METABOLISM CLINICAL AND EXPERIMENTAL 60 (2011) 591–593

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Editorial

A complex interplay of factors causes diabetic nephropathy

Diabetic kidney disease is a major public health problem. There has been a continual rise in the number of patients with chronic kidney disease and epidemic increases in the number of patients progressing to end-stage renal disease necessitating dialysis and transplantation [1]. Patients with diabetes who develop kidney disease have decreased quality of life and shorter life spans (because of increased morbidity and mortality from cardiovascular disease). In addition, there is a significant cost to society as illustrated by the fact that about 6.5% of the Medicare budget is directed to the end-stage renal disease population that comprises about 500 000 people according to 2009 statistics [1]. There have been many advances in the management of diabetic kidney disease. Yet despite these treatments, the number of cases of diabetic kidney disease continues to rise [1]. Hence, there are very compelling reasons to better understand the mechanisms underlying diabetic kidney disease to develop new treatments that can both prevent the development of kidney disease and slow or stop progression of diabetic kidney disease. As with all complications of diabetes, hyperglycemia activates a series of changes that lead to glomerular and tubular dysfunction and accelerates glomerular cell apoptosis [2]. In the following, a brief survey is presented of the various mechanisms that have been attributed to mediate hyperglycemia's adverse effects. More importantly, we will suggest changes in the relative importance of mechanisms that cause diabetic nephropathy by elevating the importance of endogenous protective factors that need to be induced using therapeutic means.

From research dating back to 1968, a number of studies have determined a number of key mechanisms responsible for the development and progression of diabetic kidney disease including glomerular hyperfiltration, transforming growth factor β (TGFβ), and advanced glycation end products (AGEs). Moreover, increases in urine albumin to levels greater than 30 mg/g (using the spot urine albumin to creatinine ratio) are also associated with increased progression of kidney disease. Increased urine albumin may be a marker and/or a pathogenic factor as well [3].

The role of glomerular hyperfiltration was elegantly demonstrated by Zatz et al [4] when they compared diabetic and nondiabetic rats over a 1-year period. Using micropuncture techniques to measure glomerular pressures in rats, the authors determined that the diabetic animals had higher glomerular blood flows. It was thought that these high glomerular flows caused eventual loss of glomerular cells and eventual nephron loss. Since the initial studies, no research in humans has definitively shown that glomerular hyperfiltration per se is pathogenic. Yet treatments that lower glomerular blood flow and pressure have had a major impact on slowing progression of diabetic kidney disease possibly by working through mechanisms that are independent of glomerular effects and hemodynamic effects. Glomerular blood flow is principally controlled by systemic blood pressure that affects flow into the glomerulus and by intrarenal angiotensin II that mainly regulates the resistance of the blood vessel (efferent arteriole) that exits the glomerulus. Treatments were designed to decrease the actions of angiotensin II. Zatz et al [4] showed that an angiotensin-converting enzyme inhibitor lowered glomerular pressures and decreased diabetic kidney disease in rats. And medications that target the renin-angiotensin-aldosterone pathway and, in particular, angiotensin II (ie, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and recently the renin inhibitor) have been shown to slow progression of kidney disease. In addition to inhibition of angiotensin II, many studies have definitively proven that lowering blood pressure (goal is at least <130/80) is one of the most important therapeutic interventions as well. Thus, the current approach to treatment of diabetic kidney disease includes a combination of tight control of blood glucose (goal is <7%), tight control of blood pressure (goal <130/80), lowering of urine albumin, and stopping of smoking (a well-established endothelial cell toxin that also affects progression of kidney disease). There has been great interest in the use of drugs that inhibit the actions of angiotensin II to prevent the onset and progression of the pathologies of diabetic kidney disease. An excellent study by Mauer and colleagues [5] that followed the development of diabetic kidney disease in patients with type 1 diabetes mellitus using serial renal biopsies reported surprising results. Normotensive patients were treated by conventional therapy (without inhibition of the actions of angiotensin II), with an ACE inhibitor, or with an angiotensin receptor blocker. The study showed that neither the ACE inhibitor nor the angiotensin receptor blocker had any impact on preventing the developing diabetic kidney disease. Thus, considering this important study and the fact that the number of cases of diabetic kidney disease continues to rise, there is a compelling need for new treatments.

The kidney consists of nephrons (glomeruli and tubules), blood vessels, and a small amount of interstitial tissue. The glomerulus is composed of 3 cell types: glomerular endothelial cells, glomerular epithelial cells (also called *podocytes*), and

mesangial cells. Many studies over the years have focused on the mesangial cell as the primary target of the effects of hyperglycemia on the kidney. However, current research has shown that the other glomerular cell types as well as tubulointerstitial cells are also targets of the effects of hyperglycemia [6]. Clearly, mechanistic information is needed to reveal the pathways that are causing podocyte apoptosis, endothelial cell dysfunction, and tubulointerstitial fibrosis [7-9].

There have been many discoveries in recent years that have elucidated hyperglycemia-induced factors that affect renal glomerular and tubulointerstitial cells. These mechanisms can be classified as deleterious factors and protective factors. Recent research has shown that both increases in deleterious factors along with decreases in protective factors are of mechanistic importance in the development of diabetic kidney disease. Deleterious factors consist of TGFβ, AGEs, protein kinase C (PKC) β, nuclear factor–κB, and increased oxidant production (mitochondrial superoxide production and NADPH oxidase); and there is a growing understanding of an important role for aldosterone. Protective factors consist of the antioxidant system (glucose 6-phosphate dehydrogenase [G6PD], catalase, superoxide dismutase, glutathione), vascular endothelial cell growth factor (VEGF), activated protein C, and possibly insulin. The following is a brief overview of the deleterious and protective factors.

Most of the studies to date have focused on hyperglycemia's toxic effects derived from its metabolites or the changes in signaling pathways resulting from its metabolism. For example, increases in TGFβ expression have been shown clearly in the glomeruli of diabetic animals and patients. Transforming growth factor β is a profibrotic factor that was determined to play a major role in diabetic kidney disease principally by several groups [9]. These reports showed in an elegant series of studies in cultured cells and animal models that hyperglycemia leads to an increase in TGFβ and that blocking TGFβ (eg, by using antibodies) is protective in animal models. There are no direct and specific inhibitors of TGFβ, but there have been trials with antifibrotic agents that to date have not yet shown efficacy as a single agent. Advanced glycation end products formed from nonenzymatic glycation process either intra- or extracellularly may lead to renal hypertrophy, apoptosis, and activation of inflammatory cytokines by binding to cellular receptors [10]. There have been various trials with AGE inhibitors that have had uncertain outcomes due to side effects. Work from our laboratory and others determined that PKC activation due to increases of diaglycerol production from glucose metabolin causes damage to renal glomerular cells by inducing the expression of cytokines such as TGFβ, extracellular proteins, and oxidant producing enzymes NADPH oxidases [8]. Preliminary studies have been ongoing with ruboxistaurin (a PKCβ inhibitor), which has shown promise with respect to diabetic kidney disease; but more research is needed [11]. Increased production of oxidants has also been determined to be associated with hyperglycemia and could be important in diabetic kidney disease [12]. There are 2 main sources of an increased oxidant production due to hyperglycemia: mitochondrial superoxide production [13] and increased NADPH oxidase activity [14]. In animal models, inhibition of these pathways leads to amelioration of diabetic kidney disease. There are ongoing trials of agents targeted against these pathways. Past clinical studies that have tried various antioxidants as a treatment of diabetic kidney disease or other vascular complications have not been effective. It is unclear what the significance is of the lack of efficacy of antioxidants in previous human studies, which many have attributed to the deficiency of specific antioxidants targeting the specific pathways of oxidation production. However, it is also possible that oxidative stress is not the most critical step in causing nephropathy. Another deleterious factor that has been underappreciated until recently is aldosterone. Elevated levels of aldosterone per se cause endothelial dysfunction and damage to kidneys. Work done in collaboration with colleagues demonstrated that aldosterone causes increased oxidative stress and decreased nitric oxide by decreasing G6PD in endothelial cells [15]. Several studies demonstrating that inhibition of aldosterone using spironolactone or eplerenone both in diabetic animal models and in patients have suggested that there is potential in treating diabetic kidney disease [16,17]. In contrast to the reports of hyperglycemia's toxic effects, studies detailing the protective responses of the tissue to hyperglycemia are important. The work by Wei et al in this issue provides another very interesting mechanism leading to cell damage, endoplasmic reticulum (ER stress). Endoplasmic reticulum stress leads to protein misfolding and cellular apoptosis. Endoplasmic reticulum stress has been implicated in pancreatic β-cell death, insulin resistance, and dysfunction of many tissues in diabetes and other chronic degenerative diseases [18]. Their studies suggest that inhibition of ER stress in rats with streptozotocin-induced diabetes protects against the development of diabetic kidney disease. However, ER stress has also been shown to occur in insulin resistance. Because the prevalence of nephropathy is much lower in insulin resistance without diabetes, it is not clear whether ER stress is a major contributor to diabetic nephropathy.

In contrast to the reports of hyperglycemia's toxic effects, studies detailing the protective responses of the tissues to hyperglycemia are infrequent. There is ample evidence that endogenous protective factors exist. Impairment in protective factors plays a mechanistic role in the development of diabetic kidney disease; and thus, enhancing protective factors can potentially be a treatment of diabetic kidney disease. A clear example of endogenous protective factors is the increase of antioxidative stress enzyme in response to oxidants. The antioxidant system is essential to regulate the hyperglycemia-induced increase in oxidants; yet work from our laboratory has illustrated that G6PD, which is the main source of the reductant NADPH (upon which the antioxidant system depends), is decreased by hyperglycemia [19]. Thus, decreased G6PD in concert with increased oxidant production is a potent combination leading to highly significant increases in cellular oxidants. Elevation of VEGF has been shown to be of major importance in the pathogenesis of proliferative diabetic retinopathy. Increases of VEGF in the renal tissue have been controversial. Evidence of VEGF protective effects has been proposed. Some patients who took the anti-VEGF agent bevacizumab developed proteinuria and renal failure [20]. Kidney biopsies showed lesions similar to mice that were bred to lack the VEGFa gene, indicating that VEGF may be important

for the survival of podocytes and endothelial cells in the glomeruli. Protein C is also involved in coagulation that is activated by thrombomodulin [21]. Recent studies in genetically altered mice demonstrated that decreases in activated protein C led to worse diabetic kidney disease, whereas mice overexpressing activated protein C appeared to have relative protection against diabetic nephropathy [21].

The role of insulin as a protective factor needs to be stressed. Insulin, in addition to controlling blood glucose, has multiple other actions (eg, it is an activator of endothelial nitric oxide synthase in endothelial cells and a stimulator of G6PD [22]). A recently published study showed that the loss of insulin receptors in the podocytes led to glomerular pathologies, suggestive of diabetic nephropathy [23]. Clinical studies such as the 50-year Medalist Study examined people who have had type 1 diabetes mellitus for more than 50 years and revealed that 20% to 30% of these diabetic patients did not exhibit significant diabetic retinopathy and nephropathy. Surprisingly, most of these patients still made insulin (measured by c-peptide levels) and had insulin-producing β-cells, suggesting that protective factors may exist in the microvascular tissues and pancreatic cells. Recently, we have reported that hyperglycemia can lead to the activation of a tyrosine phosphatase, Src homology-2 domain-containing phosphate 1, which deactivates the receptors and protective actions of platelet-derived growth factor in the retina, where platelet-derived growth factor is a potent survival factor for pericytes. Because pericyte apoptosis is a key factor of diabetic retinopathy, this effect of hyperglycemia to deactivate endogenous protective factors could be a critical action in causing nephropathy.

There are other factors that have been identified and certainly more to be identified. Much more needs to be determined about the complex interrelationships between deleterious and protective factors to develop rational strategies that will lead to treatments for the prevention and progression of diabetic kidney disease that are both effective and safe. It is likely that a combination of therapies will be needed to treat diabetic kidney disease. Decreasing hyperglycemia's toxic effects and stimulating the endogenous protective factors will be required to develop a safe and effective treatment of nephropathy and other vascular complications of diabetes.

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0026-0495/$ - see front matter

doi:10.1016/j.metabol.2010.10.004

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