Mechanisms of Disease

PATHOPHYSIOLOGY OF PROGRESSIVE NEPHROPATHIES

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IN patients with renal diseases characterized by proteinuria, the initial insult to the kidney is usually followed by a progressive decline in the glomerular filtration rate. This decline has been thought to be due to changes in renal hemodynamics initiated by the loss of nephrons.[1] When renal mass is reduced in rats, the remaining nephrons undergo sudden hypertrophy, with a concomitant lowering of arteriolar resistance and an increase in glomerular plasma flow.[2,3] Afferent arteriolar tone decreases more than efferent arteriolar tone, and therefore, the hydraulic pressure in glomerular capillaries rises[4] and the amount of filtrate formed by each nephron increases. These changes increase the filtration capacity of the remaining nephrons, thus minimizing the functional consequences of nephron loss, but they are ultimately detrimental.[5] Therapies that attenuate these adaptive changes limit the decline in the glomerular filtration rate and minimize structural damage. For example, angiotensin-converting–enzyme (ACE) inhibitors, which reduce intraglomerular capillary pressure more effectively than other antihypertensive drugs, consistently protected rats with reduced renal mass[6,7] or diabetes mellitus[8,9] from progressive renal injury.

Why should hemodynamic changes — specifically, glomerular hypertension — lead to progressive renal injury? One possible explanation is that the high glomerular capillary pressure enlarges the radius of the pores in the glomerular membrane by a mechanism that is mediated at least in part by angiotensin II.[10,11] This enlargement impairs the size-selective function of the membrane so that the protein content of the glomerular filtrate increases, which in turn increases the endocytosis of protein by tubular epithelial cells, ultimately resulting in a nephritogenic effect.[12] A vicious circle is then established in which changes in renal hemodynamics due to the loss of nephrons lead first to proteinuria and then to the loss of more nephrons.

We will review recent data suggesting that proteins filtered by the glomerulus cause injury of the tubulointerstitium, leading to parenchymal damage and, ultimately, renal scarring and insufficiency (Fig. 1). We will also review experimental and clinical evidence linking the renoprotective effects of ACE inhibitors to their ability to reduce the levels of protein in the glomerular filtrate, as well as the predictive value of the degree of proteinuria in determining the rate of progression of chronic renal diseases characterized by abnormal loss of proteins into the urine — that is, proteinuric nephropathies.

ACTIVATION OF LOCAL HORMONES AND INFLAMMATORY CHEMOKINES BY FILTERED PROTEIN

Evidence from Animal Studies

That increased glomerular filtration of protein accelerates the natural slow, progressive loss of nephrons that occurs in all chronic renal diseases was initially suggested by studies in animals. Analysis of renal-biopsy specimens from rats with doxorubicin-induced nephrosis[13] or age-related proteinuria[14] showed the accumulation of filtered proteins in the cytoplasm of proximal tubular cells, causing breaks in their basement membrane and extravasation of their contents into the interstitium, followed by an inflammatory reaction and tubulointerstitial and glomerular lesions.[12] In subsequent studies, in rats given repeated intravenous injections of albumin, glomerular filtration of albumin was consistently followed by the development of interstitial lesions and scarring.[15] The sequence of events and nature of the renal lesions were virtually identical to those in rats with toxic or immune[13] proteinuric nephropathy, suggesting a common pathway of injury.

Filtered proteins are reabsorbed by proximal tubular cells, in which they are degraded by lysosomes. Albumin is taken up by a dual pathway: a low-capacity one that reabsorbs physiologic amounts, and a high-capacity one that comes into play when the concentration in tubular fluid is high.[16]

Proximal tubular cells can change their phenotype in response to protein overload.[17] In cultured proximal tubular cells, increasing concentrations of delipidated albumin, IgG, or transferrin cause concentration-dependent increases in the rate of synthesis of

Renal injury

Decreased nephron mass

Increased generation
of angiotensin II

Glomerular-capillary
hypertension

Increased glomerular permeability
to macromolecules

Up-regulation of
$TGF\text{-}\beta 1$ gene

Increased filtration
of plasma proteins → Proteinuria

Excessive tubular
reabsorption of protein

Tubular-cell
hypertrophy

Abnormal accumulation
of protein in endolysosomes
and endoplasmic reticulum

Nuclear signals for NF-κB–dependent
and NF-κB–independent vasoactive
and inflammatory genes

Release of vasoactive and
inflammatory substances into
the interstitium

Increased synthesis
of type IV collagen

Transformation
of tubular cells to
fibroblasts

Fibroblast
proliferation

Interstitial
inflammatory
reaction
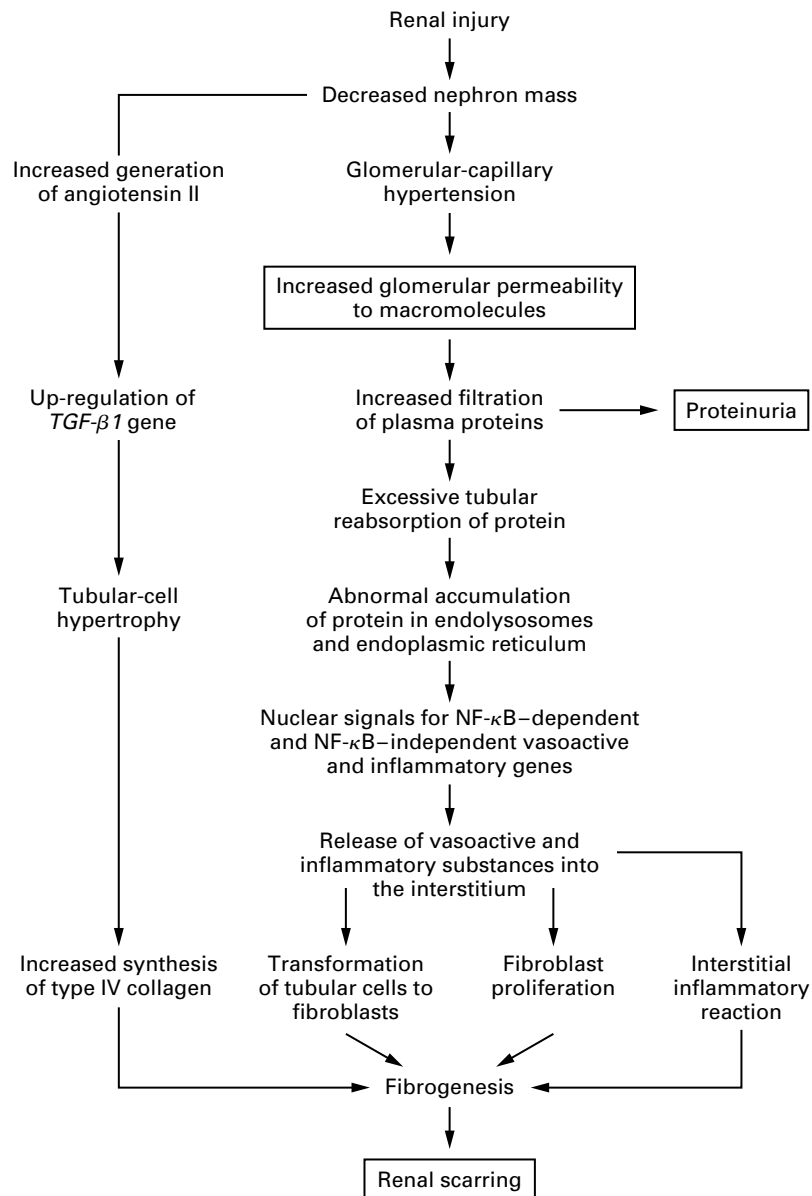
Fibrogenesis

Renal scarring

Figure 1. Effect of Increased Glomerular Permeability to Proteins on Progressive Renal Injury.

Excessive reabsorption of protein as a consequence of increased glomerular permeability results in the accumulation of proteins in proximal tubular cells and may trigger the activation of genes encoding endothelin, chemokines, and cytokines that are either dependent on nuclear factor κB (NF-κB) or independent of this factor. Excessive synthesis of these or other vasoactive and inflammatory substances contributes to the proliferation of fibroblasts and interstitial inflammation, ultimately leading to increased synthesis of extracellular matrix and renal scarring. Moreover, the increase in the synthesis of angiotensin II by the kidney as a result of injury leads to an increase in the expression of the gene for transforming growth factor β1 (*TGF-β1*) in tubular cells, eventually inducing hypertrophy of these cells and increasing the synthesis of type IV collagen (fibrogenesis).

endothelin-1.[18] Similarly, albumin and transferrin stimulate transcription of the gene for monocyte chemoattractant protein 1 in these cells,[19] an effect that can be prevented by the addition of lysine, which inhibits luminal protein uptake,[20] or dactinomycin, which inhibits gene transcription. Albumin also stimulates these cells to produce RANTES (regulated upon activation normal T-cell expressed and secreted),[21] an immunoregulatory cytokine with chemotactic properties for monocytes and memory T cells.[22]

Tubular epithelial cells in vivo are organized as a continuous polarized layer with highly specialized luminal and basolateral compartments. In cultured cells that maintain their polarized organization, endothelin-1, monocyte chemoattractant protein 1, and RANTES are mainly secreted into the basolateral compartment in response to protein overloading[18,19,21] (Fig. 2). If a similar pattern of secretion occurred in vivo, these substances could be released into the interstitium to promote the migration of macrophages and T lymphocytes.[23] The interstitial accumulation of chemokines could induce the proliferation of fibroblasts and increased synthesis of extracellular matrix as well as inflammation.

Evidence from Animal Models of Human Diseases

In rats with proteinuric renal disease, the synthesis of genes in renal tissue that encode vasoactive and inflammatory substances is consistently increased, as are the synthesis of the corresponding proteins and urinary excretion of these proteins. Thus, in rats in which renal mass is reduced by surgical removal of the right kidney and ligation of two or three branches of the left renal artery (remnant kidney)[24] or in rats with Heymann's nephritis, a form of membranous glomerulopathy in which glomerular injury is induced by heterologous antibodies against Fx1A antigen,[25] the expression and urinary excretion of renal endothelin-1 are increased, and the extent of the increases correlates with the rate of progression of renal damage. In renal tissue from rats with Heymann's nephritis, endothelin-1 messenger RNA (mRNA) is increased, proximal tubular cells contain large amounts of immunoreactive endothelin-1 (mostly basolaterally), and endothelin-1 can be detected in mononuclear cells infiltrating the interstitium.[26] Transgenic mice bearing the human endothelin-1 gene have interstitial renal disease and fibrosis.[27] Rats with protein-overload proteinuria have increased renal monocyte chemoattractant protein 1 mRNA and increased amounts of this protein in proximal tubules.[28]

Similarly, renal RANTES mRNA is increased in proteinuric mice, and the secondary interstitial inflammatory reaction is reduced by the selective receptor antagonist MetRANTES.[29,30] In other studies in rats with a remnant kidney or Heymann's nephritis, excessive reabsorption of proteins in the proximal tubules has been associated with an increase in the proinflammatory glycoprotein osteopontin in the kidneys.[31,32] Immunohistochemical studies of proximal tubules revealed osteopontin and reabsorbed IgG in the same cells.[31] Cells overloaded with IgG and osteopontin were surrounded by inflammatory infiltrates.

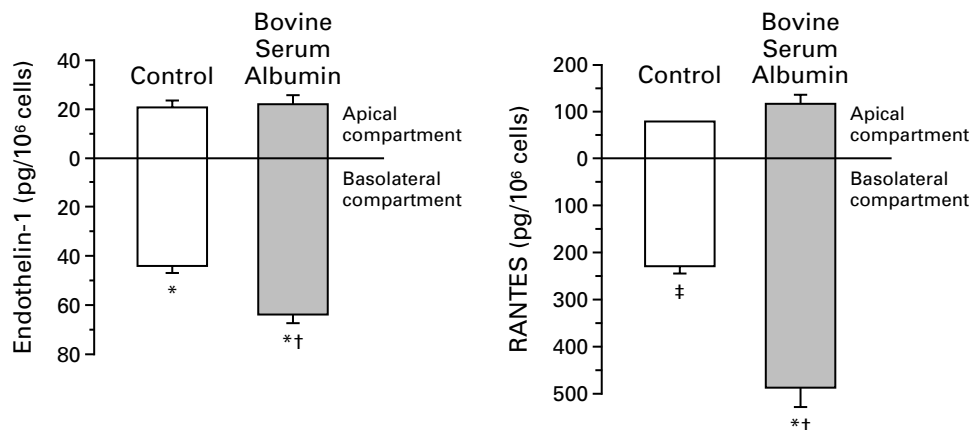How does the excessive concentration of proteins

Figure 2. Polarized Secretion of Endothelin-1 and the Immunoregulatory Cytokine RANTES (Regulated upon Activation Normal T-Cell Expressed and Secreted) by Proximal Renal Tubular Cells after Luminal Exposure to Bovine Serum Albumin.

For the study of endothelin, cells were exposed to bovine serum albumin (10 mg per milliliter) for 24 hours, and for the study of RANTES, cells were exposed for 48 hours. Values are means ±SE. Asterisks indicate P<0.01 for the difference between the apical compartment and the basolateral compartment, the daggers indicate P<0.01 for the difference between the control basolateral compartment and the bovine serum albumin basolateral compartment, and the double dagger indicates P<0.05 for the difference between the apical compartment and the basolateral compartment.

in cell cytoplasm and organelles lead to up-regulation of vasoactive and inflammatory genes? In virus-infected cells,[33] in which large amounts of viral protein are synthesized, the accumulation of viral protein in the endoplasmic reticulum activates the transcription factor nuclear factor κB (NF-κB).[34] This factor in turn activates interferon and cytokine genes, whose products have antiviral actions. A similar mechanism may be operative in tubular epithelial cells overloaded with reabsorbed proteins. In cultured proximal tubular cells, increasing concentrations of albumin cause a dose-dependent increase in NF-κB that is abolished by blocking the degradation of IκB, a cellular protein that binds and inhibits the action of NF-κB.[21] NF-κB is also increased in tubular cells incubated with tumor necrosis factor α, a potent stimulus of the expression of NF-κB–dependent inflammatory genes.[35] That protein-induced up-regulation of inflammatory genes in cultured cells is dependent on activation of NF-κB was confirmed by the finding that an antioxidant inhibitor of NF-κB activation[36] prevented the formation of RANTES in proximal tubular cells in response to proteins.[21]

Components of the complement system that are filtered by glomeruli can also cause interstitial injury, as suggested by the deposition of complement C3 on the luminal surface of proximal tubules in rats with protein-overload nephropathy.[15] Similarly, in rats with aminonucleoside-induced nephrosis, C3 and the complement complex C5b–9 were found on the luminal side of proximal tubular cells as early as seven days after injection of the aminonucleoside (puromycin).[37] The complement complex C5b–9 has chemotactic properties, and accumulation of C5b–9 on the plasma membrane of proximal tubular cells is followed by the generation of oxygen free radicals[38] and the recruitment of inflammatory cells into the interstitium. These cells are then activated to secrete transforming growth factor β[39] (Fig. 1). Transforming growth factor β binds to specific receptors on interstitial fibroblasts and induces them to proliferate and secrete matrix components, contributing to interstitial remodeling and scarring.[39,40] Endothelin-1 has similar actions on interstitial fibroblasts.[41]

Remodeling of the interstitial architecture may also occur as a result of transformation of tubular cells, as occurs in mice with anti–basement membrane disease,[42] in which tubular epithelial cells are converted into fibroblasts at the site of injury and induced to express fibroblast-specific proteins. Fibroblast-specific protein 1, a member of the superfamily of intracellular calcium-binding proteins,[42] promotes epithelial and mesenchymal transformation of tubular cells.[43] Angiotensin II, acting through angiotensin II type 1 receptors, also induces hypertrophy in tubular cells by up-regulating the gene for transforming growth factor β1[44] (Fig. 1), which in turns leads to increased synthesis of type IV collagen.[45] In rats, re-

peated infusions of angiotensin II cause interstitial fibrosis and lead to the deposition of type IV collagen[46] — a process that illustrates the morphogenic effect of angiotensin II on tubulointerstitial structures.[47]

Angiotensin II also stimulates the secretion of other peptides with chemotactic properties.[46,48] Conversely, in rats with experimentally obstructed kidneys, angiotensin II antagonists lower concentrations of transforming growth factor β1 mRNA in renal tubular cells and prevent interstitial fibrosis.[49] In addition, when glomerular selectivity is lost, as in proteinuric renal diseases, protein-bound inflammatory cytokines may be filtered, dissociate from their binding proteins because of the low pH of tubular fluid, and cause a second wave of inflammation.[50,51]

LIMITING PROTEIN FILTRATION

If the interstitial inflammatory reaction and consequent fibrosis in chronic proteinuric nephropathies are caused by protein overloading, limiting protein filtration and reabsorption should prevent activation of the tubular cells and renal injury. This is what happens in animals given drugs that improve the size-selective function of the glomerular membrane. After ACE inhibitors were found to have a greater renoprotective effect than other antihypertensive drugs in rats with a remnant kidney,[7] most studies in rats with diabetes mellitus,[8] puromycin-induced and doxorubicin-induced nephropathies,[52] age-induced changes,[53] and Heymann's nephritis[25] confirmed the antiproteinuric and renoprotective properties of this class of compounds. In two studies, however, ACE inhibitors failed to reduce proteinuria or protect the kidneys from injury.[54,55] The fact that ACE inhibitors may not limit renal damage when no substantial antiproteinuric effect is achieved suggests that the renoprotection conferred by this class of drugs is a result of their effect on membrane permeability rather than a result of the morphogenic action of angiotensin II.

In rats with Heymann's nephritis, ACE inhibitors, besides reducing proteinuria and renal damage, also decrease urinary endothelin-1 excretion to levels similar to those in normal rats.[26] In these rats blockade of the synthesis or biologic activity of angiotensin II reduces both the level of transforming growth factor β mRNA in the kidney and the expression of genes for extracellular matrix proteins, such as type IV collagen, laminin, and fibronectin.[56] Limiting protein excretion and the consequent activation of tubular epithelial cells are instrumental in protecting the kidney from further damage in animals.

PROTEIN-DEPENDENT INTERSTITIAL INFLAMMATION IN NEPHROPATHIES

Since Cameron and coworkers[57] reported in 1978 that patients with focal segmental glomerulosclerosis and the nephrotic syndrome had a poor prognosis,

evidence has accumulated that in humans with nephropathy, more severe proteinuria means more rapid progression of disease.[58-61] For example, among 400 patients with nondiabetic proteinuric renal diseases, those with higher rates of urinary protein excretion had more rapid progression of renal disease, independent of its specific cause.[58] Furthermore, there is compelling evidence that in humans with IgA, membranous, or membranoproliferative glomerulopathy, focal glomerulosclerosis, or diabetic nephropathy, increased urinary protein excretion is associated with tubulointerstitial infiltration of monocytes and T lymphocytes,[62-64] which in turn is an indicator of the decline in renal function.[64-66]

In the case of minimal-change disease,[67] glucocorticoid-induced regression of proteinuria prevents interstitial inflammation and deterioration in renal function. Similarly, patients with this disease and the nephrotic syndrome who have only a few relapses are protected from progressive renal damage,[68] whereas focal glomerulosclerosis tends to develop in those who have an initial response to a glucocorticoid but who have frequent relapses.[69-74] In these latter patients, the failure of cytotoxic drugs such as cyclophosphamide to ameliorate proteinuria is associated with an increased risk of renal failure.[74] Finally, in patients who have the nephrotic syndrome due to minimal-change disease that is resistant to treatment and who have permanent proteinuria, renal function inevitably deteriorates over time.[75] In the few such patients who have had repeated renal biopsies, the biopsies showed increasing glomerulosclerosis, with interstitial inflammation and fibrosis.[76] Thus, even though nephrologists have sometimes considered patients with the nephrotic syndrome and minimal-change disease to be an exception to the rule that protein excretion contributes to progressive renal failure, there is ample evidence that the opposite is true. Minimal-change disease is in fact a good example of the close relation between the remission of proteinuria and renoprotection, rather than between the failure to reduce urinary protein excretion and the progression of renal failure.

Among a series of base-line tests, measurement of urinary protein excretion was a strong and independent predictor of renal outcome in 409 patients with type 1 diabetes mellitus and nephropathy[77] and in 840 patients with nondiabetic renal disease at enrollment in the Modification of Diet in Renal Disease Study.[78] Furthermore, the occurrence of proteinuria in 10 to 30 percent of patients with chronic hypertension or diabetes after 10 to 15 years of normal renal function invariably predicts a subsequent decline in the glomerular filtration rate.[79,80] Our group found that regardless of the nature of the underlying disease, base-line urinary protein excretion was the best single predictor of disease progression and end-stage renal disease in 274 patients with nondiabetic proteinuric nephropathies.[81] Over a three-year follow-up period, few patients with urinary protein excretion of less than 2.5 g per 24 hours at base line had a decline in the glomerular filtration rate, whereas those with proteinuria in the nephrotic range (protein excretion, >4.3 g per 24 hours) at base line had a decrease in the glomerular filtration rate of more than 10 ml per minute per 1.73 m² of body-surface area per year.

RATIO OF URINARY PROTEIN TO CREATININE AS A PREDICTOR OF END-STAGE RENAL DISEASE

In patients with chronic proteinuric nephropathies, the ratio of protein to creatinine in a single morning urine specimen correlated with 24-hour urinary protein excretion and predicted the rate of decline in the glomerular filtration rate and the progression to end-stage renal disease better than measurement of 24-hour urinary protein excretion.[82-85] For example, a urinary protein:creatinine ratio of 1.0 or more distinguished patients in whom renal disease subsequently worsened from those in whom it did not (Fig. 3). Patients with a urinary protein:creatinine ratio of less than 1.0 had no decline in the glomerular filtration rate and no end-stage renal disease, whereas those with a ratio of 1.0 or more had a decrease in the glomerular filtration rate during a one-year follow-up period. In this group the higher the ratio, the more rapid the rate of decline in the glomerular filtration rate and the higher the risk of end-stage renal disease. The risk exceeded 30 percent per year in patients with a urinary protein:creatinine ratio of more than 4.0. For these patients, the expected time to dialysis was less than one year (Fig. 3).

EFFECT OF REDUCING PROTEIN EXCRETION ON THE DECLINE IN THE GLOMERULAR FILTRATION RATE

In patients with various renal diseases, reducing urinary protein excretion slows the rate of decline in the glomerular filtration rate,[86] and reducing blood pressure slows the decline in the glomerular filtration rate more in patients with a high rate of urinary protein excretion at base line than in those with a low rate.[78] In patients with diabetic nephropathy, lowering blood pressure with an ACE inhibitor decreases urinary protein excretion and slows the decline in the glomerular filtration rate more than does lowering blood pressure to similar levels with beta-blockers and diuretics.[87] In a study of patients with type 1 diabetes and some renal failure, captopril preserved renal function better than did diuretics, beta-blockers, or other antihypertensive drugs and halved the need for dialysis and transplantation.[88] During follow-up, the reduction in blood pressure was similar in the two groups; urinary protein excretion decreased in the patients treated with captopril and increased in the other groups.[89]
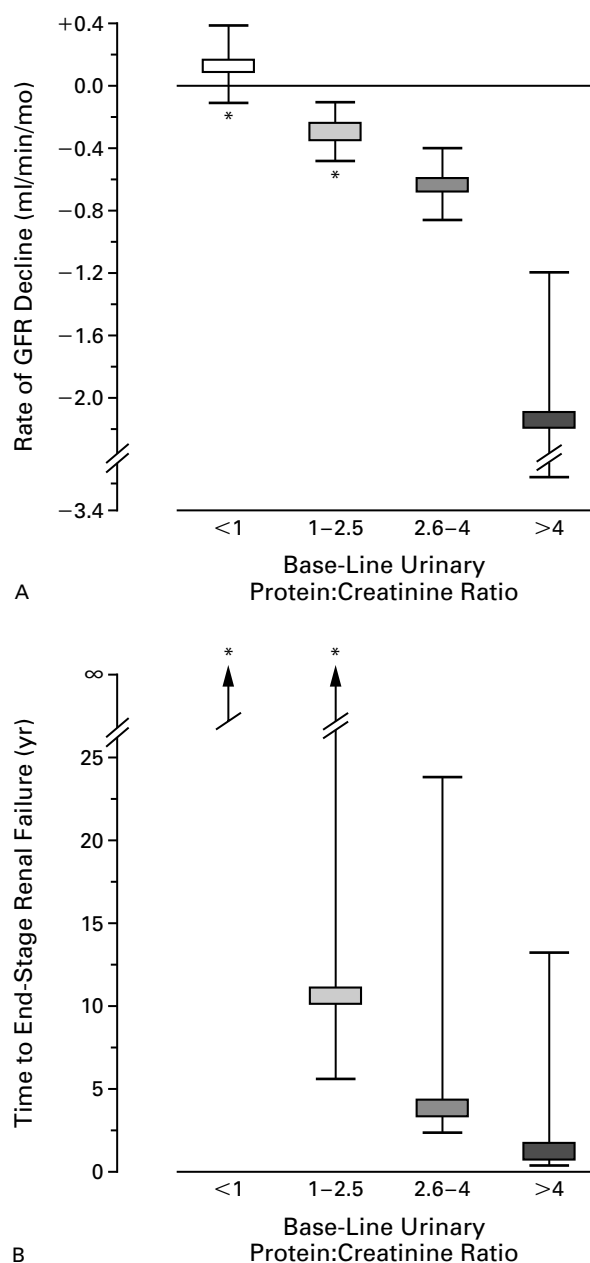
In the Angiotensin-Converting–Enzyme Inhibition

Figure 3. Mean (±SE) Rate of Decline in the Glomerular Filtration Rate (GFR) per Patient per Month (Panel A) and Mean Predicted Time to End-Stage Renal Disease (Panel B), According to Base-Line Ratio of Urinary Protein to Creatinine in 98 Patients with Chronic Nondiabetic, Proteinuric Nephropathies.

Asterisks indicate a significant difference (P<0.05) from a base-line urinary protein:creatinine ratio above 4. The I bars in Panel B are ranges.

in Progressive Renal Insufficiency Study,[90] which included 583 patients with renal insufficiency due to various nephropathies, the risk of a doubling of the serum creatinine concentration was lower in patients who received an ACE inhibitor than in those who received a placebo, but the blood pressure of pa-

tients in the placebo group was also higher. This finding leaves unanswered the question of whether the renoprotective effect of the ACE inhibitor was related to its antiproteinuric effect or to its antihypertensive effect. The majority of the patients in this trial had a urinary protein excretion of less than 2 g per 24 hours and a remarkably slow rate of progression of renal failure. These patients did not benefit from ACE-inhibitor therapy, and its renoprotective value was confined to patients with urinary protein excretion of more than 3 g per 24 hours at base line and in whom urinary protein excretion decreased during treatment.

The Ramipril Efficacy in Nephropathy Study was designed to address formally whether excessive ultrafiltration of proteins influenced the progression of renal disease and whether an ACE inhibitor (ramipril) was superior to placebo and other antihypertensive drugs, at similar levels of blood-pressure control, in reducing proteinuria, limiting the decline in the glomerular filtration rate, and preventing end-stage renal disease in patients with hypertension (87 percent of the patients) and in those without hypertension.[91] In this trial, the mean rate of decline in the glomerular filtration rate in the 90 patients whose urinary protein excretion was 1 to 3 g per 24 hours at base line was one third of that in the 87 patients whose urinary protein excretion was more than 3 g per 24 hours.[92] Among the patients with base-line urinary protein excretion of more than 3 g per 24 hours, the mean (±SE) rate of decline in the glomerular filtration rate was significantly lower in the ramipril group than in the control group (-0.53 ± 0.08 vs. -0.88 ± 0.13 ml per minute per month), and the risk of reaching the combined end point of a doubling in the base-line serum creatinine concentration or end-stage renal disease was also significantly lower in the ramipril group. Systolic and diastolic blood pressures were similar in the two groups at base line and during follow-up (with differences consistently less than 2 mm Hg), providing evidence that ramipril slowed the decline in renal function through a renoprotective effect that was largely independent of changes in blood pressure. On the other hand, the finding that the ramipril-induced reduction in urinary protein excretion was the only time-dependent covariate that predicted a slower rate of decline in the glomerular filtration rate and progression to end-stage renal disease clearly indicated that renoprotection is linked to a reduction in protein excretion.

ROLE OF BLOOD-PRESSURE REDUCTION

The Modification of Diet in Renal Disease Study found a correlation between higher mean blood pressures (for values of more than 98 mm Hg in patients with base-line urinary protein excretion of 0.25 to 3 g per 24 hours and of more than 92 mm Hg in patients with base-line protein excretion of more than

3 g per 24 hours) during follow-up and a faster decline in the glomerular filtration rate that depended on the level of urinary protein excretion at base line. In addition, the decline in the glomerular filtration rate during the study was more effectively slowed by tight blood-pressure control in patients with higher rates of urinary protein excretion at base line than in those with lower rates.[78] On the basis of these findings, the authors recommended that the target blood pressure should be less than 125/75 mm Hg in patients with urinary protein excretion of more than 1 g per 24 hours and less than 130/80 mm Hg in those with urinary protein excretion of 1 g per 24 hours or less.

In patients with lower rates of urinary protein excretion, the glomerular filtration rate is less likely to decline with time, regardless of which antihypertensive drug is given.[93] For example, a meta-analysis showed that in patients with diabetic nephropathy, non-nifedipine calcium-channel blockers and ACE inhibitors had similar effects on the rate of decline in the glomerular filtration rate when urinary protein excretion was reduced to a similar extent.[94,95] On the other hand, treatment with other antihypertensive drugs was less effective in decreasing urinary protein excretion and preventing a decline in the glomerular filtration rate.

CONCLUSIONS

Progression to irreversible renal parenchymal damage and end-stage renal disease is the final common pathway of chronic proteinuric nephropathies and is relatively independent of the type of initial insult. In animals, a reduction in nephron mass exposes the remaining nephrons to adaptive hemodynamic changes that are intended to sustain renal function but may be detrimental in the long term. High glomerular capillary pressure impairs glomerular permeability to proteins, which are then filtered in excessive quantities and reach the lumen of the proximal tubule. The secondary process of reabsorption of filtered proteins can contribute substantially to renal interstitial injury by activating intracellular events, including up-regulation of vasoactive and inflammatory genes. The corresponding molecules formed in excessive amounts by the renal tubules cause an interstitial inflammatory reaction that normally precedes renal scarring and correlates with declining renal function.

In several studies, the increase in urinary protein excretion correlated with the tendency of the renal disease to progress more than with the underlying renal disease itself. Whenever urinary protein excretion is reduced, the decline in the glomerular filtration rate slows or stops. Thus, ACE inhibitors and other antihypertensive drugs, to the extent that they lower the rate of urinary protein excretion, effectively limit the progressive decline in the glomerular filtration rate.

Quantification of urinary proteins helps predict the risk of disease progression and the need for dialysis, and measurement of the protein:creatinine ratio in a single morning urine specimen is an excellent alternative to measurement of protein in a 24-hour urine collection. Physicians can now predict the risk of dialysis for individual patients.

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