Interstitial inflammation and fibrosis in obstructive nephropathy; the role of ACE inhibitors and nitric oxide

S. Klahr* and J. J. Morrissey* [1]
From the *Departments of Medicine and [1]Cell Biology and Physiology. Washington University School of Medicine and Barnes-Jewish Hospital. St. Louis, Missouri (USA).

SUMMARY

In this work we review the fundamental mechanisms leading to the establishment of obstructive nephropathy from the cell biology perspective. In particular, the implication of macrophages and infiltrating leukocytes in post-obstructive tubulo-interstitial changes are analyzed. Also, the involvement of specific cytokines such as TGF-β and TNF-α, as well as metalloproteinases and the transcription factor NF-kB is detailed. Finally, we comment upon the effect of pharmacological intervention with ACE inhibitors on the development of obstructive nephropathy.

Key words: ***Citokines, NF-kB, ACE inhibitors, nephropathy.***

INFLAMACION INTERSTICIAL Y FIBROSIS EN LANEFROP ATIA OBSTRUCTIVA; EL PAPEL DE LOS IECA Y DEL OXIDO NITRICO

RESUMEN

En este trabajo se revisan los mecanismos fundamentales conducentes al desarrollo de nefropatía obstructiva desde el punto de vista de la biología celular. En particular se analiza la implicación de los macrófagos y leucocitos infiltrantes en los cambios túbulo-intersticiales post-obstructivos. Además se detalla el papel de determinadas citocinas como el TGF-β y el TNF-α., así como de las metaloproteinasas y un factor de transcripción NF-kB en estos cambios. Finalmente se revisa el efecto de la intervención farmacológica con inhibidores de la ECA sobre el desarrollo de nefropatía obstructiva.

Palabras clave: ***Citocinas, NF-kB, inhibidores de la ECA, nefropatía.***

«Obstructive nephropathy» refers to the renal disease caused by impaired flow of urine or tubular fluid. «Obstructive uropathy» refers to the structural or functional changes in the urinary tract that impede the normal flow of urine[1]. Obstructive nephropathy may be manifested clinically as a sudden or as a gradual and insidious decrease of renal function. The decrease may be halted and even reversed if the obstruction is relieved. Obstruction may be due to anatomical or functional abnormalities of the urethra, bladder, ureters or renal pelvis[1]. These abnormalities may be congenital or acquired. Obstructive uropathy can also occur during the course of diseases extrinsic to the urinary tract[1].

Correspondencia: Saulo Klahr, M.D.
Department of Medicine
Barnes-Jewish Hospital
216 South Kingshighway
St. Louis, Missouri 63110

INCIDENCE AND CAUSES

The incidence, prevalence and cost of obstructive uropathy are difficult to estimate because this entity

occurs in the setting of a variety of diseases that may warrant hospitalization or surgical intervention. Obstructive uropathy has a bimodal distribution in humans. It is common in childhood, due mainly to congenital anomalies of the urinary tract. It declines with age until late adulthood. At age 60 to 65 years the incidence rises, predominantly in men, due to prostatic hyperplasia or cancer[2]. In 1985 in the United States 397,100 hospital discharges were coded as obstructive uropathy. About 166 patients per 100,000 population had a presumptive diagnosis of obstructive uropathy on admission to hospitals in the United States[2]. Among male patients with kidney and urologic disorders, obstructive uropathy ranked fourth at hospital discharge (242 patients/100,000 discharges). In females with kidney and urologic problems, obstructive uropathy ranked sixth as a diagnosis at hospital discharge (94 patients/100,000 discharges).

From 1989 to 1993, a span of 5 years, 4,869 patients with the diagnosis of obstructive nephropathy began treatment for end-stage renal disease (ESRD) in the United States[3].

During this period, obstructive nephropathy accounted for 2% of the patients being treated under Medicare regulations for ESRD[3]. An additional 355 patients (0,1%) with the diagnosis of «congenital obstructive uropathy» were treated for ESRD during the same period[4]. Among the 4,869 patients with obstructive nephropathy being treated for ESRD, 6.9% were younger than 20 years of age, 35.7% were between the ages of 20 and 64 years and 57.4% were older than 64 years. Males comprised 73.8% of patients with obstructive nephropathy being treated for ESRD. In terms of racial origin, 80.5% of the patients were white, 16.4% African Americans, 1.7% of Asian descent and 0.8% Native Americans[3].

Obstructive uropathy is a common cause of ESRD in children[4]. Obstruction of the urinary tract in early gestation may cause renal dysplasia, while obstruction occurring in late gestation or after birth can cause irreversible loss of renal function[4]. New ultrasound techniques have made possible the diagnosis of obstructive uropathy in the fetus. In the adult, the incidence and causes of obstructive uropathy vary with the sex and age of the patient. In young and middle-aged males, acute obstruction due to renal stones is common but temporary. In females of this age group pelvic cancer is an important cause of obstructive uropathy. In the older age group, obstructive uropathy is more common in males, due to prostatic diseases (hyperplasia, cancer).

TUBULOINTERSTITIAL CHANGES IN OBSTRUCTIVE NEPHROPATHY

The tubulointerstitium occupies approximately 80% of total kidney volume. Renal tubular epithelial cells represent the major compartment of the kidney. The interstitium is surrounded by vascular and tubular compartments, which make it specially vulnerable to pathologic events. Structural derangements of the tubulointerstitial compartment occur in a variety of renal diseases. Interstitial fibrosis is characterized by the accumulation of matrix proteins in the renal interstitium. The matrix deposition leads to tubular atrophy and a reduction of peritubular capillaries, and is accompained by a macrophage infiltation of the interstitium and an increased number and transformation of fibroblasts.

Several studies indicate that tubulointerstitical changes, not glomerular pathology, correlate better with decrements in GFR in a variety of renal diseases[5-8]. In addition, renal tubular cells produce an array of growth factors, vasoactive peptides, and cytokines[9], the action of which may in turn be modulated by the extracellular matrix. Adherence of cells to matrix can induce cells to make cytokines, which in turn may lead to alterations which matrix[10]. Such interactions may lead to alterations of the cell to cell contact that have an important role in inflammation and scarring.

Obstructive nephropathy can cause major changes in the tubulointerstitial compartment of the kidney[11]. Renal interstitial fibrosis is a common consequences of long-standing obstructive uropathy. Fibrosis very likely develops due to an imbalance between extracellular matrix synthesis and deposition and matrix degradation. Several investigators have examined the mechanisms underlying the fibrogenesis of obstructive uropathy. Nagle et al.[12] reported a widened interstitial space after 7 days of ureteral obstruction in rabbits, with an increase in collagen fibers and fibroblasts. By day 16 of obstruction, collagen was greatly increased and was arranged in large bundles. Nagle and Bulger[13] also described a mononuclear cell infiltrate and proliferation of interstitial cells in the renal parenchyma of rabbits with chronic unilateral ureteral obstruction. Sharma et al.[14] described interstitial fibrosis and thickening of the tubular basement membrane after unilateral obstruction in the rabbit. There was increased deposition of several extracellular matrix components (collagen types I, III and IV), fibronectin and heparin sulfate proteoglycans in the renal interstitium of rabbits with ureteral obstructive of 3, 7 and 16 days duration.

MECHANISMS UNDERLYING THE RECRUITMENT OF MACROPHAGES

A leukocyte infiltrate, comprised predominantly of macrophages, was found as early as 4 weeks after the onset of ureteral obstruction in rats but its peak response occurred after 24 hours. The signals responsible for recruiting macrophages and suppressor T cells into the renal interstitium after ureteral ligation appear to be specific for these cells, since neutrophils were not detected in this compartment in the initial few hours after the onset of obstruction. However, the factors involved in cell recruitment into the obstructed kidney are not well characterized.

Supernatants prepared from the renal cortex of rats with unilateral ureteral obstruction had greater chemotactic activity for rat peritoneal macrophages than did supernatants from the contralateral kidney of the same rats[15]. This activity for macrophage migration peaked between 4 and 12 hours of obstruction and declined after longer periods of time (24 to 72 hours). The activity was heat-stable and extractable with methanol, suggesting the presence of a lipid. Monocyte chemoattractant peptide-1 (MCP-1), which is expressed in tubular epithelium at 12 hours after ureteral ligation and persists to 96 hours, may participate in macrophage recruitment[16]. Other substances such as transforming growth factor β (TGF-β), osteopontin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), which are overexpressed in the obstructed kidney, may also have a role in macrophage recruitment[17-19].

PATHOPHYSIOLOGIC ROLE OF INFIL TRATING LEUKOCYTES

The invasion of the renal interstitium by macrophages and T lymphocytes in rats with obstructive nephropathy coincides with a decline in renal blood flow and GFR. After the onset of obstruction, there is a transient increase in renal blood flow mediated by prostaglandins.

Three to five hours after the onset of obstruction renal blood flow decreases, and by 24 hours the values are 40-70% of those observed before obstruction. The decrease in renal blood flow is due in part to increased release of thromboxane A_2, a powerful vasoconstrictor. Obstructed kidneys display an enhanced metabolism of arachidonic acid and the activities of both cycloxygenase and thromboxane synthase are increased. Administration of thromboxane synthase inhibitors increases GFR and partially reverses the renal vasoconstriction that occurs after acute ureteral obstruction. The role of infiltrating macrophages in decreasing renal plasma flow and GFR in obstructive uropathy was studied by subjecting rats to total body irradiation before obstruction[20]. Total body irradiation, or the administration of ACE inhibitors, arginine, probucol or N-acetyl cysteine markedly decreased the infiltration of the obstructed kidney by leukocytes.

Thromboxane excretion in the urine decreased in rats irradiated prior to ureteral obstruction and both renal plasma flow and GFR increased in the postobstructed kidney when compared to levels in non-irradiated rats. Thus, infiltrating leukocytes account in part for the decline in GFR and renal plasma flow seen after obstruction, most likely via the production of thromboxane A_2. However, elimination of the leukocyte infiltrate from the renal interstitium did not restore the function of the postobstructed kidney to «normal», suggesting that factors other than leukocyte infiltration likewise have a role. Increased production of thromboxane A_2 by intrinsic renal cells also contributes to the vasoconstriction of obstructive nephropathy.

CHANGES OF THE TUBULOINTERSTITIUM COMPARTMENT IN OBSTRUCTIVE UROP ATHY

The relative volume of the cortical interstitium was increased in the ligated kidney after 3 days of unilateral ureteral obstruction in the rat[21]. There was increased deposition of collagen types I, III and IV in the tubulointerstitium by the third day of obstruction. In addition, the level of mRNA for collagen α1 (IV) was significantly greater in the obstructed kidney at this time interval. Thus, events leading to interstitial fibrosis occur promptly after the onset of obstruction. By contrast, we found that the amounts of collagens I, III and IV did not change in the glomeruli of the obstructed kidney after 5 days of ureteral obstruction. These results are consistent with the finding that glomeruli appear normal by light microscopy through 7 days of obstructive nephropathy. The issue of whether or not interstitial collagen types I and III are localized in the glomeruli of the normal kidney is controversial. However, we found collagen types I and III in the glomeruli of our specimens of rat kidneys when fixed with Histochoice as well as when the sections were treated with trypsin to uncover these epitopes[21]. Both interstitial collagen, types I and III, and the basement membrane collagen, type IV, were deposited in the interstitial space of the obstructed kidney. Collagen types I and III were increased in the interstitial space only, while collagen IV was deposited both in the interstitium of the obstructed kidney and in the tubular basement membrane. Furthermore, we found that collagen IV increased to a greater degree than other collagens in the renal cortex of the adult rate after unila-

teral ureteral obstruction. The increase in collagen IV in both the interstitial space and in the basement membrane of renal tubules may contribute to alterations in tubular function in the obstructed kidney.

Renal tubular cells may contribute to the increased production and deposition of collagen IV in tubular basement membranes and interstitium. Renal tubular cells in culture produce collagen types I, III and IV. The expression of collagen α1 (IV) mRNA is increased substantially in the tubules of the obstructed kidney. It was reported[13] that fibroblasts migrated to and proliferated in the interstitium of the obstructed kidney during unilateral ureteral obstruction. In addition, Kuncio et al.[22] found that several cytokines secreted by infiltrating macrophages and T lymphocytes act as chemoattractants and stimulate fibroblasts proliferation and that interstitial fibroblasts produced collagen types I, III and IV. The substantial increase in collagen types I and III found in the interstitium of the obstructed kidney at 3, 4 or 5 days after ureteral obstruction is consistent with the increased cellularity due to fibroblast proliferation and infiltrating mononuclear cells. Thus, interstitial fibroblasts may contribute to the increase in collagen production in the obstructed kidney of rats with unilateral ureteral obstruction.

Our group[23] and others[24] have reported that α smooth muscle actin (SMA) mRNA and protein and the intermediate filament desmin are upregulated in the obstructed kidney of rats with unilateral ureteral ligation. The overexpression of α-SMA and desmin indicates myofibroblast modulations.

PATHWAYS AND SIGNALS OF INTERSTITIAL FIBROSIS IN OBSTRUCTIVE UROPATHY

A number of cytokines, vasoactive compounds, chemoattractant molecules and growth factors are upregulated after the onset of obstructive uropathy (table I). Several of our studies[23, 25, 26] and other reports[27] suggest than the renin-angiotensin system is activated after ureteral obstruction. Increasing levels of angiotensin II may in turn upregulate the expression of other factors: TGF-β1, tumor necrosis factor-α (TNF-α), osteopontin, VCAM-1, nuclear factor kB (NF-kB), etc.

TRANSFORMINGGROWTH FACTOR β

Transforming growth factor β (TGF-β), a multifunctional cytokine, plays an important role in embryonic development and in regulating repair and regeneration following tissue injury[28]. It is also involved in angiogenesis, regulation of inflammation, integrin expression, protease activity and apoptosis[29]. Five distinct isoforms of TGF-β have been described and three of these, TGF-β1, TGF-β2 and TGF-β3, are found in all mammalian tissues. TGF-β1 is synthesized as a 391-amino acid precursor molecule that is cleaved to a 112-amino active subunit. Biologically active TGF-β1 as a 25-kD dimeric protein composed of two subunits linked by a disulfide bond, and is secreted in an inactive (latent) form that requires processing before it can exert its effect. Latent TGF-β1 is stored at the cell surface and in the extracellular matrix and is converted to active TGF-β1 by mechanisms not well understood[30]. Active TGF-β1 has a major role in tubulointerstitial fibrosis (fig. 1). It increases matrix synthesis, inhibits matrix degradation, upregulates the integrin-matrix adhesion factors, and it is also a chemoattractant for macrophages and fibroblasts. TGF-β1 affects a wide variety of proteins found in the extracellular matrix including fibronectin, collagen types I, II, III, IV and V, thrombospondin, osteopontin, tenacin, elastin, hyaluronic acid, osteonectin, SPARC, and proteoglycans, such as biglycan and decorin. TGF-β1 inhibits matrix degradation by increasing the activity of tissue inhibitors of metalloproteinases and decreasing the activity of metalloproteinases. It also stimulates the synthesis of receptors for ECM proteins (fig. 1).

Furthermore, TGF-β1 is a chemoattractant for fibroblasts and stimulates fibroblast proliferation[22]. Thus, this cytokine has a major role in the accumulation of ex-

Tabla I. Factors with increased expression in the ureteral obstructed kidney

1. Transforming growth factor β (TGF-β1)
   - Protein 53 (p53)
   - Protein 21 (p21, WAF-1)
   - Tissue inhibitor of metalloproteinases-1 (TIM-1)
   - Decorin
2. Nuclear factor KB9 NF-KB)
3. Tumor necrosis factor α (TNF-α)
4. Chemoattractants
   - Monocyte chemoattractant peptide 1 (MCP-1)
   - Osteopontin
5. Adhesion Proteins
   - Intercellular adhesion molecule-1 (ICAM-1)
   - Vascular cell adhesion molecule 1 (VCAM-1)
   - Fibronectin alternate splice forms
6. Proto-oncogenes
   - c-fos, c-jun, jun B, c-myc, cH-Ras
7. Growth factors
   - Interleukin-6 (IL-6)
   - Platelet activating factor (PAF)
   - Basic fibroblast growth factor (BFG)
8. Vasoactive compounds
   - Angiotensinogen
   - Angiotensin II
   - Endothelin
   - Thromboxane A_2
   - Prostaglandins
9. Proteins involved in apoptosis
   - Clusterin (SGP-2)
   - Osteopontin
10. Matrix/basement membrane proteins
    - Collagen types I, III and IV

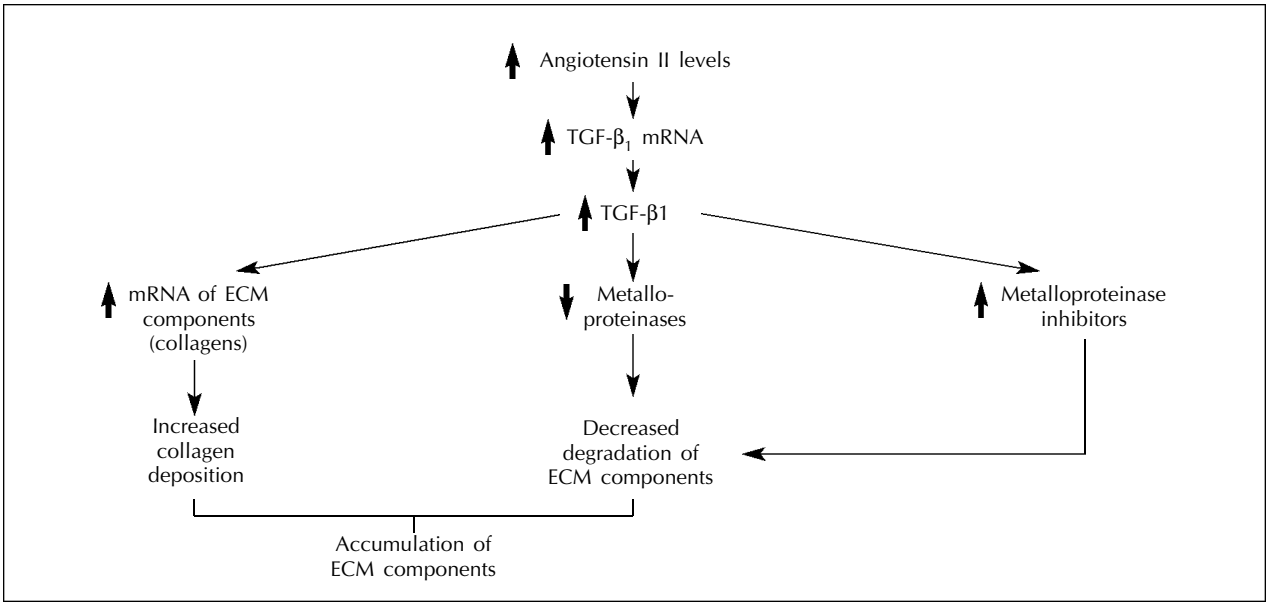

Fig. 1.—Proposed pathogenesis of interstitial fibrosis in obstructive nephropathy. Abbreviations are: TGF-β1, transforming growth factor-β1; ↑, increase; ↓, decrease; ECM, extracellular matrix. Adapted from Klahr et al.[28].

tracellular matrix protein in the renal interstitium. While several cytokines may initiate fibrogenesis, TGF-β1 is considered to be a major stimulating factor. Angiotensin II increases the expression of TGF-β1 mRNA in rat aortic smooth muscle cells[31]. Therefore, we examined the expression of TGF-β1 mRNA in the renal cortex of rats with unilateral ureteral obstruction to determine whether or not angiotensin II increases TGF-β1 mRNA[20]. TGF-β1 mRNA levels did not change significantly during 14 days of obstruction in the contralateral kidneys of rats with unilateral ureteral obstruction, whereas in the obstructed kidneys TGF-β1 mRNA levels were increased significantly after 3 days as compared to the levels in control (unoperated rats) kidneys. The increase in TGF-β1 mRNA in the obstructed kidney cortex was confined to tubular cells. An angiotensin-converting enzyme (ACE) inhibitor (enalapril) significantly blunted but did not completely abrogate the increase of TGF-β1 mRNA[17]. These data indicate that in obstructive uropathy, TGF-β1 is increased at the transcriptional level and thus may play an important role in initiating fibrogenesis in this model of renal disease. In turn, angiotensin II appears to have an important role in stimulating TGF-β1 expression in obstructive uropathy.

METALLOPROTEINASES AND TISSUE INHIBITORS OF METALLOPROTEINASES

The extracellular matrix is a dynamic compartment undergoing both synthesis and degradation. Matrix metalloproteinases (MMP) are enzymes that can degrade collagen and non-collagenous components of the extracellular matrix[9]. The activity of MMP is partially controlled by tissue inhibitors of metalloproteinases (TIMP). The family of TIMPs comprises three proteins designated TIMP-1, TIMP-2 and TIMP-3. Our laboratory[32] and that of Diamond[33] have examined the expression of TIMP mRNA in the setting of obstructive uropathy.

There was a significant increase in TIMP-1 mRNA expression in the obstructed kidney of the rat after 12 hours of obstruction. After 4 days of obstruction there was a 30-fold increase in TIMP-1 mRNA in the experimental kidney when compared to the contalateral unobstructed kidney. TGF-β may promote fibrosis by inhibiting extracellular metalloproteinases and upregulating TIMP-1 mRNA and its resulting protein. Engelmeyer et al.[33] reported a significant decrease in TIMP-3 mRNA in the cortex of the obstructed kidney, but the biological implications of this finding have not been elucidated.

PROTEINS 53, 21 (WAF-1) AND GADD45

Several pathologic settings requiring DNA replication and/or repair are dependent upon the expression of protein 53 (p53) and at least one of two other p53-dependent proteins, p21 (also known as WAF-1) and GADD45. An interesting common effect of p53 and TGF-β is that they independently induce the

production of p21, an inhibitor of cyclin-dependent kinases. We found a progressive increase in the amount of p53 mRNA and p21 mRNA at 1, 3, 5 and 8 days of ureteral obstruction[34]. The amount of GADD45 did not change. Thus, during unilateral ureteral obstruction the p53 and p21 (WAF-1) genes are activated. Protein 53 is associated with cell proliferation, DNA repair, the maintenance of DNA integrity and the regulation of apoptosis[35].

TUMOR NECROSIS FACTOR-α

Tumor necrosis factor-α (TNF-α) is a proinflammatory peptide (17 kDa-157 amino acids) produced by monocytes-macrophages and resident renal cells[36, 37]. TNF-α, which originates from a precursor polypeptide that is anchored to the plasma membrane, has a molecular weight of 26 kDaB (TNF-R1) and the other with a molecular weight of 75 kDa (TNF-R2)[38]. Binding of TNF-α to its receptors activates a number of signal transduction pathways that result in the expression of a variety of transcription factors, cytokines, growth factors, receptors, cell adhesion molecules, mediators of inflammatory processes, acute phase proteins, and major histocompatibility complex proteins[38]. Lipopolysaccharide (LPS)-induced renal injury is associated with increased expression of TNF-α by renal cells. Proximal tubular cells express TNF-α when stimulated with interleukin-1α or LPS[39]. Also, mRNA transcripts of TNF-α occur in cortical tubules of mice injected with LPS. The above data indicate that resident renal cells, that is, glomerular mesangial cells and tubular epithelial cells, are sources of TNF-α production in renal injury. In normal rats TNF-α mRNA was more abundant in glomeruli that in renal cortical tubules[40]. We measured TNF-α mRNA in the renal cortex of rats at different times after the onset of unilateral ureteral obstruction and determined whether angiotensin II inhibition or total body irradiation affected the mRNA levels of TNF-α[40]. Cortical tubules obtained from the kidney with ureteral obstruction showed a remarkable increase in TNF-α mRNA expression, whereas the glomeruli obtained from the same kidneys did not. Thus, upregulation of TNF-α expression was confined to renal tubular cells of the obstructed kidney. It must be stressed that in this study, we only determined changes in TNF-α mRNA content. It remains to be determined if these changes in mRNA correlate with similar changes in the expression of TNF-α protein. Levels of TNF-α mRNA increased significantly in the obstructed kidney at 1 hour (x 2), 2 hours (x 2.7), 4 hours (x 3.6), 24 hours (x 2.7), 72 hours (x 1.8) and 120 hours (x 2.8) after ureteral ligation when compared to the contralateral kidney of the same animals or to the control kidney of normal rats. Treatment with enalapril, an ACE inhibitor, before and after the onset of unilateral ureteral obstruction decreased TNF-α mRNA levels in the obstructed kidney by about 40% at 4 hours after the onset of obstruction, but at 120 hours there was no difference in TNF-α levels between the obstructed kidneys of treated or untreated animals. Total body irradiation, which prevents the migration of macrophages to the obstructed kidney, did not affect the upregulation of TNF-α mRNA expression at 4 hours after unilateral ureteral obstruction. Thus, TNF-α may have a role in initiating tubulointerstitial injury in the obstructed kidney. Leukocytes, infiltrating the renal insterstitium of the obstructed kidney, do not appear to contribute to the increased expression of TNF-α mRNA. Angiotensin II may contribute, at least in part, to the early increase in the expression of TNF-α mRNA in the obstructed kidney.

Tumor necrosis factor-α participates in the recruitment of inflammatory cells in animal models of glomerular injury. It stimulates the production of chemotactic factors by resident cells and upregulates MCP-1 mRNA in human mesangial cells. Wolf et al.[41] demonstrated that TNF-α increases RANTES mRNA in a murine mesangial cell line and in vivo in rat kidneys perfused with TNF-α. Mulligan et al.[42] reported that anti-TNF-α or soluble recombinant human TNF receptor 1 blocked the upregulation of intercellular adhesion molecule 1, endothelial leukocyte adhesion molecule 1, and vascular adhesion molecule 1 in nephrotoxic nephritis. The above data support the concept that TNF-α contributes to the increased macrophage migration into the renal interstitium of the affected kidney. We have shown previously that macrophages infiltrated the interstitium of the obstructed kidney cortex at 4 hours after the onset of unilateral ureteral obstruction, and at 24 hours the influx was at a level approximately 10-fold greater than normal. Taken together, these observations suggest than an early increase in TNF-α after ureteral obstruction of the kidney upregulates the production of a chemoattractant(s) for monocytes and contributes to the infiltration of the obstructed kidney by leukocytes.

The binding of TNF-α to a cell surface receptor results in intracellular metabolic changes that mediate apoptotic and necrotic cell death[43, 44]. Although the mechanism of the cytocidal action of TNF-α has not been completely elucidated, TNF-α receptors have been shown to have a sequence similar to Fas antigen receptor, which is considered to mediate apoptosis[45]. Some investigators have reported apoptosis of renal tubular cells in the obstructed kidney one week after ureteral ligation[46, 47]. It remains to be determined whether or not an increase in TNF-α in the obstructed kidney induces apoptosis of tubular cells and results in tubular damage.

NUCLEAR FACTOR-KB

Nuclear factor-KB (NF-kB) participates in the transcriptional regulation of a number of genes in diverse tissues, including the kidney[48, 49]. NF-kB is located in the cell cytoplasm in an inactive form, complexed to an inhibitor (IkB). The activated form of NF-kB is a heterodimer, composed of two proteins, a p65 (also called rel A) and a p50 subunit. Certain compounds [TNF-α, lipopolysaccharide (LPS) or phorbol myristate acetate (PMA)] induce the dissociation of the NF-kB•IkB complex, with subsequent translocation of NF-kB to the nucleus. NF-kB in the nucleus binds to DNA motifs present in the promoter of various genes, particularly those associated with an inflammatory or immune response[49]. NF-kB is activated during experimental ureteral obstruction[50, 51]; however, administration of an ACE inhibitor, enalapril, significantly decreased the activation of NF-kB in the obstructed kidney of rats with ureteral ligation. We found that enalapril significantly decreased the ability or protein extracted from the nucleus to bind to an NF-kB consensus oligonucleotide compared to similar extracts obtained from the kidneys of untreated animals. Both AT_1 and AT_2 receptor antagonists decreased NF-kB activation in the obstructed kidney[52]. It is likely that the macrophage infiltration of the obstructed kidney, mediated by angiotensin II, is dependent on the activation of NF-kB which in turn regulates the synthesis of MCP-1. NF-kB also regulates the synthesis of other proteins, including TNF-a, interleukins (-1α, -2 and -6), inducible nitric oxide, cyclooxygenase-2, adhesion molecules, 5-lipoxygenases and receptor molecules. Thus, NF-kB may have a major role in the interstitial fibrosis of obstructive uropathy. Angiotensinogen (the precursor of angiotensin II) transcription in the liver is increased by TNF-α and by an NF-kB-like protein binding to an inducible enhancer called the acute phase response element[53]. Transcriptional control of angiotensinogen in hepatocytes is an important regulator of circulating concentrations of this precursor of angiotensin II.

EFFECT OF PHARMACOLOGIC INTERVENTIONS ON THE INTERSTITIAL FIBROSIS OF OBSTRUCTIVE NEPHROPATHY

Both an ACE inhibitor (enalapril) and an angiotensin II receptor (AT_1) antagonist (SC51316) ameliorated the increase in interstitial volume and attenuated the increased expression of TGF-β1 in tubular cells, the increased production of extracellular matrix protein, the activation of NF-kB, the proliferation of fibroblasts and the conversion of their phenotype to myofibroblasts (table II)[52]. A monocyte/macrophage infiltrate was present in the obstructed kidney of untreated rats and the obstructed kidney of rats treated with the angiotensin II receptor antagonists. By contrast, this infiltrate was markedly decreased in the obstructed kidney of rats treated with the ACE inhibitor. The reason for this difference appears to be the greater generation of nitric oxide due to increased levels of bradykinin as a consequence of ACE inhibition[54]. In fact, cotreatment of rats with unilateral obstruction with an ACE inhibitor and L-NAME (an inhibitor of nitric oxide formation) reversed the beneficial effect of enalapril in the obstructed kidney. Administration of L-arginine in the drinking water significantly blunted the increases in interstitial volume, monocyte infiltration, interstitial collagen IV and α-smooth muscle actin expresión[54, 55]. However, in contrast to ACE inhibitor, arginine administration did not decrease the expression of TGF-β1 mRNA in the obstructed kidney of rats with unilateral ureteral ligation[54]. We also found a 10-fold increase in TIMP-1 mRNA in the obstructed kidney. Administration of an ACE inhibitor blunted this increase, by 40% ($p < 0,001$). The addition of L-NAME to the ACE inhibitor prevented the decrease in TIMP-

Tabla II. Effect of ACE inhibitors or angiotensin II receptor antagonists on various parameters associated with renal interstitial fibrosis in obstructive nephropathy

Parameter	Untreated	ACE Inhibitor	AT-1 Receptor Antagonist	AT-2 Receptor Antagonist
Interstitial volume	++++	+	+	++++
Monocytes/Macrophages	++++	+	++++	++++
Transforming growth factor β1	++++	+	+	+++
Fibroblast proliferation	++++	+	+	++++
Myofibroblast phenotype	++++	+	+	+
Clusterin	+++	+	++++	+
NF-kB activation	++++	+	+	++

Values represent a semiquantitative assessment of response during obstructive nephropathy. A value of (++++) is the maximum response. A value of (0) would indicate no change from normal; however, in each instance there is an increase in that parameter from normal.

1 mRNA. A common denominator of the beneficial effects of enalapril and arginine and the deleterious effects of L-NAME cotreatment with enalapril may be the generation of nitric oxide[54].

In many of the above studies the ACE inhibitor was administered prior to or concomitant with the onset of ureteral obstruction. We also have examined the effects of inhibiting angiotensin II formation in rats after 4 or 6 days of unilateral ureteral obstruction. Delayed administration of an ACE inhibitor was shown to slow and in several instances to halt the progression of fibrosis in the tubulointerstitium of the kidney with ureteral ligation[26].

Some transcription factors, such as NF-kB, may be influenced by the reduced/oxidized glutathione level of the cell. The possibility that in obstructive nephropathy, oxidants generated by infiltrating leukocytes and intrinsic renal cells may account for some of the functional and morphologic changes observed should be considered. Modi et al.[55] found that kidneys from rats with bilateral ureteral obstruction had higher levels of malondialdehyde, an index of lipid peroxidation, a greater number of infiltrating leukocytes in the cortex, decreased levels of reduced flutathione and increased levels of oxidized glutathione. In contrast, the kidneys of rats with ureteral obstruction given probucol, an antioxidant and lipid-lowering agent, had significantly higher levels of reduced gluthatione and a lesser number of infiltrating leukocytes[55]. Ricardo et al.[56] found a significant decrease in the mRNA for copper, zinc superoxide dismutase (Cu, Zn-SOD) and catalase with increased levels of superoxide anion and hydrogen peroxide at 24 and 96 hours in kidney slice cultures after the onset of ureteral ligation. There was decreased staining for Cu, Zn-SOD and catalase protein in the tubules of the obstructed kidney compared with specimens of the contralateral kidneys. Also, stimulation of isolated proximal tubules with exogenous TGF-β1 and interleukin-1 was found to decrease catalase mRNA when compared with control proximal tubules. This decrease in catalase activity, coupled with an increase in oxidant stress in the renal cortex after ureteral ligation may contribute to the inflammation and subsequent tubulointerstitial fibrosis of obstructive nephropathy[56]. In preliminary experiments we also found[57] that N-acetylcysteine administration to rats with ureteral obstruction blunted the activation of NF-kB seen in the obstructed kidney. In addition, N-acetylcysteine administration significantly decreased MCP-1 and VCAM-1 mRNA expression and the infiltration of monocytes in the obstructed kidney[19].

Bibliografía

1. Klahr S: Urinary tract obstruction, in *Disease of the Kidney* (6th ed), edited by Schrier RW, Gottschalk CW, Boston, Little, Brown, 709-738, 1997.
2. National Kidney and Urologic Diseases Advisory Board: The scope and impact of kidney and urologic diseases, in *Long-Range Plan*, Chapter 1, Washington DC, National Institutes of Health, NIH Publication 90-583, 7-35, 1990.
3. United States Renal Data System 1996 Annual Data Report II: Incidence and prevalence of ESRD. *Am J Kidney Dis* 18 (Suppl. 2): S34-S47, 1996.
4. Peters CA: Obstruction of the fetal urinary tract. *J Am Soc Nephrol* 8: 653-663, 1997.
5. Striker GE, Schainuck LI, Cutler RE, Benditt EP: Structural-functional correlations in renal diseases I. A method for assaying and classifying histopathologic changes in renal disease. *Hum Pathol* 1: 615-630, 1970.
6. Schainuck LI, Striker GE, Cutler RE, Benditt EP: Structural-functional correlations in renal disease II: The correlations. *Hum Pathol* 1: 631-641, 1970.
7. Bohle A, Von Gise H, Mackensen-Haen S, Stark-Jakob B: The obliteration of the postglomerular capillaries and its influence upon the function of both glomeruli and tubuli. *Klin Wochensch* 59: 1043-1051, 1981.
8. Bohle A, Mackensen-Haen S, Gise H y cols.: The consequences of tubulointerstitial changes for renal function in glomerulopathies, in *tubulointerstitial Nephropathies*, edited by Amerio A, Cortelli P, Massry SE, Boston, Kluwer 29-40, 1991.
9. Eddy AA: Molecular insights into renal interstitial fibrosis. *J Am Soc Nephrol* 7: 2495-2508, 1996.
10. Brady HR, Adler S: Cell-cell and cell matrix interactions, in *The Kidney*, edited by Brenner BM, 5th ed, Philadelphia, WB Saunders, 193-210, 1996.
11. Nath KA: Tubulointerstitial changes as a major determinant in the progression of renal damage. *Am J Kidney Dis* 20: 1-17, 1992.
12. Nagle RB, Bulger RE, Cutler RE, Jervis HR, Benditt EP: Unilateral obstructive nephropathy in the rabbit I. Early morphologic, physiologic and histological changes. *Lab Invest* 28: 456-467, 1973.
13. Nagle RB, Bulger RE: Unilateral obstructive nephropathy in the rabbit II. Late morphologic changes. *Lab Invest* 38: 270-278, 1978.
14. Sharma AK, Mauer SM, Kim Y, Michael AF: Interstitial fibrosis in obstructive nephropathy. *Kidney Int* 44: 774-780, 1993.
15. Rovin BH, Harris KPG, Morrison A, Klahr S, Schreiner GF: Renal cortical release of a specific macrophage chemoattractant in response to ureteral obstruction. *Lab Invest* 63: 213-220, 1990.
16. Diamond JR, Kees-Folts D, Ding G, Fyre JE, Restrepo NC: Macrophages, monocyte peptide-1, and TGF-β1 in experimental hydronephrosis. *Am J Physiol* 266: F926-F933, 1994.
17. Kaneto H, Morrissey J, Klahr S: Increased expression of TGF-β1 mRNA in the obstructed kidney of rats with unilateral ligation. *Kidney Int* 44: 313-321, 1993.
18. Diamond JR, Kees-Folts D, Ricardo SD, Pruznak A, Eufemio M: Early and persistent up-regulated expression of renal cortical osteopontin in experimental hydronephrosis. *Am J Pathol* 136: 1455-1466, 1995.
19. Duan L, Morrissey J, McCracken R, Klahr S: Regulation of MCP-1, ICAM-1 and VCAM-1 during unilateral ureteral obstruction (abstract). *J Am Soc Nephrol* 7: 1697, 1996.
20. Harris KPG, Scheriner GF, Klahr S: Effect of leukocyte depletion of the function of the post-obstructed kidney in the rat. *Kidney Int* 36: 210-215, 1989.

21. Kaneto H, Morrissey J, McCracken R, Rayes A, Klahr S: Enalapril reduces collagen type IV synthesis and expansion of the interstitium in the obstructed kidney. *Kidney Int* 45: 1637-1647, 1994.
22. Kuncio GS, Neilson EG, Haverty T: Mechanisms of tubulointerstitial fibrosis. *Kidney Int* 39: 550-556, 1991.
23. Ishidoya S, Morrissey J, McCracken R, Reyes A, Klahr S: Angiotensin II receptor antagonist ameliorates renal tubulointerstitial fibrosis caused by unilateral ureteral ligation. *Kidney Int* 47: 1285-1294, 1995.
24. Diamond JR, Van Goor H, Ding G, Engelmeyer E: Myofibroblasts in experimental hydronephritis. *Am J Pathol* 146: 121-129, 1995.
25. Klahr S, Ishidoya S, Morrissey J: Role of angiotensin II in the tubulointerstitial fibrosis of obstructive nephropathy. *Am J Kidney Dis* 26: 141-146, 1995.
26. Ishidoya S, Morrissey J, McCracken R, Klahr S: Delayed treatment with enalapril halts tubulointerstitial fibrosis in rats. *Kidney Int* 49: 1110-1119, 1996.
27. Pimentel JL, Martínez-Maldonado M, Wilcox JN, Wang S, Luo C: Regulation of renin-angiotensin system in unilateral ureteral obstruction. *Kidney Int* 44: 390-400, 1993.
28. Border WA, Ruoslahti E: Transforming growth factor β in disease: the dark side of tissue repair. *J Clin Invest* 90: 1-7, 1992.
29. Munger JS, Harpel JG, Gleizes PE, Mazzieri R, Nunes I, Rifkin DB: Latent transforming growth factor β: structural features and mechanisms of activation. *Kidney Int* 51: 1376-1382, 1997.
30. Böttinger EP, Letterio JJ, Roberts AR: Biology of TGF-β in knockout and transgenic mouse models. *Kidney Int* 51: 1355-1360, 1997.
31. Gibbons GH, Pratt RE, Dzau VJ: Vascular smooth muscle hypertrophy. Autocrine transforming growth factor β1 expression determines growth response to angiotensin II. *J Clin Invest* 90: 451-461, 1992.
32. Morrissey J, Ishidoya S, McCracken R, Klahr S: The effect of ACE inhibitors on the expression of matrix genes and the role of p53 and p21 (WAF1) in experimental renal fibrosis. *Kidney Int* 49 (Suppl. 54): S83-S87, 1996.
33. Engelmeyer E, Van Goor H, Edwards DR, Diamond JR: Differential mRNA expression of renal cortical tissue inhibitor of metalloproteinase 1, -2, -3 in experimental hydronephrosis. *J Am Soc Nephrol* 5: 1675-1683, 1995.
34. Morrissey J, Ishidoya S, McCracken R, Klahr S: Control of p53 and p21 (WAF1) expression during unilateral ureteral obstruction. *Kidney Int* 50 (Suppl. 57): S84-S92, 1996.
35. Hartwell LH, Kastan MB: Cell cycle control and cancer. *Science* 26: 1821-1828, 1994.
36. Egido J, Gómez-Chiarri M, Ortiz A, Bustos C, Alonso J, Gómez-Guerrero C, Gómez-Garre P, López-Armada MJ, Plaza J, González E: Role of tumor necrosis factor-α in the pathogenesis of glomerular diseases. *Kidney Int* 43 (Suppl. 39): S59-S64, 1993.
37. Baud L, Fouqueray B, Philippe C, Amram A: Tumor necrosis factor alpha and mesangial cells. *Kidney Int* 41:600-603, 1992.
38. Ortiz A, Bustos C, Alcázar A, López-Armada MJ, Plaza JU, González E, Egido J: Involvement of tumor necrosis factor α in the pathogenesis of experimental and human glomerulonephritis, in *Advances in Nephrology*, edited by Grunfeld JP, Bach JF, Kreis H, Maxwell MH, St. Louis, Mosby, 53-77, 1995.
39. Wuthrich RP, Glimcher LH, Yui MA, Jevnikar AM, Dumas SE, Kelley VE: MHC class II antigen presentation and tumor necrosis factor in renal tubular epithelial cells. *Kidney Int* 37: 783-792, 1990.
40. Kaneto H, Morrissey J, McCracken R, Ishidoya S, Reyes A, Klahr S: The expression of mRNA for tumor necrosis factor α increases in the obstructed kidney of rats soon after unilateral ureteral ligation. *Nephrology* 2: 161-166, 1996.
41. Wolf G, Aberle S, Thaiss F, Nelson PJ, Kaensky AM, Neilson EG, Strahl RA: TNF-α induces expression of the chemoattractant cytokine RANTES in cultured mouse mesangial cells. *Kidney Int* 44: 795-804, 1993.
42. Mulligan MS, Johnson KJ, Todd RF, Issekutz TB, Miyasaka M, Tamatani T, Smith CW, Anderson DC, Ward PA: Requirements for leukocyte adhesion molecules in nephrotoxis nephritis. *J Clin Invest* 91: 577-587, 1993.
43. Larrick JW, Wright SC: Cytotoxic mechanism of tumor necrosis factor-α. *FASEB J* 4: 3215-3223, 1990.
44. Laster SM, Wood JG, Gooding LR: Tumor necrosis factor can induce both apoptotic and necrotic forms of cell lysis. *J Immunol* 141: 2629-2634, 1988.
45. Itoh N, Yonehara S, Ishii A, Yonehara M, Mizushima SI, Shameshima M, Hase A, Seto Y, Nagata S: The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell* 66: 233-243, 1991.
46. Gobe GC, Axelsen RA: Genesis of renal tubular atrophy in experimental hydronephrosis in the rat. Role of apoptosis. *Lab Invest* 56: 273-281, 1987.
47. Kennedy WA II, Stenberg A, Lackgrew G, Hensle TW, Sawezuki IS: Renal tubular apoptosis after partial ureteral obstruction. *J Urol* 152: 658-664, 1994.
48. Baeurle PA, Henkel T: Function and activation of NF-kB in the immune system. *Annu Rev Immunol* 12: 141-179, 1994.
49. Barnes PJ, Karin M: Nuclear factor-kB—a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 336: 1066-1071, 1997.
50. Wendt T, Zhang YM, Bierhaus A, Kriegsmann J, Deng Y, Waldherr R, Teske T, Luther T, Fünfstuk R, Nawroth PP, Stein G: Tissue factor expression in an animal model of hydronephrosis. *Nephrol Dial Transplant* 10: 1820-1828, 1995.
51. Morrissey J, Klahr S: Enalapril decreases nuclear factor kB activation in the kidney with ureteral obstruction. *Kidney Int* 52: 926-933, 1997.
52. Klahr S, Morrissey J: Comparative study of ACE inhibitors and AII receptor antagonists in interstitial scarring. *Kidney Int* 52 (Suppl. 63): S111-S114, 1997.
53. Brasier AR, Li J, Wimbish KA: Tumor necrosis factor activates angiotensinogen by the Rel A transactivator. *Hypertension* 27: 1009-1017, 1996.
54. Morrissey J, Ishidoya S, McCracken P, Klahr S: Nitric oxide generation ameliorates the tubulointerstitial fibrosis of obstructive nephropathy. *J Am Soc Nephrol* 7: 2202-2212, 1996.
55. Modi KS, Morrissey J, Shah SV, Schreiner GF, Klahr S: Effects of probucol on renal function in rats with bilateral ureteral obstruction. *Kidney Int* 38: 843-850, 1990.
56. Ricardo SK, Ding G, Eufemio M, Diamond JR: Antioxidant expression in experimental hydronephrosis: a role of mechanical stretch and growth factors. *Am J Physiol 272 (Renal Fluid Electrol Physiol 41)* F789-F798, 1997.
57. Morrissey J, Duan L, Klahr S: N-acetylcysteine treatment alters nuclear transcription factor activity and monocyte infiltration during ureteral obstruction (abstract). *J Am Soc Nephrol* 7: 1830, 1996.