Growth Factors and Apoptosis in Neonatal Ureteral Obstruction

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ABSTRACT
Renal Insufficiency as a result of congenital obstructive nephropathy is a consequence of impaired renal growth: chronic unilateral ureteral obstruction (UUO) results in greater injury to the immature kidney than to the adult kidney. The neonatal kidney responds to UUO by marked activation of the renin-angiotensin system, which contributes to severe vasoconstriction and progressive interstitial fibrosis of the obstructed kidney. The latter results in part because of activation of transforming growth factor-beta 1 by angiotensin II. Chronic UUO in the neonatal rat delays maturation of the obstructed kidney, possibly in part through suppressed expression of epidermal growth factor. In addition to affecting growth factors, UUO stimulates apoptosis in the obstructed kidney, which is quantitatively greater in the neonate than in the adult. In contrast, expression of clusterin, a glycoprotein that may play a protective role in the response to UUO, is greater in the adult than in the neonatal obstructed kidney. The response of the developing kidney to UUO is similar in a number of respects to cystic kidney disease. This includes a reduction in epidermal growth factor, and increased apoptosis that may result from suppression of bcl-2, an oncoprotein that inhibits apoptosis. Improved knowledge of the cellular and molecular basis for cystic renal disorders should lead to specific intervention in fetuses and infants with congenital obstructive nephropathy, thereby improving renal growth and development.

Key Words: Renal development, ureteral obstruction, apoptosis, transforming growth factor-beta 1, clusterin

Congenital obstructive nephropathy is a primary cause of renal failure in infants and children (1). The developing kidney is more susceptible to injury from urinary tract obstruction than that of the adult (2). After relief of temporary unilateral ureteral obstruction (UOO), growth and function of the neonatal guinea pig kidney remain impaired, whereas recovery of RBF in the adult is complete, with no decrease in kidney weight (Figure 1) (3).

HEMODYNAMICS
On the basis of a series of studies in the neonatal guinea pig, we originally concluded that a decrease in RBF resulting from UUO was a central factor leading to the arrested growth of the obstructed kidney (4) (Figure 2). Renal vasoconstriction after ipsilateral UUO is associated with reduced glomerular volume and a decreased number of perfused glomeruli (4). The effects of altered growth factors, tubular atrophy, and interstitial fibrosis were considered secondary. Removal of the intact opposite kidney at the time of partial left ureteral obstruction in the neonatal guinea pig abrogates the effects of UUO on ipsilateral renal growth, RBF, glomerular perfusion, and glomerular volume (4) (Figure 2).

RENIN-ANGIOTENSIN SYSTEM
Similar to contralateral nephrectomy, chronic treatment of the neonatal guinea pig with enalapril also reduces renal vascular resistance of the obstructed kidney (5), and prevents the reduction in number of perfused glomeruli and the early reduction in glomerular volume (4,5) (Figure 3). These findings indicate an important role for the renin-angiotensin system (RAS) in mediating the renal hemodynamic effects of UUO.

However, the effects of angiotensin-converting enzyme inhibition on the intact opposite kidney are different: compensatory renal growth is prevented (4) (Figure 3). These results underscore the dependence of normal renal growth on an intact RAS (discussed later).

The renal RAS is highly activated in early development, with greater renin gene expression and more widespread microvascular renin distribution in the neonate than in the adult rat (6). In the neonatal rat, complete UUO increases ipsilateral renal renin gene expression, and leads to persistence of a fetal pattern of microvascular immunoreactive renin distribution, with renin extending along afferent and interlobular arteries, rather than being restricted to the juxtaglomerular region (7). Moreover, renal renin content is increased in the obstructed kidney (7), and additional cells are recruited in the renal cortex to secrete renin (8). Most recently, we have found that after 4 wk of UUO, expression and binding of the angiotensin Type 1 (AT1) receptor is increased in the ipsilateral kidney (9). El Dahr et al. have shown in the adult rat that renal angiotensinogen, angiotensin-converting enzyme, and angiotensin are also increased in the ob-
The recognition of the renal effects of a number of such compounds in recent years has turned our attention from the hemodynamic component of UUO (Figure 2) to growth factors themselves. In addition, factors mediating the progression of interstitial fibrosis have come under increased scrutiny, as has the importance of interstitial fibrosis in the kidney with chronic UUO (17).

The effects of maturation on the renal growth response to UUO have been demonstrated in models of UUO in the rat. As in the guinea pig (Figure 1), partial UUO in neonatal rats impairs renal growth, whereas partial UUO in 6-wk-old animals does not (18,19). After complete UUO in the neonate, the normal maturation increase in DNA content is prevented.
Figure 3. Effect of 3 wk of left partial unilateral ureteral obstruction (UUO) on left kidney glomerular volume (A) and right kidney weight (B) in neonatal guinea pigs. Although enalapril prevents glomerular contraction in the obstructed kidney (A), compensatory growth of the intact opposite kidney is also prevented (B). Solid bars, control group; hatched bars, enalapril-treated group. *P < 0.05 versus sham (same treatment group). (Data from Reference 4.)

What accounts for the dramatic effects of renal maturation on the response to ureteral obstruction? The increase in renal DNA content of the adult kidney subjected to ipsilateral UUO may be the result, in part, of a marked interstitial infiltrate of macrophages and monocytes, which may in turn be responsible for release of cytokines that contribute to the development of interstitial fibrosis (21). The stimulation of osteopontin by UUO may contribute to this process (22) (Figure 4). Partial UUO in the neonatal rat increases interstitial cellularity only after 6 to 9 wk of age (23), whereas interstitial collagen deposition is apparent by 3 to 4 wk (17).

One prominent characteristic of the neonatal rat kidney is widespread interstitial distribution of the contractile protein aSM-actin (24). Whereas this pattern of actin distribution normally disappears in the rat by 2 wk of age, ipsilateral ureteral ligation markedly prolongs immunoreactive aSM-actin distribution in the interstitium (20), an effect that may be associated with the transformation of fibroblasts to myoblasts (25). Myofibroblasts, in turn, may contribute to extracellular matrix accumulation (discussed later).

Epidermal growth factor (EGF) does not appear in the murine kidney until after birth (26). We have found that although EGF expression in the kidney normally increases linearly during the first month of life, ipsilateral ureteral obstruction suppresses the normal increase in EGF expression (20). Whereas EGF is abundant in distal tubular cells of the intact kidney of 14-day-old rat pups, it is suppressed in those of the obstructed kidney (Figure 5A and B). Because angiotensin II can potentiate the mitogenic effect of EGF (27), it is possible that suppressed levels of EGF in the obstructed neonatal kidney contribute to impaired growth of the kidney despite stimulation by high levels of angiotensin II. Alternatively, EGF may function as a differentiation factor in the developing kidney (28), and suppressed expression of EGF may in turn contribute to the delayed maturation of the obstructed kidney.

As shown in Table 1, neonatal ureteral obstruction in the rat impairs the normal increase in renal growth and DNA content, results in persistence of an immature pattern of interstitial aSM-actin distribution, and suppresses the normal increase in renal epidermal growth factor expression. In a recent preliminary report, we have demonstrated that chronic obstruction of the ureter or of individual nephrons results in apical epithelial cell expression of the EGF receptor (29). This cellular distribution characterizes the immature fetal tubule before epithelial polarization (30).

Renal renin mRNA remains elevated in the obstructed neonatal kidney, and the fetal pattern of microvascular renin distribution persists well into the neonatal period. This is associated with enhanced renin content and renin secretion, which also characterize the fetal kidney. Finally, the mRNA and binding of AT1 receptor are increased by chronic neonatal
Figure 5. (A) Immunoreactive epidermal growth factor (EGF) distribution in the kidney of a neonatal rat subjected to 14 days' ipsilateral unilateral ureteral obstruction (UUO). There are scattered brown-staining distal tubules indicating the presence of EGF immunoreactivity. (B) EGF distribution in the kidney from a 14-day-old sham-operated rat. There is heavy brown staining of numerous distal tubules. (C) Immunoreactive transforming growth factor-beta 1 (TGF-β1) distribution in the kidney of a neonatal rat subjected to 14 days' ipsilateral UUO. There is heavy brown staining of the luminal side of tubular epithelial cells. (D) TGF-β1 distribution of the intact opposite kidney from the same animal. There is faint luminal tubular epithelial staining. (E) Distribution of apoptotic cells in the kidney of a 3-day-old neonatal rat subjected to ipsilateral UUO. Brown-staining cells are identified by the TUNEL (Tdt-uridine-nick-end-labeling) technique, and are characterized by condensed nuclei with cytoplasmic "blebs." (F) Kidney from a 3-day-old sham-operated rat contains no identifiable apoptotic cells.
TABLE 1. Evidence that neonatal ureteral obstruction slows renal maturation

| Renal growth and DNA content remain low (20) |
| Immature interstitial α-smooth muscle actin persists (20) |
| Renal epididymal growth factor expression remains suppressed (20) |
| Apical localization of tubular epithelial cell epidermal growth factor receptor (30) |
| Immature intrarenal renin-angiotensin system persists |
| Increased steady-state renin mRNA (20) |
| Persistent fetal microvascular renin distribution (20) |
| Increased renal renin content (7) |
| Increased number of renin-secreting cells (8) |
| Increased mRNA and binding of angiotensin Type 1 receptors (7) |

UO (9). Taken together, these data suggest that ureteral obstruction in early development may interfere with growth of the kidney by impairing normal maturation. The mechanism for this remains to be elucidated.

Ureteral ligation in the neonatal rat results in markedly increased renal interstitial fibrosis after 2 wk (20). This is associated with a progressive increase in TGF-β1 expression in the obstructed kidney during the postnatal period, which contrasts with a gradual decrease in TGF-β1 expression in the normal kidney (20). Immunoreactive TGF-β1 appears on the luminal surface of tubular cells, and is significantly enhanced by 14 days of ipsilateral UO in the rat pup (Figure 5C and D). Because TGFβ1 is known to stimulate extracellular matrix synthesis and inhibition of matrix degradation (31), it is likely that increased production of this cytokine contributes to the progressive fibrosis of the obstructed kidney. We have shown that inhibition of AT1 receptors by losartan in neonatal rats with UO reduces expression of TGF-β1 in the obstructed kidney, but did not affect expression of the cytokine in normal kidneys (12). However, administration of losartan did not restore the reduced DNA content of the obstructed kidney, and, in fact, reduced the normal increase in renal DNA content of the unobstructed kidney (12). This is consistent with the dependence of normal renal development on endogenous angiotensin II (11). Thus, although angiotensin II inhibition has been shown to ameliorate renal fibrosis caused by UO in the adult rat (32), any potential beneficial effects in the neonate are outweighed by impairment of normal renal development.

APOPTOSIS

The process of growth is the result of a balance between cell proliferation and cell death. Thus, factors that may influence the rate of cellular destruction should be evaluated along with those responsible for proliferation. Recent studies in adult animals have shown that UO leads to distal tubular programmed cell death or apoptosis (33,34). Partial UO in the post-weaned rat results in progressively increasing apoptosis over a 3-wk period (34). We have found that after 2 wk of ipsilateral complete UO, distal tubular apoptosis is activated in the obstructed kidney, and the number of apoptotic cells is twofold greater in neonatal than in adult kidneys (35). After only 3 days of UO, apoptotic cells appear in the tubules of the ipsilateral kidney, but not in the contralateral kidney (Figure 5E and F).

In view of the likely role of apoptosis in contributing to tubular atrophy in the obstructed kidney, attention has turned to factors that may mediate apoptosis itself or modulate the consequences of apoptosis. One molecule that has stimulated considerable interest is the large dimeric glycoprotein clusterin. This molecule is also known as sulfated glycoprotein-2, dimeric-acidic glycoprotein, testosterone-repressed prostate message-2, and apolipoprotein J. Clusterin is activated by ipsilateral UO, and is expressed in the obstructed kidney (34,36). Clusterin is normally present in the condensing nephrogenic mesenchyme, and is turned off early in glomerular differentiation such that the only nephron segment that normally continues to express it in the mature kidney is the distal tubule (37). In addition to UO, clusterin expression is increased in other forms of renal injury, including collecting duct cysts in a mouse model of polycystic kidney disease (37). As its name implies, clusterin contributes to cell aggregation (38), and may contribute to preservation of epithelial integrity in the face of tubular injury (39).

In addition to its putative roles in organogenesis and cell adhesion, clusterin has been associated with apoptosis (40,41). There is recent evidence that clusterin gene expression may actually be confined to surviving cells that are surrounded by cells undergoing apoptosis (42). This is consistent with a recent report of the effects of UO in the young rat, in which clusterin (sulfated glycoprotein-2) gene expression decreased progressively during 3 wk of complete or partial UO, whereas the apoptotic nuclear count increased (34). Although clusterin expression increases in areas of apoptosis in various types of renal injury (43,44), clusterin can be expressed by either viable or apoptotic tubular epithelial cells (35,44). The precise relationship of clusterin to apoptosis remains to be elucidated.

After unilateral ureteral ligation in the neonatal rat, ipsilateral renal clusterin gene expression increases markedly by 24 h and continues to increase over the first month of life (Figure 6). We have also found that inhibition of AT1 receptors in neonatal rats leads to marked increases in clusterin expression in intact as well as obstructed kidneys (12). This suggests that renal clusterin expression is normally inhibited by endogenous angiotensin II. Of particular interest, we found that although renal clusterin expression is markedly increased after ipsilateral UO in both neonatal and adult rats, mRNA levels are significantly
higher in adult than neonatal obstructed kidneys (35). In contrast, 14 days' UUO causes marked increase of renin gene expression in the neonate but no significant increase in the adult (20). It is therefore possible that the reduced clusterin activation in the neonatal obstructed kidney compared with that of the adult may be attributed at least in part to greater activation of the RAS in the neonate.

To further explore the regulation of apoptosis in the kidney subjected to chronic ureteral obstruction, we also investigated the role of bcl-2, an oncoprotein discovered as a balanced translocation in human B cell lymphomas. Bcl-2 is a 26-kd integral membrane protein localized to the outer mitochondrial membrane, perinuclear membrane, and smooth endoplasmic reticulum. Bcl-2 has been shown to inhibit many forms of programmed cell death. Knock-out of bcl-2 by homologous recombination in the mouse results in diffuse renal apoptosis in polycystic kidneys, with an appearance similar to that of the severely hydronephrotic rat kidney (45). In preliminary studies, we have found uniform bcl-2 immunostaining of tubular cells in the intact kidneys but no staining of epithelial cells in dilated tubules of the obstructed adult kidney (46). In the neonate, there was widespread bcl-2 tubular nuclear staining in the intact kidney, but none in the obstructed kidney (46). These results suggest that bcl-2 normally inhibits renal tubular apoptosis, and that by suppressing bcl-2, UUO increases renal epithelial cell apoptosis. Although the action of bcl-2 may be through protection against oxidative stress (47), this does not appear to be the only mechanism (48).

**Figure 6.** Steady-state ratio of renal clusterin to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA quantitated by dot-blot hybridization in rats 1 to 28 days of age subjected to ipsilateral unilateral ureteral obstruction within the first 48 h of life (dashed line) or sham operation (solid line). Renal GAPDH expression does not change with maturation. *P < 0.05 versus sham.

**TABLE 2.** Similarities between the renal response to neonatal ureteral obstruction and cystic kidney disease

<table>
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<th>Similarity</th>
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<tr>
<td>Increased microvascular renin distribution (50)</td>
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<tr>
<td>Decreased epithelial growth factor production (51)</td>
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<tr>
<td>Apical epithelial cell localization of epithelial growth factor receptors (30)</td>
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<tr>
<td>Increased tubular apoptosis (52)</td>
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<tr>
<td>Increased tubular clusterin production (53)</td>
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<tr>
<td>Decreased tubular bcl-2 production (45)</td>
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<td>Decreased PKD-1 production (54)</td>
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**RENEAL CYSTIC DISEASE AND OBSTRUCTIVE NEPHROPATHY**

In summary, the molecular and cellular consequences of UUO in the neonate include a delay in ipsilateral renal maturation, marked activation of the RAS, decreased expression of EGF, increased renal tubular cell apoptosis, increased tubular clusterin expression, and decreased bcl-2 production. Interestingly, Fetterman et al. reported focal tubular dilatation of the distal tubule in fetal rabbit kidneys subjected to chronic ureteral obstruction (49). There are a number of corollaries between the hydronephrotic immature kidney and inherited or acquired cystic kidney disease (Table 2). Graham and Lindop described renin accumulation along the afferent arteriole in kidneys from patients with autosomal dominant polycystic kidney disease (50). Gattone et al. found markedly reduced renal tubular EGF immunostaining in the CPK mouse with inherited polycystic kidney disease (51), whereas Orellana et al. demonstrated apical localization of EGF receptors in cystic collecting tubules in this model (30). Woo has shown increased renal tubular apoptosis in the PCY mouse with inherited polycystic kidney disease (52). Rosenberg et al. have described increased renal tubular clusterin immunostaining in rats with phenol-induced cystic kidney disease (53). Finally, as noted above, Vets et al. have shown that the bcl-2 knockout mouse develops cystic kidney lesions along with a marked increase in renal apoptosis (45). Most recently, preliminary studies indicate that the newly discovered polycystic (PKD1) gene is suppressed in the hydronephrotic kidney resulting from UUO (54).

**THE FUTURE**

It is likely that improved knowledge of the cellular and molecular basis for renal cystic disease will lead to significant advances in the diagnosis and treatment of congenital obstructive nephropathy. Some of these may eventually be accomplished by gene therapy. Although inhibition of angiotensin has been shown to reverse renal interstitial fibrosis in the adult rat with ipsilateral UUO (32), the dependence of normal renal development on an intact RAS precludes this approach in the fetus and neonate. Such treatment, however, may prove beneficial in older infants and children.
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