Evidence that inhibition of tubular cell apoptosis protects against renal damage and development of fibrosis following ureteric obstruction

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INTRODUCTION TO URETERIC OBSTRUCTION

Ureteric obstruction causes impedance to the flow of urine in the ureter. The obstruction can be partial or complete, unilateral, or bilateral. The etiology is variable and wide-ranging. Classification is according to cause, duration, degree, and level. Most commonly, obstruction occurs at the ureteropelvic junction. The broader term “obstructive uropathy” can be used to indicate any obstruction to urinary flow occurring between the renal pelvis and the urethra, which causes a developing hydrenephrosis and associated renal impairment. Urethral strictures and benign prostatic hypertrophy are cases in point (Table 1).

As a large body of experimental evidence from animal models of unilateral ureteric obstruction (UOO) exists and forms the basis of our review, we will briefly highlight some of the clinical aspects of this pathology. The incidence of UOO is reported as 1/1,000 in adults. Ureteric calculi are the most common cause, and acute obstruction typically presents with significant renal colic as the major symptom. Relief of all types of obstruction generally leads to the normalization of any deterioration in renal function noted at diagnosis. However, some evidence from animal models suggests that obstruction can cause progressive deleterious effects on renal function and blood pressure control, especially in the presence of preexisting pathologies such as essential hypertension. The last 10 years have seen a proliferation of studies in rodents wherein complete unilateral ureteric obstruction has been used as a model of renal fibrosis. However, the relevance of the findings to human obstructive uropathy has, in many cases, not been the primary aim. In this review, we outline the major events linking damage to the renal parenchyma and cell death to the evolution of fibrosis following obstruction. Special focus is given to the role of apoptosis as a major cause of cell death during and post-complete ureteric obstruction. Several interventions that reduce tubular apoptosis are discussed in terms of their ability to prevent subsequent progression to end-organ damage and fibrosis.

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response. Tubular cell death during UUO has been shown to provide the stimuli for the generation of the inflammatory processes known to cause cell injury and death. The affected cells may constitutively activate in response to extracellular and intracellular death-inducing signals. The active form, in turn, activates pathways, resulting in the cleavage of genomic DNA, causing cells to die in an organized fashion.

Studies in vitro show that tubular cell death in response to mechanical stretch occurs via caspase-dependent apoptosis linked to increases in oxidative stress. Periods of mechanical stretch of proximal tubular epithelial cells of up to 24 h caused downregulation of the expression of the mRNA for the endogenous antioxidant catalase compared with unstretched control cultures. Depletion of cellular catalase activity logically leads to decreased detoxification of hydrogen peroxide. Acalasmin mice show an increase in tubular apoptosis following UUO, indicating that hydrogen peroxide accumulation occurs and indeed contributes to oxidative stress-related cell death in this model. In an in vitro model of wound healing using injured alveolar epithelial cells, hydrogen peroxide has been shown to cause cell death via caspase-dependent apoptosis.

The previously mentioned decrease in the RBF, in itself contributes to tubular cell stress and death by generating a hypoxic environment within the kidney. Death of primary and immortalized tubular epithelial cells proceeds via apoptosis in in vitro models of hypoxia.

Stressed cells also produce mediators which act in an autocrine and paracrine manner to induce apoptosis. Fas-Fas ligand (FasL) interactions, increases in ANG II, and transforming growth factor-β1 (TGF-β1) activity have all been implicated in the autonomous generation of apoptotic cell death in the tubular cells early after UUO. The production of ANG II in the obstructed kidney is thought to contribute to tubular cell death via the induction of TGF-β1 and increases in oxidative stress.

The presence of TGF-β1 overexpression in biopsies from human obstructive uropathy further confirms its role in the pathophysiology of renal damage.

Injured tubular cells also secrete proinflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) and FasL. IL-1 and TNF-α are chemotactic for monocytes/macrophages and neutrophils, which catalyzes the production of toxic prooxidant substances including hypochlorous and nitrous acids. These cytokines constitute the major receptor-triggered apoptotic signals in the nephron following UUO.

Support for the importance of apoptotic cell death during animal models of UUO-induced tubular atrophy has been provided by immunohistochemical detection of markers of apoptosis (p53, Bax, activated caspase-3) and histochemical

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<th>Table 1. Causes of ureteric obstruction</th>
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<td><strong>Cause</strong></td>
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<td>Retroperitoneal fibrosis/malignancy</td>
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<td>Local tumor extension</td>
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<td>Vesical tumor (involving the ureteric orifice)</td>
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Outline of the causes of ureteral obstruction presenting in primary care urology in adults and children.

evident by 3 days and well advanced within 10–14 days (49, 85, 88). This parallels what happens to the kidney in cases of human obstructive uropathy, presenting with long-standing severe or complete obstruction. Accumulated evidence from animal models now permits the generation of a global picture of the major pathophysiological events leading to renal fibrosis following complete UUO. In response to a range of cellular stresses, alterations in renal cell death and proliferation and the development of renal inflammation act coordinately to give rise to the tubulointerstitial lesion.

What are the principal stresses affecting the tubular epithelium during UUO? Mechanical stretching and desquamation of the tubular epithelium are early events occurring as a consequence of peristaltic retrograde pressure transfer and urinary stasis in the obstructed ureter (36). Early increases in intratubular pressure are likely to be further promoted by early increases in renal perfusion and filtration following local increases in nitric oxide (NO) generation. An in vitro model examining the effects of direct pressure (130 mmHg) on tubular epithelial cell cultures demonstrated increased NO generation and inducible nitric oxide synthase (iNOS) expression within 60 min of the initiation of pressure transfer (23). Both iNOS and constitutive NO synthase (eNOS) enzyme activity is increased throughout the injured tubular epithelium (67, 70). NO dose dependently relaxes the afferent arterioles, leading to increases in the glomerular filtration rate (GFR), thereby compounding the urine-pooling effect (103).

Histological sections of the kidney early after obstruction show dilatation of the tubules, confirming the importance of the urinary pooling effect. The transferred pressure causes the epithelial cells lining the nephron to have a flattened appearance (85). The cessation of this primary insult relies on increased compliance in the ureteropelvic region and the shunting of pooled urine to local lymphatics over the course of the first 24 h postobstruction (76). Later in the course of obstruction, the renal blood flow (RBF) markedly decreases, preventing further increases in the volume of pooled urine by eliciting a reduction in the GFR (36, 71). The net effect of these processes is to reduce the intratubular pressure from its maximal value of 50–70 mmHg, occurring at 3 h after obstruction, to a near-normal value of 30 mmHg by 24 h postobstruction.

These initial stress stimuli in the tubular compartment are known to cause cell injury and death. The affected cells provide the stimuli for the generation of the inflammatory response. Tubular cell death during UUO has been shown to occur principally via programmed cell death or apoptosis. The major effectors of apoptosis are the cystine aspartate-specific proteases (caspases). These are enzymes that are proteolytically activated in response to extracellular and intracellular death-inducing signals. The active form, in turn, activates pathways, resulting in the cleavage of genomic DNA, causing cells to die in an organized fashion.
detection of DNA strand breaks characteristic of the apoptotic process (TUNEL assay) (109).

PROAPOPTOTIC CYTOKINES HAVE OVERLAPPING PROFIBROTIC EFFECTS

The previous section highlights the fact that the increased presence of a number of cytokine species in the obstructed kidney occurs in conjunction with increases in tubular cell apoptosis. Some of these mediators show certain duplicity in their range of activities. In addition to its role in the priming of apoptosis in UUO (16), TGF-β1 is well documented as representing one of the most important profibrotic cytokines in renal damage (6, 7, 49). Basic fibroblast growth factor (bFGF) is another profibrotic cytokine, which both induces and is induced by TGF-β1, and has been shown to be of importance in post-UUO fibrosis (45, 81, 95). We will use TGF-β1 activity post-UUO as a classic example of the sources and modes of action of a cytokine with proapoptotic and profibrotic effects.

TGF-β1 MEDIATES BOTH APOPTOSIS AND FIBROSIS FOLLOWING UUO

There are two principal origins of TGF-β1 following UUO. It forms a component of early autocrine events occurring in the damaged tubule, as demonstrated by the upregulation of its mRNA along the length of the nephron of rat kidneys with downstream obstruction (24). Additionally, post-UUO inflammation provides a major source of TGF-β1, following its release from macrophages (2, 61).

TGF-β1 is known to promote apoptosis following renal injury in both in vivo and in vitro models (16, 69). Proapoptotic effects of ANG II in renal cells have been shown to be mediated, at least in part, via the induction of TGF-β1 (4). It has been demonstrated that TGF-β1 does not directly induce renal tubular cell apoptosis but rather primes for apoptosis in response to other death signals. Stimulation of an immortalized renal tubular cell line with TGF-β1 led to increased apoptotic sensitivity to the protein kinase C inhibitor staurosporine (16). The same paper explored the mechanism underpinning the priming effect. TGF-β1 accelerated the cleavage and activation of pro-caspase-9, followed by activation of caspase-3. This is due to cross talk between the MAP kinase and Smad signaling pathways downstream of TGF-β1 receptor stimulation. The reliance of this effect on p38 MAP kinase activation was demonstrated using the specific chemical inhibitor SC68376. (16).

TGF-β1 is regarded as the profibrotic mediator par excellence. Through its interaction with its receptors on epithelial cells, mesangial cells, and fibroblasts, it can induce increases in the deposition of extracellular matrix proteins (e.g., collagens I, III, and IV and fibronectin) (8, 91). This can occur both directly and indirectly via the induction of connective tissue growth factor (CTGF) (28). Additionally, TGF-β1 upregulates the expression of various matrix metalloproteases (MMPs) in both epithelial cells and fibroblasts (31, 60) as well as that of some of their endogenous inhibitors [e.g., tissue inhibitor of metalloproteinases-1 (TIMP-1)] (3). TGF-β1 also induces the upregulation of α- and β-subunits of matrix-interacting integrin molecules (37, 57). The net effect of this is to cause an increase in fiber production to occur in tandem with changes in cellular motility and invasiveness. This gives rise to the phenomenon of compartment-specific, pathological matrix remodeling and the generation of scarring. TGF-β1 induces these effects via stimulation of its receptor complex and downstream mobilization of various intracellular pathways, principally the Smad pathway (98, 105).

As well as promoting apoptosis, ANG II is also documented as having a role to play in fibrosis, especially in the kidney, where it is believed to promote the fibrotic response principally via the activation of TGF-β1 (104, 106). Aside from its pathological role in apoptosis and fibrosis, TGF-β1 has some beneficial effects post-UUO. An example is the positive role of its induction of fibronectin and collagen IV expression in injured tubular epithelial cells (8, 29). This assists in the restoration of tubular basement membrane integrity. Additionally, TGF-β1 possesses potent anti-inflammatory effects that are likely to have an impact on acute renal injury. It is expressed by all leukocyte lineages and acts in both an autocrine and paracrine manner to limit the duration of white blood cell activation and induce tolerance to antigenic stimuli (reviewed in Ref. 62).

One theory currently popular on the origins of renal fibrosis is the suggestion that under the influence of certain growth factors a proportion of tubular epithelial cells undergo a trans-differentiation process known as epithelial-mesenchymal transdifferentiation (EMT), whereupon the cells migrate to the interstitium and synthesize excess matrix. EMT is characterized by the loss of certain epithelial markers such as E-cadherin following the switching off of cytokines such as bone morphogenetic protein-7 (BMP-7), which favor maturation of the epithelial phenotype. Additionally, cells gain the expression of fibroblast (vimentin) and smooth muscle (α-smooth muscle actin) markers. These changes lead to an intermediate phenotype known as the myofibroblast, which is motile, invasive, and fiber producing. The process of EMT and its implications for fibrosis in renal disease have been dealt with in a number of excellent articles (46, 63, 97, 108). Stimuli relevant in UUO, such as hypoxia and mechanical stretch, have been shown to induce this transition in cultured cells (66, 89). Hypoxia induces TGF-β1 in cultured cells of various types (22, 51, 87), whereas TGF-β1 is known to directly induce EMT in cultured tubular epithelial cells, most particularly following combined treatment with EGF in a mouse tubular epithelial cell line (77). This would suggest that physiological stresses cause EMT to occur by a TGF-β1-dependent mechanism.

SUMMARY OF HOW INJURY AND CELL DEATH LEAD TO FIBROSIS

It appears clear that apoptosis in the nephron following UUO is dependent on the responses elicited by environmental stimuli such as stretch, hypoxia, and oxidative stress. These cause cell death, promote an inflammatory response, and result in the instigation of matrix synthesis and deposition by native renal cells. It is known that cytokines such as TGF-β1 have a key role to play in both the apoptotic and fibrotic responses.

CONSEQUENCES OF DESCRIBED EVENTS ON RECOVERY OF RENAL FUNCTION FOLLOWING RELIEF OF OBSTRUCTION

In terms of functional impairment during UUO, global renal clearance is acutely reduced. However, this is then compensated for by tubuloglomerular feedback-mediated increases in
the single-nephron filtration rate of the contralateral nonobstructed kidney (5). In the absence of recovery of the obstructed rat kidney, the contralateral kidney undergoes a compensatory hypertrophy (21), and in the case of congenital obstruction, both hypertrophy and hyperplasia also occur (80).

Despite these adaptive changes that act to maintain renal function, evidence from animal studies demonstrate that the fibrotic response persists, even in the event of the relief of obstruction, and that this correlates with a progressive loss of function in the affected kidney (9, 40). Following the relief of a 5-day UUO, the fibrotic process was already in place and continued to progress (40). The relief of a 3-day UUO in rats led to acute improvements in the RBF and GFR at 14 days postrelief relative to values of these parameters found during obstruction (9). However, these authors highlighted that in the longer term the continued fibrotic response pushes the kidney toward the development of chronic failure. The fact that relief of obstruction does not always reverse the fibrotic process may have profound repercussions for certain individuals with systemic diseases predating the obstruction that are known to be linked to renal dysfunction (e.g., essential hypertension) or in patients with only a single functioning kidney before obstruction. Indeed, in the spontaneously hypertensive rat, UUO induces an accelerated rate of the hypertensive state and the incidence of stroke compared with nonobstructed littersmates (38). Removal of the affected kidney reduced plasma renin activity, demonstrating that the elevation in systemic blood pressure had been worsened by the obstruction. In the human context, transient increases in blood pressure are frequently observed in cases of UUO and usually normalize following relief of the obstruction (44). However, the impact of a UUO-induced elevation of a preexisting hypertensive state has not been examined in humans. One would expect that if obstruction occurred in patients with a single functioning kidney and persisted long enough to induce significant cell death, then activation of the subsequent fibrotic cascade would present with a progressive descent into chronic renal failure.

PREVENTING PRIMARY TUBULAR CELL DEATH PREVENTS PROGRESSION TO FIBROSIS

Background

Tubular cell apoptosis is an early event that occurs before the onset of frank fibrosis, and thus it can be hypothesized that reducing initial cell death may prevent progression to fibrosis. Direct inhibition of caspase activity in a rat model of renal ischemia-reperfusion injury caused decreases in tubular cell death by apoptosis and a prevention of subsequent inflammation and fibrosis (15). This suggests that inhibition of apoptosis could have a similar impact in UUO and prompts an evaluation of the available literature on the subject. We hypothesized that limiting tubular cell death in response to early stress stimuli would indeed prevent the generation of fibrosis.

Effect of Modifying Activity of Mediators of Cell Signaling on Post-UUO Apoptosis and Fibrosis

Increases and decreases in the expression levels and activities of certain mediators in the damaged kidney can have profound effects on tubular apoptosis following UUO. Many lead to the activation of caspases and the induction of apoptosis, as described above for TGF-β1. Thus they represent potential targets for novel therapies to prevent primary renal injury and subsequent fibrosis.

Animal models of complete UUO would appear to support this hypothesis. Administration of a TGF-β1-neutralizing antibody to rats 1 day before, and at subsequent 2-day intervals for up to 13 days after UUO, reduced the severity of TIF (69). In the early stage of this experiment, the neutralizing antibody greatly reduced tubular apoptosis coincidently with increases in tubular reproliferation rates. The reduction in apoptosis was associated with the preservation of mitochondrial Bcl-2 localization and a reduction in p53 expression compared with untreated controls. In the same paper, mechanical stretch significantly induced apoptosis in NRK-52E cells, but this response was completely inhibited in the presence of the TGF-β1-neutralizing antibody. Inhibition of the direct profibrotic role of TGF-β1 is also likely to have occurred in this study. Both antiapoptotic and direct antifibrotic effects of the neutralizing antibody are likely to have synergized to prevent the development of TIF.

Another cytokine believed to play a causative role in tubular cell death following UUO is TNF-α. Prevention of the biological activity of TNF-α via the administration of a pegylated form of soluble TNF-α receptor type 1 significantly reduced obstruction-induced caspase activity and tubular cell apoptosis (68). Interestingly, TNF-α receptor type 1 knockout mice show reduced tubulointerstitial fibrosis following UUO, suggesting that a link is likely to exist between apoptosis and fibrosis via the activity of TNF-α (30).

Renal ANG II generation is increased in the obstructed kidney, and in vitro studies have shown it to induce apoptosis of tubular epithelial cell cultures when administered in pathophysiologically equivalent doses (4). Indeed in animal models of UUO, the use of angiotensin-converting enzyme inhibitors and ANG II type 1 receptor deletion causes a reduction in early apoptosis and late fibrosis (43, 88). This poses an interesting question regarding patients who have been receiving ANG II antagonists as part of an antihypertensive regime and coincidentally present with obstruction. Do such individuals show better preservation of ipsilateral kidney function and architecture via alterations in renal cell death and fibrosis? To our knowledge, to date, no such information has been published.

Conversely, the increased provision of certain factors has been shown to ameliorate the development of UUO-related fibrosis subsequent to reductions in tubular apoptosis. A qualitatively similar effect as described for the TGF-β1-neutralizing antibody has been observed in rats with a novel hepatocyte growth factor (HGF) transgene-based therapy (25). These rats demonstrated enhancement of cellular proliferation and an inhibition of tubular apoptosis following complete UUO. As with the TGF-β1-neutralizing antibody, Bcl-2 expression in the mitochondria was preserved in the HGF-transfected UUO rats. HGF has been shown to antagonize TGF-β1 signaling, and this may explain the similar results obtained with HGF overexpression and TGF-β1 neutralization.

The administration of insulin-like growth factor-1 (IGF-1) has been shown to ameliorate the renal injury resulting from UUO. Here, 2-day-old rats were subjected to complete UUO or sham operation and then administered IGF-1 or saline for up to 7 days after the procedure. IGF-1 treatment reduced tubular...
BMP-7 is a growth factor associated with tubular development during nephrogenesis and the maintenance of the differentiated state of renal tubular epithelial cells in the mature kidney, being downregulated in acute renal injury and during the course of diabetic nephropathy (93, 102). Evidence exists to suggest that the activity of BMP-7 is inhibited in renal cells via a TGF-β-dependent mechanism. This is based on the fact that tubular epithelial cell cultures treated with TGF-β1 showed reduced BMP-7 and Alk3 expression and increased expression of gremlin, an endogenous antagonist of BMP-7 (102). The same paper noted that the loss of BMP-7 expression per se led to increased profibrotic gene expression [fibronectin, collagen III (α1)]. Administration of BMP-7 at the time of UUO and every other day thereafter blocked increases in the rates of tubular apoptosis. Subsequent tubular degeneration and atrophy were inhibited and importantly so was the progression of tubulointerstitial inflammation and fibrosis (35). BMP-7 has also been shown to reverse EMT, TIF, and renal functional impairment using nephrotoxic serum nephritis (NTN), as a model of progressive chronic renal injury (108). The implementation of a BMP-7 treatment regime following the release of a 3-day UUO was also seen to reduce fibrosis and protect the GFR and RBF when examined at 7 days post-UUO (73).

Studies centered on the manipulation of the level and activity of EGF and its receptor (EGF-R) have revealed that, for the most part, stimulation of this pathway has a protective effect against the degree of apoptosis and later appearance of fibrosis in animal models of UUO. EGF is generally known to lead to increased proliferation and decreased susceptibility to apoptosis via Akt pathway activation (84). A decrease in EGF mRNA expression and protein levels has been demonstrated in the renal tubules following UUO (94). However, the administration of recombinant EGF to adult Sprague-Dawley rats following UUO led to a reduction in levels of markers of apoptosis, including DNA laddering and apoptotic body formation at 3 days post-UUO (50). In neonatal rats of the same strain subjected to UUO plus or minus EGF treatment, analysis of tubular cell death and proliferation was examined at 7 days postobstruction in tandem with assessment of the fibrotic response. EGF markedly enhanced tubular repopulation by 76% in combination with an 80% reduction in tubular apoptosis, compared with levels found in the ligated kidneys of vehicle-treated rats. This occurred in association with markedly less evidence of tubular atrophy and dilation, reduced levels of TGF-β1, and a 50% reduction in the quantitative measurement of TIF (13). As the effect of EGF treatment was studied during UUO in both these studies, the potential of EGF to reverse the apoptotic and fibrotic processes following the relief of UUO remained an interesting and unanswered question. This was addressed in a study using neonatal rats, where the effects of EGF were evaluated following a 7-day administration protocol initiated at the time of obstruction, with relief of the obstruction at 5 days (14). Renal tissue was then examined at 28 days postobstruction. EGF treatment was associated with significant reductions in levels of vimentin and clusterin, both markers of tubular injury, and a 50% decrease in tubular atrophy compared with saline-treated rats.

This last point indicated that less tubular cell death had occurred, although apoptosis itself was not directly quantified. The deposition of collagen and the interstitial expression of α-smooth muscle actin were reduced by 50% in the postobstructed kidney, demonstrating amelioration of the fibrotic response. The potential of these results to indicate the clinical use of EGF in cases of adult obstruction is limited by the fact that the study used neonates and that EGF treatment was initiated from the start of UUO. This last point begs the question of whether the protection observed was indeed true reversal.

Evidence of the likely mechanisms involved in EGF-induced resistance to apoptosis has been elucidated using a combined in vivo and in vitro approach (53). Stretch-induced apoptosis in immortalized rat tubular epithelial cell cultures was associated with a 50% decrease in the phosphorylation of BAD. Hypophosphorylation of BAD facilitates its translocation to the mitochondria, initiating the release of proapoptotic factors into the cytoplasm. EGF treatment during stretch prevented the hypophosphorylation of BAD and apoptosis, and this effect was blockable via the use of specific kinase inhibitors. Following UUO, reduced tubular apoptosis in the obstructed kidneys of rats treated systemically with EGF was associated with hyperphosphorylation of BAD. A neutralizing antibody to EGF or EGF-R abolished these effects. Although a quantification of fibrosis was not made in this paper, consideration of the results in combination with the previously observed antifibrotic effects of EGF treatment during UUO in rats supports a link between EGF-induced reductions in apoptosis and subsequent inhibition of fibrosis.

One potential issue regarding EGF activity centers on evidence suggesting that it may have species-specific effects in UUO. The studies mentioned above were all carried out in rat models of UUO. Somewhat contradictory evidence has been reported in mice. The transgenic expression of a dominant negative EGF receptor under the control of a proximal tubular-specific promoter has been shown to reduce tubular atrophy and fibrosis following prolonged renal ischemia. This occurred in conjunction with a reduction in postinjury tubular proliferation. Interestingly, increases in tubular proliferation following UUO in the rat were proposed to represent one of the beneficial effects of EGF. Despite the fact that in this study a quantification of apoptosis was not carried out, the implication of the results is to suggest that the EGF activity in the injured proximal tubule may promote subsequent tubular atrophy and fibrosis (99). This is supported by observations in cultured murine proximal tubular epithelial cells treated with a combination of TGF-β1 and EGF, which show optimal transdifferentiation to the fiber-producing myofibroblast phenotype (77). Studies in fetal rat hepatocytes have shown that the process of EMT leads to a resistance to the proapoptotic effects of TGF-β1 (101). It is interesting to speculate as to whether mediators such as EGF could play a similar role in protecting transdifferentiating renal cells from apoptosis, without compromising the phenotypic change. To date, the exogenous administration of EGF in a murine model of UUO, or any other renal pathology linked to apoptosis and fibrosis, has not been comprehensively undertaken.

Osteopontin is a glycoprotein upregulated in rat renal tubular cells during UUO (19, 20, 47) and in vitro in a rat tubular epithelial cell line following stimulation with ANG II (20) or a combination of TGF-β1 and EGF (64). Aside from its macrophage chemoattractant properties, it has been shown to activate
and potentiate signaling from the EGF receptor to increase human prostate cancer cell proliferation (1) and human mammary epithelial cell migration (100). A decrease in apoptosis was observed in serum-starved mouse tubular epithelial cell cultures following the addition of osteopontin (78). Following UUO, osteopontin null mice show increased tubular apoptosis in conjunction with reductions in macrophage influx, TGF-β1 expression, and interstitial deposition of collagen I and IV (78). This again suggests, in mice, the possibility of an inverse relationship existing between certain antiapoptotic signals and the development of fibrosis. The relationship between osteopontin and EGF signaling may explain these effects. The fact that the osteopontin null genotype may reduce the peritubular accumulation of inflammatory cells may explain why in these mice, contrary to our hypothesis, increased apoptosis can occur without worsening of the fibrotic response.

The role of NO in renal injury remains somewhat controversial. Conflicting reports concerning whether it plays a protective or deleterious role have appeared, due to the fact that it can exert both cytoprotective and prooxidant/proapoptotic effects as well as proinflammatory and anti-inflammatory effects (reviewed in Ref. 27). The protective effect of an angiotensin-converting enzyme inhibitor on UUO-induced renal injury was blockable using an inhibitor of NOS activity. At the same time, provision of the NOS substrate L-arginine protected against the development of TIF (74). L-Arginine reduced apoptosis, whereas NO synthesis inhibitors increased apoptosis in an in vitro model of cyclic stretch using rat tubular epithelial cells (70). In the same study, the authors observed increased rates of tubular apoptosis occurring up to 14 days post-UUO in iNOS null mice compared with those seen in their wild-type littermates. An independent study reported that the obstructed kidneys of iNOS null mice showed increased interstitial fibrosis (increased tissue collagen and TGF-β1 content, interstitial volume, and macrophage infiltrate) at 2 wk postobstruction compared with wild-type mice with UUO (32), thus linking the increase in apoptosis phenotype to a downstream increase in fibrosis. The importance of iNOS in protection from UUO-related renal damage has been shown in a rat model (41). Here, retrograde ureteric transfection of the kidney with a plasmid containing the iNOS gene led to a better preservation of RBF and GFR at 24 h postobstruction. Although this result suggests a protective effect and a potential for this type of gene therapy in maintaining renal perfusion via increases in iNOS activity, the consequences for the apoptotic and fibrotic responses were not examined. However, with the data from the iNOS null mouse borne in mind, it is conceptually feasible that iNOS transfection might exert antiapoptotic and antifibrotic effects. Potential primary antiapoptotic effects of increased NO production include preservation of Bcl-2 localization in the mitochondria of tubular epithelial cells (69) and the induction of stress survival factors such as heat shock proteins, which are known to inhibit apoptosis and are proposed to be involved in iNOS mediation of the preconditioning effect (65, 79).

**Effect of Reducing Oxidative Stress on Post-UUO Apoptosis and Fibrosis**

As previously mentioned, mechanical stretch of tubular epithelial cell cultures leads to the generation of both oxidative

![Fig. 1. Summary of mediators and mechanisms shown to block tubular cell apoptosis following unilateral ureteral obstruction (UUO). Figure details the likely mechanisms of action of antiapoptotic strategies utilized in the inhibition of tubular cell apoptosis in animal models of UUO. Blunt-ended blue lines and green arrow-tipped lines indicate the site/stage of antiapoptotic effect. CLP, cardiolipin; NO, nitric oxide; BMP-7, bone morphogenic protein-7.](http://ajprenal.physiology.org/Downloadedfromhttp://ajprenal.physiology.org)
stress and apoptosis. Evidence exists to suggest that the primed release of proapoptotic factors from the mitochondria is dependent on the establishment of a prooxidant environment within the cell. The release of cytochrome c from the mitochondria is a crucial step in the intrinsic pathway of apoptosis and is believed to rely on both changes in mitochondrial permeability and the oxidation of the cytochrome c-binding partner cardiolipin on the outer mitochondrial membrane (42). Thus antioxidants could potentially act as inhibitors of apoptosis in stressed tubular cells.

Fundamental evidence to suggest a link among oxidative stress, tubular apoptosis, and the development of renal fibrosis comes from studies in mice that lack the expression of a functional catalase gene (96). At 7 days after UUO, the obstructed kidneys of these mice had increased levels of markers of oxidative stress-related lipid peroxidation compared with normal mice with obstruction. At day 4, significantly higher rates of tubular apoptosis correlated with higher levels of active caspase 9, and evidence of tubulointerstitial fibrosis was more severe in catalase-deficient mice at 7 days after obstruction.

Support for the potential of reducing oxidative stress as a method of protecting tubular cells from undergoing apoptosis exists from studies involving the administration of antioxidants to animals with obstruction. In one study, rats treated with the bioflavonoid quercetin were shown to have reductions in the UUO-induced upregulation of proapoptotic markers such as Fas and FasL at 7 days postobstruction (43). This may have been, in part, through its anti-inflammatory activity, which could act to reduce the post-UUO levels of inflammatory cell-derived Fas ligand. A number of other strategies such as Fluvastatin and α-tocopherol (vitamin E) supplementation have been shown to reduce oxidative stress and to prevent the development of tubulointerstitial fibrosis following complete UUO, although in these cases their effect on the apoptotic response was not examined (58, 72, 86).

CONCLUDING REMARKS

Tubular cell death following UUO is promoted by a number of stimuli, inducing the activation of cytokines and the production of unfavorable prooxidant conditions, which cause the cells to die. The extent of tubular cell apoptosis would appear to correlate with the later generation of TIF in a number of studies, and such a relationship has been proven in a model of renal ischemia-reperfusion. In the case of UUO, a number of studies have shown that reducing initial cell death by either neutralizing the activity of apoptosis-inducing molecules or supplementing with prosurvival factors, effectively prevents inflammation, further cell death, and progression to fibrosis. The prevention of fibrosis is of paramount importance in ensuring that acute injuries, such as complete UUO of short duration, do not lead to long-term loss of renal function. Although no hard evidence exists, we suggest that the initiation of initial apoptosis is likely to limit the generation of signals from dying cells that would subsequently attract and promote the inflammatory response. This, in turn, would prevent the renal accumulation of a number of proapoptotic and profibrotic cytokines as well as limit oxidative stress. In such a model, the processes and mediators necessary for progression to fibrosis would not be in place and end-organ damage would not occur. The collated evidence for such a model is presented in Fig. 1.

In this review, we have shown evidence from the literature to support our hypothesis that inhibiting tubular cell death by apoptosis can lead to the maintenance of a functioning, nonfibrotic kidney post-UUO. However, the extrapolation of these findings to the clinical setting may contain some caveats. For example, it is likely that such strategies would need to be implemented early after complete UUO, before the renal tubular cells suffered significant stress resulting in the induction of apoptosis. Overall, the evidence presented here demonstrates an apparent interplay between early apoptosis and subsequent fibrosis in UUO, and this could have profound repercussions not only for UUO but also for renal diseases of diverse etiology.

GRANTS

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F12 RELATIONSHIP BETWEEN APOPTOSIS AND FIBROSIS IN UUO

Invited Review

Peters CA, Gaertner RC, Carr MC, and Mandell J.

Naber KG and Madsen PO.


Liu Y.

Phillips AO, Topley N, Morrisey K, Williams JD, and Steadman R.


Moody TE, Vaughn ED Jr, and Gillenwater JY.


