 days of age, mitotic activity was increased in the obstructed kidney throughout the cortex, particularly in the subcapsular nephrogenic zone (Fig. 2A). Apoptosis was prominent in the walls of dilated collecting ducts of the obstructed kidney (Fig. 2, C and D). After 7 days, mitosis decreased within the obstructed kidney, while apoptosis persisted in collecting ducts, but was largely absent from the proximal
tubules. In the contralateral kidney, mitosis was also present in the nephrogenic zone, with little activity in the medulla (Fig. 2B); there was scattered apoptotic activity in the cortex and medulla (Fig. 2, E and F). Both mitotic and apoptotic indexes decreased with further duration of obstruction (Fig. 2, G and H), but greater activities remained in the obstructed kidneys.

After 7 days of UUO, nephrons in the obstructed kidney contained numerous PAX-2-positive, primitive-appearing nephron profiles within the outermost cortical zone, indicating delayed maturation (Fig. 3, A and C). In contrast, sham-operated (not shown) and contralateral kidneys had progressed past the nephrogenic stage of S-shaped nephrons, so that the outermost cortex contained numerous spherical glomeruli (Fig. 3, B and D). Megalin, a marker of proximal tubule maturation, appeared in S-shaped nephrons of the obstructed kidney (Fig. 3E), with strong staining in the maturing nephrons of the contralateral kidney (Fig. 3F).

By 14 days of age, many nephrons in obstructed kidneys remained aberrant in form, with contorted initial proximal segments (Fig. 4A); some of their epithelial cells—although cuboidal or columnar in profile—lacked Lotus-lectin positivity (Fig. 4, B-D). Despite these abnormalities, serial sections (example shown in Fig. 4, A-D) of 37 glomeruli from obstructed kidneys (from three 14-day-old mice) in every case revealed intact glomerulotubular junctions with attached proximal tubules. The glomeruli in contralateral kidneys often contained tall, Lotus-positive epithelial cells as part of their capsules, with similar cells in the contiguous proximal tubules, identical in appearance to those in sham-operated kidneys (example shown in Fig. 5A).

After 21 days of obstruction, the nephron population had become heterogeneous, with normal-appearing nephrons mingled with abnormal ones (Fig. 4, E-G). After 28 days of obstruction, thick proximal tubules, when present, remained connected to glomeruli, but they were reduced to small concentrations in the kidney wall; even these nephrons displayed abnormal morphology (Fig. 4H). The rest of the parenchyma was filled with glomeruli that often remained attached to profiles of withered proximal tubules that lacked luminal patency (Fig. 4, I and J). Serial section analysis of 10 series of these nephrons showed the tubules to be blind-ended, indicating that their glomeruli are atubular. The majority of glomeruli were found in clusters and lacked Lotus positivity in their capsules, which consisted of multiple layers of epithelial cells expressing both α-SMA and vimentin (Fig. 4, K and L).

As shown in Fig. 5A, the fraction of Lotus-lectin-stained glomeruli (an index of both maturation and obstructive injury) progressively decreased in the obstructed kidney from 7 to 28 days of age. In the sham-operated kidney, the fraction of Lotus-lectin-stained glomeruli increased from 14 to 28 days of age, whereas in the contralateral kidney the fraction did not change. At 21 and 28 days, the fraction of Lotus-positive glomeruli in contralateral kidneys was less than that of sham-operated mice, consistent with a delay in proximal tubular maturation. Glomerular growth was arrested in the obstructed kidney, whereas average glomerular area progressively increased in the sham-operated and contralateral kidney (Fig. 5B). In the obstructed and sham-operated kidneys, the fraction of glomerular profiles having juxtaglomerular apparatus containing identifiable immunoreactive renin increased from 7 to 14...
RENAL MATURATION AND INJURY ARE DELAYED AFTER NEONATAL UUO

By 14 days of age, evidence of necrosis had largely disappeared (Fig. 7C), and proximal tubules, along with their glomeruli, continued to differentiate in both kidneys with further maturation of mitochondria. Evidence of oxidative stress was present in the form of 4-hydroxynonenal immunopositivity in both obstructed (Fig. 7, D and E) and contralateral kidneys (Fig. 7, F and G), staining for which was absent from sham-operated kidneys (not shown). Little evidence of either apoptosis or autophagy was present.

By 28 days of age, although proximal tubule structure was normal in the contralateral kidney (Fig. 7K), the majority of tubules in the obstructed kidneys had undergone severe degenerative changes, to become shrunken, fragmented profiles lacking a lumen and containing autophagic bodies (Fig. 7, H, I, J). Hydroxynonenal expression and diformazan deposition were now concentrated in the autophagic bodies (Fig. 7, I and J). Hydroxynonenal expression persisted in proximal tubules of the contralateral kidneys, revealing ongoing oxidative stress.

days and then decreased through 28 days (Fig. 5C). The fraction of renin-positive glomeruli was suppressed in the contralateral kidney of 7- and 14-day-old mice (Fig. 5C). In the obstructed kidney, immunoreactive renin extended down the afferent arteriole of 14-day-old mice, but in the contralateral kidney it was restricted to the juxtaglomerular region (Fig. 5C).

Proximal tubules: development and response to oxidative injury. Fractional proximal tubule mass of the obstructed kidney failed to increase during the first 14 days, and progressively decreased thereafter (Fig. 6). Fractional proximal tubule mass increased in the contralateral kidney from 7 to 14 days and remained unchanged through 28 days (Fig. 6B). Fractional proximal tubule mass in the sham-operated kidney increased progressively from 7 to 28 days, whereas that of the contralateral kidney lagged behind that in the sham-operated animals at 28 days (Fig. 6B). The morphology of contralateral kidney proximal tubules was indistinguishable from that of the sham-operated (not shown).

At 7 days of age, proximal tubules having a normal structural appearance (including small immature mitochondria) were found in both obstructed (Fig. 7A) and contralateral kidneys (not shown). However, scattered profiles, limited to the cortex of the obstructed kidney, showed extensive cytoplasmic vacuolation within the epithelial cells (Fig. 7B). The presence of these vacuoles, representing swollen mitochondria, plus the retention of a brush border, identified such profiles as necrosing proximal tubules.

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Fig. 2. Distribution of mitosis (A, B, G) and apoptosis (C, D, E, F, H) following UUO in neonatal mice. In both the obstructed (A) and contralateral 7-day kidney (B), phospho-histone immunostaining shows mitotic activity concentrated in the subcapsular cortex, associated with developing nephrons. Although proliferation is increased in the obstructed kidney, there is a progressive decrease with age regardless of obstruction. Apoptosis revealed by nuclear terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick-end labeling (TUNEL) reaction staining is also more abundant in the obstructed kidney (C) where it is associated with collecting ducts (CD; examples indicated by CD and shown in detail in D), and peaking at 14 days. Apoptosis in the contralateral kidney is scattered (E; cortex; F; medulla, arrows). Morphometric measurements of mitosis and apoptosis are shown in G and H, respectively. Solid black bars, obstructed kidney; open white bars, contralateral kidney; shaded bars, sham-operated kidney (values are means ± SE). †P < 0.05 obstructed vs. sham-operated. ‡P < 0.05 obstructed vs. contralateral. Scale bar in B = 250 μm and applies to A and B; scale bar in F = 100 μm and applies to C, E, and F; scale bar in D = 25 μm.

Fig. 3. UUO delays nephron maturation in 7-day neonatal mice. A, C, E: obstructed kidney. B, D, F: contralateral kidney. A, B: PAX-2 staining is an indicator of immature epithelial cells in the forming nephrons. Whereas PAX-2 staining is present in numerous dilated profiles in the outer cortex of the obstructed kidney (A), staining is largely restricted to CD10 in the contralateral kidney (B). C, D: semithin plastic sections showing dilated S-shaped immature nephron in obstructed kidney (C) and maturing glomerulus with proximal tubule (PT) in contralateral kidney (D). E, F: similar profiles in which megalin immunostaining indicates proximal tubular cells in S-shaped nephron in the obstructed kidney (E) and in a more mature nephron in the contralateral kidney (F). Scale bar in B = 100 μm and applies to A and B; scale bar in F = 50 μm and applies to C–F.
Despite this, blue diformazan crystals in the interstitium indicate functioning mitochondria (Fig. 7, L and M). Maturation of the interstitium and response to obstruction. The renal interstitium was expanded in the obstructed kidney, with increasing volume fraction of /H\textsubscript{9251}-SMA, fibronectin, and collagen by 14–21 days of age (Fig. 8, A-C). By 28 days of obstruction, collagen contribution and interstitial /H\textsubscript{9251}-SMA were both increased, while the relative contribution of fibronectin decreased after 21 days (Fig. 8, G-I). Chronic UUO significantly increased expression of /H\textsubscript{9251}-SMA within fusiform or stellate interstitial cells (“myofibroblasts”) that wind among medullary collecting ducts of the obstructed kidney (Fig. 8, A). As shown in serial sections (Fig. 8, A-C), the pattern of deposition of picrosirius-positive collagen (Fig. 8C), often used as an indicator of fibrosis, does not correspond closely to that of either /H\textsubscript{9251}-SMA or fibronectin. There was widespread distribution of interstitial /H\textsubscript{9251}-SMA in both contralateral and sham-operated neonatal kidneys (Fig. 8D), which decreased with age (Fig. 8, D and G). The matrix protein fibronectin, although widespread in these kidneys (Fig. 8, B, E, H), was not consistently associated with these interstitial cell complexes. The distribution of the interstitial proteins differs between the two kidneys, with a tendency to concentrate in the inner medulla of the obstructed kidney and a more generalized pattern observed in the contralateral kidney.

DISCUSSION

Over the past 30 years, surgical UUO in a variety of experimental animals has become established as a reproducible animal model of progressive kidney disease (8). Because of its relative technical simplicity, complete ureteral ligation has been employed in most studies, and the majority of reports based on this model utilize mice, in which genetically engineered mutants are most readily available (1). Although attention has been focused largely on interstitial cellular proliferation and fibrosis as end-points for study (8), we recently highlighted the effects of injury to the proximal tubule in adult mice subjected to 7–14 days of complete UUO (16, 17). These studies showed the proximal tubule to be the primary target of injury.
obstructive injury and oxidative damage, responding with multiple forms of cell death (necrosis, apoptosis, and autophagy) that lead to dramatic loss of proximal tubular mass after 7 days, with 40% of glomeruli becoming atubular after 14 days of UUO. This model of accelerated renal injury continues to be widely used despite the rarity of chronic complete ureteral obstruction in clinical practice. To more closely parallel most cases of obstructive nephropathy, we developed a model of reversible, variable partial UUO in the neonatal mouse, which leads to a more gradual progression of renal cellular responses (37). The purpose of the present study was, however, to determine the impact of ongoing nephron development and maturation on the most widely employed model of murine UUO. The results of the present study may prove relevant also to the evolution of unilateral multicystic-dysplastic kidney, which is thought to develop as a result of ureteral atresia in the human fetus (41).

Other animal models have been developed to study congenital obstructive nephropathy, including fetal sheep and primates (30). Neonatal models of obstructive nephropathy include the pig, in which nephrogenesis continues after birth (19). Although specific molecular effects of obstruction have been explored in these studies, they have not focused on the response by the proximal tubule.

Chronic UUO impairs the maturation of both obstructed and contralateral neonatal kidneys. The neonate subjected to UUO is undergoing rapid somatic growth: UUO slows growth and nephron maturation of the obstructed kidney (Fig. 9A). In both obstructive injury and oxidative damage, responding with multiple forms of cell death (necrosis, apoptosis, and autophagy) that lead to dramatic loss of proximal tubular mass after 7 days, with 40% of glomeruli becoming atubular after 14 days of UUO. This model of accelerated renal injury continues to be widely used despite the rarity of chronic complete ureteral obstruction in clinical practice. To more closely parallel most cases of obstructive nephropathy, we developed a model of reversible, variable partial UUO in the neonatal mouse, which leads to a more gradual progression of renal cellular responses (37). The purpose of the present study was, however, to determine the impact of ongoing nephron development and maturation on the most widely employed model of murine UUO. The results of the present study may prove relevant also to the evolution of unilateral multicystic-dysplastic kidney, which is thought to develop as a result of ureteral atresia in the human fetus (41).

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obstructed and contralateral kidneys of neonatal mice, mitoses are numerous for the first 2 wk, an expected characteristic of rapid growth. The rate of human renal growth also changes markedly with age, decreasing over 10-fold from 3.1 mm/mo at birth to 0.25 mm/mo at 7 mo of age, and remaining constant thereafter (32). Microdissection studies of human kidneys reveal that postnatal renal growth is primarily the result of rapid growth of proximal tubules (14). Whereas the maturing renal cortex normally grows circumferentially, the medullary region grows longitudinally (5), a process impaired by hydronephrosis. While in the mouse the papilla never forms in the obstructed kidney, contralateral kidney papillary growth is delayed compared with sham-operated kidneys (Fig. 9, A and B). There are at least two potential mechanisms responsible for this transient hydronephrosis in the contralateral neonatal kidney. The first is an increase in urine flow resulting from contralateral complete UUO: papillary hypoplasia develops also in polyuric 14-day-old congenital progressive hydronephrosis mice with diabetes insipidus because of a mutation in aquaporin-2 (31). The second is suppression of renin production by the contralateral kidney in the first 2 wk following UUO (Fig. 5C); plasma renin concentration is correlated with the number and intensity of renin-positive glomeruli (4). Signaling by angiotensin II through activation of the AT1 receptor is necessary for normal renal papillary growth ex vivo as well as in vivo (35), an effect independent of urine flow or intrapelvic pressure. Targeted deletion of renin, angiotensin-converting enzyme, or angiotensin AT1 receptors results in a similar phenotype in the postnatal mouse, characterized by failure of papillary elongation (25, 36, 39). However, in contrast to persistent lack of angiotensin production in these knockout mice, suppression of renin production in the contralateral kidney is transient and partial. Although blunted, growth of the papilla in the maturing intact kidney continues despite the persistence of contralateral UUO.

Fig. 7. Proximal tubular oxidative stress and mitochondrial injury following neonatal UUO. A, B: 7-day kidneys, semithin plastic sections of obstructed kidney. A: intact PT, composed of cuboidal epithelial cells with large round nuclei and small immature mitochondria (seen in detail in inset). B: example of scarce early necrotic degeneration, indicated by multiple vacuoles derived from swollen mitochondria. Inset: remaining brush border (arrowheads), identifying this profile as a proximal tubule. C, D, E, F, G: 14-day kidneys, distinguished as Ob for obstructed kidney or CL for contralateral kidney. C: semithin section of obstructed kidney equivalent to the field in A. Inset: detail of PT, with low columnar epithelium and mitochondria that are more rod-shaped than those of the younger kidney. D–G: kidneys perfused with nitroblue tetrazolium (NBT), which is metabolized by mitochondria to blue diformazan crystals, localizing superoxide anion surrounding PTs. Brown-staining immunoreactive 4-hydroxynonenal (HNE) localizes oxidative stress that concentrates in mitochondria. Both the obstructed (D; detail shown in E) and contralateral kidneys (F; detail shown in G) contain metabolically active mitochondria despite oxidative stress. Arrows in G indicate concentrations of HNE immunostaining. H–I: 28-day obstructed kidney. H: over the course of prolonged ureteral obstruction, the proximal tubules have undergone atrophy and fragmentation, with severe cell shrinkage and accumulation of lipid bodies (yellow-gold coloration, inset), indicating autophagy of cell components, primarily mitochondria. I: obstructed kidney PT profile similar to that shown in H, following NBT perfusion and stained for HNE. Diformazan is not visualized in PT due to overlapping HNE reactivity concentrated in cytoplasmic bodies. J: obstructed kidney with PAS staining; NBT perfusion results in diformazan deposition within atrophic PTs. K–M: 28-day contralateral kidney. K: contralateral kidney continued to mature, forming thick PTs composed of columnar epithelium with abundant fusiform mitochondria (detail in inset). L: contralateral kidney with NBT perfusion and HNE staining; PTs show persistent HNE staining, but peritubular diformazan crystals indicate functioning mitochondria. M: contralateral kidney, PAS staining, and NBT perfusion. Plane of section grazes the outer surface of a PT, showing concentration of diformazan particles (blue) at the basal portions of epithelial cells. Scale bars in B and its inset each = 10 μm and apply to corresponding fields in A, C, H, and K. Scale bar in F = 10 μm and applies to both D and F; scale bar in G = 5 μm and applies to E and G. Scale bar in M = 10 μm and applies to H–M.

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Maturation of the renal cortex is also delayed in the contralateral kidney, as revealed by persistence of a lower fraction of Lotus-lectin-positive glomeruli and proximal tubules.

Chronic UUO induces delayed proximal tubular injury in the neonate. Although scattered proximal tubules undergo necrosis after 7 days of UUO, most tubules continue to mature with functioning mitochondria through 14 days of obstruction (Fig. 9A). With more prolonged obstruction, the fraction of Lotus-lectin-positive glomerular capsules is reduced and many proximal tubules undergo apoptosis and autophagy, losing their lumina and becoming blind-ended, thus creating glomeruli that are functionally atubular (Fig. 4). In neonatal mice subjected to partial UUO, proximal tubular injury is minimal throughout the first 21 days (up to the time of weaning), but...
of the neonatal mouse kidney, proximal tubular mitochondrial morphology changes from spheroidal at birth to fusiform at 4 wk, and there is a doubling of cytochrome oxidase activity from 7 to 28 days (22). It is therefore likely that a shift from glycolysis to oxidative metabolism associated with mitochondrial growth accounts for the increasing susceptibility of the proximal tubule to obstructive injury from birth to adulthood.

Progressive accumulation of 4-hydroxynonenal immunoreactivity indicates oxidative stress localized to proximal tubules, which likely leads to mitochondrial damage in the obstructed kidney. Notably, preservation of proximal tubules in the contralateral kidney despite their immunohistochemical staining for 4-hydroxynonenal (which is not present in sham-operated animals) reveals the action of endogenous antioxidants in this kidney (Fig. 9B). In addition, compared with the sham-operated kidney, increased interstitial fibronectin in the contralateral kidney at 21 days may reflect a response to this stimulus (Fig. 8H). These findings underscore the effects of neonatal UUO on the “intact” contralateral kidney, which could have implications for the contralateral kidney in infants with severe unilateral ureteropelvic junction obstruction or multicystic renal dysplasia (41).

The role of autophagy in renal disorders is complex and can either contribute to cell survival (by elimination of damaged organelles such as mitochondria) or may respond to more severe cellular stress by acting in concert with apoptosis to promote cell death (34). Autophagy may also be activated as a self-clearance mechanism in cells committed to die by apoptosis or necrosis (29). In the context of UUO, autophagy appears to play a salutary role in the obstructed kidney, as suggested by aggravation of tubular apoptosis following inhibition of autophagy by 3-methyladenine (28). Although autophagy is widespread in the obstructed kidney of the adult mouse subjected to 7–14 days of UUO (16, 17), the present study reveals that autophagy is rare in neonates through the first 2 wk of UUO, but it becomes widespread in proximal tubules of 21- and 28-day animals, commensurate with the attainment of a more mature form.

Expression of α-SMA and collagen by the obstructed kidney parallels renal maturation but not obstructive cellular injury. Progressive accumulation of interstitial myofibroblasts and collagen in the obstructed kidney is well-established in the obstructed kidney and both are widely utilized as end-points of injury in this model (8). Whereas α-SMA in the contralateral kidney of adult mice is mainly located in arteriolar smooth muscle cells, in neonatal mice both the contralateral and obstructed kidneys exhibit numerous extravascular α-SMA-containing cells in regions that lack any collagen accumulations. Thus, there is a lack of correlation between α-SMA and collagen deposition. α-SMA is also expressed in renal interstitial fibroblasts in the neonatal rat kidney, and normally decreases to undetectable levels by 14 days of age, but it persists in the obstructed kidney (10). Therefore, in both rats and mice, α-SMA expression is normally abundant in certain interstitial cells (myofibroblasts) in the immature kidney and is further augmented by the stimulus provided by UUO. Fibronectin, like α-SMA, is extensively distributed within neonatal kidneys, but is a truly interstitial protein. However, unlike α-SMA, its distribution and contribution actually decrease in the obstructed kidney after 21 days of UUO.

Fig. 9. Scheme showing contrasting effects of UUO on the obstructed and contralateral kidneys during the first 28 days of life. UUO in the neonatal mouse delays maturation of both obstructed and contralateral kidneys. A: in the obstructed kidney, maturation is delayed, with persistence of immature glomeruli and PTs, and increased renin expression along the afferent arteriole. Initially, tubular proliferation is increased, and proximal tubular integrity is maintained despite oxidative stress, likely due to greater dependence of the immature tubule on anaerobic metabolism. There is also failure of papillary growth. However, with further maturation and persistent UUO (14–28 days), there is progressive damage to the PT, with mitochondrial injury, decreased generation of ATP, reduced cellular proliferation, and cell death by autophagy, apoptosis, and necrosis. These factors result in proximal tubular atrophy and formation of atubular glomeruli with relative expansion of the interstitial space. Thus, there is a lack of correlation between proximal tubular mitochondrial dysfunction and collagen staining in the contralateral kidney despite their immunohistochemical staining for 4-hydroxynonenal (which is not present in sham-operated animals) reveals the action of endogenous antioxidants in this kidney (Fig. 9B). In addition, compared with the sham-operated kidney, increased interstitial fibronectin in the contralateral kidney at 21 days may reflect a response to this stimulus (Fig. 8H). These findings underscore the effects of neonatal UUO on the “intact” contralateral kidney, which could have implications for the contralateral kidney in infants with severe unilateral ureteropelvic junction obstruction or multicystic renal dysplasia (41).
In human congenital obstructive nephropathy, fibrosis appears in children older than one year of age (26), and children with collagen deposition have demonstrably greater renal histologic damage (33). The deposition of interstitial collagen has traditionally been viewed to be itself injurious to the kidney (13); however, an alternative school of thought, which is steadily gaining advocacy, contends that that fibrosis is rather the by-product of a reparative process (27). Thus, decreasing renal function may be more closely linked to progressive proximal tubular damage rather than to any intrinsically toxic effects of interstitial collagen or other matrix substances (40).

Neonatal vs. adult UUO. In contrast to the adult mouse kidney, in which growth and development are complete, the neonatal kidney continues to undergo nephrogenesis in the first postnatal week, and its growth must accommodate the extrarenal environment. Following UUO in adult mice, there is rapid degeneration of the glomerulotubular junction and the adjacent proximal tubule segment, leading within 7 days to the formation of atubular glomeruli and fragmented proximal tubules (16). By contrast, in the obstructed neonatal kidney, cell death is initially largely limited to apoptosis in the collecting duct, and fractional proximal tubular loss is delayed beyond 14 days, with formation of atubular glomeruli delayed beyond 21 days (Fig. 9A). This initial preservation of proximal tubular mass in the neonate is likely related to the greater reliance on glycolytic metabolism and the greater cellular proliferative response by the immature kidney. Another notable difference concerns the contralateral kidney, which undergoes minimal change in the adult (17), but which in the neonatal animal shows suppression of juxtaglomerular renin and a hydronephrotic response, with retardation of papillary development (Fig. 9B). The rapidity of proximal tubular damage, together with formation of atubular glomeruli in the adult obstructed kidney, appears to be a primary determinant of renal parenchymal loss, with fibrosis being a secondary event. Although maturation of glomeruli and proximal tubules is delayed in the neonatal obstructed kidney, cortical development proceeds while medullary development is permanently arrested, with complete lack of papillary formation. With ongoing maturation, however, the remaining cortex also succumbs to the continued stress of persistent UUO. The present study argues for early intervention in cases of congenital urinary tract obstruction, before proximal tubular maturation can lead to irreversible injury and before the development of potentially maladaptive responses by the contralateral kidney. By manipulating the stage of maturation and duration of obstruction, the future application of renal histochemical and morphometric analysis to the UUO model in mutant mice is likely to provide additional insight in the progression of renal disease. In addition to surgical release of urinary tract obstruction, the targeting of antioxidants to proximal tubular mitochondria may lead to new therapeutic approaches in the management of congenital obstructive nephropathy, the leading cause of renal failure in infants and children.

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AUTHOR CONTRIBUTIONS