The importance of diabetic nephropathy in current nephrological practice

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Abstract

Background. Diabetic nephropathy has become the major cause of end-stage renal disease (ESRD) in the western world and is forecast to become the most frequent cause of ESRD in the African continent and in developing countries in other areas.

Methods. A discussion to achieve a consensus on key points relating to diabetic nephropathy.

Results. Given the catastrophic consequences of diabetes not only for renal function but also for the cardiovascular system, major efforts should be aimed at prevention. The cornerstone of primary prevention (development of microalbuminuria) is a tight control of blood pressure and blood glucose. Although ACE inhibitors have proved effective in preventing the development of microalbuminuria in normotensive patients, this is not the case, in comparison with other classes of antihypertensive drugs, in those who are hypertensive but normoalbuminuric. Secondary prevention (transition to overt nephropathy) and tertiary prevention (progression of established nephropathy to ESRD) benefit from the use of inhibitors of the renin-angiotensin system, whilst the role of tight glycaemic control is more controversial at these stages. Therapeutic lifestyle changes are also important. They should include body weight control combined with regular physical exercise, cessation of smoking and reduced salt intake. The pathogenesis of diabetic nephropathy and its association with hypertension, accelerating renal damage, is complex. It involves genetic factors, altered renal sodium handling with sodium retention, metabolic disturbances and oxidative stress with the formation of advanced-glycation end products (AGEs) and reactive oxygen species.

Conclusions. Although the awareness of the importance of normalizing blood pressure levels and tight glycaemic control have allowed improved survival of diabetic patients, the mortality excess remains unacceptably high in patients with diabetic nephropathy. New treatment strategies are under investigation, including inhibitors of AGE formation, protein kinase C inhibitors, antioxidants, glycosaminoglycans, PPAR-γ agonists and COX-2 inhibitors.

Keywords: advanced glycation end-products; diabetic nephropathy; glycosaminoglycans; microalbuminuria; oxidative stress; prevention; treatment

Introduction

End-stage renal disease (ESRD) in patients with diabetes mellitus, particularly type 2 which is much more frequent than type 1 diabetes, has been referred to as a silent epidemic that has not been sufficiently noted by the non-nephrological community. In many countries, ESRD in patients with type 2 diabetes, be it due to diabetic glomerulosclerosis or other causes, has become the single most frequent cause of ESRD. It is increasing all over the world, particularly so in the developing countries with their more limited financial resources. Evidence has accumulated in the past decade that the frequency of microangiopathic lesions of hyperglycaemia can be significantly diminished by strict glycaemic control and, even more impressively, by intensive antihypertensive treatment. Although most of the available evidence on the prophylaxis of diabetic nephropathy is limited to individuals with type 1
diabetes, it is likely that in principle the same considerations are valid for those with type 2 diabetes, particularly because the renal risk is similar for the two types of diabetes. Although survival has greatly improved and diabetic patients are no longer excluded from renal replacement therapy (RRT) programmes, patients with diabetes receiving RRT continue to do worse with respect to survival and medical rehabilitation than patients without diabetes. The burden of co-morbid conditions due to generalized micro- and macroangiopathy is severe, the quality of life is often poor and the costs of medical treatment and social assistance are very high. These considerations have recently led to the definition of ESRD in diabetic patients as ‘a medical catastrophe of worldwide dimensions’ [1]. The complex and not yet fully unravelled mechanisms leading from chronic hyperglycaemia to microangiopathy are under intensive investigation in the search for new therapeutic tools. Irrespective of these basic research aspects, diabetes mellitus and diabetic nephropathy are, above all, public health problems. Therefore, great attention should be paid to preventive measures, beginning with lifestyle modifications (body weight control with regular physical exercise, avoidance of sodium overload, cessation of smoking). This requires intensive educational programmes at the population level.

The most relevant and also some open issues relating to diabetic nephropathy will be presented and discussed in this report.

**Diabetic nephropathy: a public health problem**

To interpret the rising tide of diabetes mellitus in the population at large, we need to look at this phenomenon with the approach of systemic epidemiology (The epidemiologic transition supercourse. Available at http://www.pitt.edu/~super1/lecture/lec0022/index.htm). During the last century mortality due to infectious diseases has declined to a very low level in the western world. In parallel, life expectancy has climbed to 75 years, a huge gain. The trade-off of longer life expectancy is the emergence of chronic degenerative diseases, such as diabetes and coronary heart disease. On a world scale, in 1994 there were 120 million diabetics. Recent projections forecast that the number of diabetics will reach 240 million in 7 years, by 2010 [2]. If we look at this in more detail, we see that the forecasted number is 28 million in Europe, while it is 138 million in Africa. This constitutes a tantalizing problem because Africa is the poorest continent on earth.

We know that type 2 diabetes is by far more frequent than type 1 diabetes (over 90% of cases are type 2). Nephropathy has a rather predictable frequency, of ~30–40% in type 1 diabetes. In type 2 diabetes the frequency of renal complications depends very much on race. It is rather low in Caucasians (~5%), but extremely high in Pima Indians (60%). In the USA, there are about 15 million diabetics and ~100 000 of these are on dialysis (0.7%). In Europe the problem is very similar, with 22.5 million diabetics of whom ~100 000–120 000 are on dialysis (0.5%). In the USA, health expenditures due to diabetes mellitus and diabetic complications was estimated to amount to 100 billion dollars in 1995. This is greater than the total health budget in 2001 for a country like Italy. Remarkably, in the USA as much as 2 billion dollars is spent on dialysis treatment. In a situation where health expenditures are alarming and the phenomenon continues to be on the rise, prevention is an absolute priority. It should be aimed first to prevent the onset of diabetes mellitus by means of effective lifestyle modifications, which should be the topic of intensive educational programmes.

The natural course of diabetic nephropathy has been described in detail. At the onset of the disease, glomerular filtration rate (GFR) is high. It starts to decline after ~5 years. The decline is sluggish, but over a time scale of ~20–25 years it inexorably leads to ESRD. The appearance of microalbuminuria, which may occur after 10 years, heralds early renal damage. The evolution to frank proteinuria is a critical phase because it accelerates renal damage, leading to ESRD within a few years.

Primary prevention of diabetic nephropathy focuses on diabetic patients with normoalbuminuria and aims to prevent the occurrence of incipient renal disease (i.e. microalbuminuria). Of the risk factors for diabetic nephropathy, two are modifiable, namely hypertension and hyperglycaemia. Concerning the importance of blood pressure control, there are three randomized, placebo-controlled studies dealing with primary prevention of incipient diabetic nephropathy, i.e. studies performed in patients with normoalbuminuria and normotension. The study by Ravid et al. [3] was carried out in the most common type of diabetes, i.e. non-insulin dependent type 2 diabetes, and enrolled 156 patients. In patients on placebo, albumin excretion was ~12 µg/24 h at baseline and rose to 26 µg/24 h at the end of follow-up. In contrast, in the group treated with enalapril, albumin excretion was quite stable. Of note, 19.5% of patients developed microalbuminuria in the placebo group, but only 6.5% in the enalapril group. Thus, the reduction of risk of microalbuminuria attributable to enalapril was 66%.

Three other studies were devoted to primary prevention in normoalbuminuric diabetics with hypertension, namely FACET, ABCD and UKPDS [4–6]. In these reports, the effect of ACE inhibitors was not superior to that of other classes of antihypertensive agents, including amlodipine [4], nisoldipine [5] and atenolol [6].

Improving glycaemic control is another cornerstone of primary prevention. In seven studies, conventional glycaemic control was compared with intensive control. In all studies better glycaemic control was associated with more favourable results. The odds ratio for microalbuminuria was less than one. In some of these studies the confidence intervals crossed the line of equivalence, indicating non-significance. However, the
improvement.

Moving to secondary prevention, i.e. prevention of the transition from incipient to overt nephropathy, there are 12 trials for type 1 diabetes. The risk of progression to gross albuminuria was uniformly reduced even though risk reduction did not achieve statistical significance in all of them. The pooled estimate was highly significant [8] and indicated that treatment with ACE inhibitors is associated with a 60% risk reduction. In type 2 diabetes there are no randomized controlled trials of secondary prevention with ACE inhibitors. The most important study is based on the angiotensin II-receptor antagonist irbesartan [9]. In this study, there was a dose-dependent reduction in the incidence of overt nephropathy. Indeed over 2 years the evolution to diabetic nephropathy was 15% in the placebo arm of the study, 10% in patients taking a 150 mg/day dose of irbesartan and 5% in those taking 300 mg/day. As to glycaemic control, it is disappointing to note that five randomized studies comparing the renal effect of intensified conventional glycaemic control in type 1 diabetics with microalbuminuria failed to show a clear benefit. However, the follow-up was too short in these studies. Furthermore, studies by Fioretto et al. [10] have suggested that the excellent glycaemic control obtained after pancreas transplantation led to a remarkable regression of renal lesions in patients with diabetic nephropathy.

Tertiary prevention aims to retard the progression of established nephropathy to ESRD. In type 1 diabetes, the groundbreaking study by Lewis et al. [11] showed a clear benefit from ACE inhibitors in terms of both evolution to ESRD and cardiovascular events. The incidence of a combined end-point, including doubling serum creatinine and starting dialysis or being transplanted, was much lower in patients on captopril than in those treated with other classes of antihypertensive drugs. We do not yet have studies with ACE inhibitors in type 2 diabetes. However, angiotensin II-receptor antagonists are certainly protective in these patients. In the IDNT study, irbesartan caused a marked reduction in the incidence of the combined endpoint in comparison to amlodipine and placebo [12]. Similarly, Brenner et al. [13] found that in 1513 patients with type 2 diabetes and overt nephropathy, treatment with losartan resulted in a 16% reduction in the risk of the primary composite endpoint, namely doubling of baseline serum creatinine, ESRD and/or death, compared to placebo.

Going beyond the kidney, it is important to remember the tremendous improvement in the life expectancy of diabetic patients which has been achieved with the judicious use of antihypertensive drugs. In the seventies, the prognosis was gloomy in diabetics with nephropathy since ~90% of them died within 14 years of the appearance of gross proteinuria. At present, antihypertensive treatment has led to a reduction in mortality by >50%, and there is much room left for improvement.

Moreover, new treatment modalities for diabetic nephropathy are under investigation, including advanced-glycation end-product (AGE) inhibitors, protein kinase C (PKC) inhibitors, antioxidants, glycosaminoglycans (GAGs), COX-2 inhibitors, PPAR-γ agonists and arginine-vasopressin (AVP) blockade.

### Diabetes and salt handling

The pathogenesis of hypertension accompanying diabetes mellitus involves various mechanisms, such as sodium retention and genetic predisposition. Total exchangeable sodium has been found to be increased up to 10% in type 1 and type 2 diabetes mellitus and plays a central role in the pathogenesis of blood pressure changes [14]. Several studies have shown that diabetic patients have an impaired ability to excrete a NaCl load [15,16]. Among various natriuretic hormones, locally formed intrarenal dopamine has attracted interest in the last years [16]. Dietary sodium appears to be the major regulating factor in the control of renal dopamine mobilization, which in turn regulates Na⁺-K⁺ ATPase activity in the proximal tubule and the thick ascending limb of the loop of Henle [17]. As diabetic patients have an impaired ability to excrete sodium, it has been hypothesized that defective renal tubular dopamine mobilization may be responsible for this [16]. A defective renal dopamine system has been shown to occur early in type 1 diabetic patients and in individuals with familiar hypertension [18]. Moreover, Tsuchida et al. [19] demonstrated that diabetic rats fed with a high salt diet developed salt-sensitive hypertension due to a defective dopaminergic system in the kidney that failed to inhibit Na⁺-K⁺ ATPase activity.

The ability of insulin to reduce urinary sodium excretion has been recognized for at least 70 years. It is well established at present that insulin mediates antinatriuresis without altering GFR, by a distal tubular anti-natriuretic effect [19]. The significance of insulin-mediated sodium reabsorption has not been fully understood as not all conditions associated with insulin resistance and hyperinsulinaemia are characterized by sodium retention. In view of the significant acute anti-natriuretic effects of insulin in the distal tubule it is obvious that to avoid sodium retention following an ordinary meal, counter-regulatory mechanisms must be operative in healthy subjects in response to hyperinsulinaemia. By using the lithium clearance method, it has been found that euglycaemic hyperinsulinaemia causes a time- and dose-dependent fall in proximal tubular sodium reabsorption, which could represent such a physiological ‘escape mechanism’ [20]. As insulin increases renal plasma flow but not GFR in humans, it was speculated that renal vasodilation may be the cause of the observed fall in proximal tubular sodium reabsorption. In this respect, it is of interest that a blunted renal vasodilator action and lesser decrease of proximal tubular sodium reabsorption following euglycaemic insulin infusion was found in patients with insulin resistance [21]. Thus,
as the acute distal anti-natriuretic effects of insulin remain intact even in insulin resistant patients, a blunted renal vasodilatory action of insulin may predispose these patients to sodium retention and hypertension. Taken together, these results support a role for renal vasodilation as a physiological counter-regulatory mechanism against the acute distal anti-natriuretic effects of insulin. As insulin-mediated increases in renal plasma flow are impaired in insulin-resistant subjects [22], this may explain why insulin-resistant states are often associated with sodium retention. It is notable that renal vasodilatation caused by insulin is mediated by the release of nitric oxide (NO) from the endothelium, a process that could be abolished by injection of L-NAME into the renal artery [23]. One could therefore speculate that mechanisms aimed at restoring NO availability and the renal vasodilator effects of insulin may prevent the development of sodium retention and hypertension. Indeed, ACE inhibitors, which have been shown to improve basal and stimulated NO-dependent endothelial function [24], also normalize the natriuretic response to a sodium load in type 1 diabetic patients [25].

Another theory to explain deranged renal sodium handling in diabetes mellitus is focused on a tubulocentric view, rather than on the renal vasculature, and is based on the physiological concept of tubulo-glomerular feed-back (TGF) [26]. A notable phenotype in early diabetes is renal growth (both hyperplasia and hypertrophy), which is mostly accounted for by proximal tubules. If we admit an increased proximal sodium reabsorption to be the primary consequence of renal growth, it derives that more of the glomerular filtrate is reabsorbed and less reaches the macula densa at the end of Henle’s loop. This causes GFR to increase consequently to the inhibition of the TGF. In other words, tubular hypertrophy would be the cause of glomerular hyperfiltration via proximal hyper-reabsorption. To support this hypothesis, an increased fractional reabsorption in the nephron segments upstream from the macula densa has been described in studies in hyperfiltrating patients with type 1 diabetes mellitus [27] and in hyperfiltrating rats with streptozocin-induced diabetes [28]. This could explain the ‘salt paradox’, observed both in human [29] and experimental studies [30], that a low-salt diet further increases renal blood flow, GFR and kidney weight, rather than reducing them, as expected if the vascular changes induced by the neurohumoral pressure-natriuresis systems were predominant. In fact, if we accept the TGF predominance, we realise that low sodium intake further inhibits the TGF via a lesser degree of sodium delivered to the macula densa, with secondary further increased GFR. The salt paradox is worth considering, as it questions the advice of salt intake restriction in early diabetes, as recommended by the American Diabetes Association [31]. However, we have to keep in mind that the salt paradox, with its pathophysiological basis, has been characterized in patients and experimental models with type 1 diabetes mellitus, whilst fewer data have been acquired on the early renal dysfunction in type 2 diabetes. Moreover, a high-salt diet would only benefit a diabetic patient to the extent that the salutary effect of preventing hyperfiltration was not offset by nefarious consequences of sodium-dependent hypertension. The balance of these two effects cannot be predicted a priori, and it may actually be different in type 1 and type 2 diabetes.

In conclusion, different pathophysiological mechanisms of deranged sodium handling have been described in diabetic patients. It is therefore reasonable to suppose that the effect of dietary sodium intake on GFR, and eventually on diabetic nephropathy, is not univocally determined, but may differ according to the pathophysiological alterations predominant in a particular patient. Hence, the necessity to dissect the complex phenotype diabetes into intermediate and more precise phenotypes at the whole-kidney, tissue, cellular, molecular and genetic level, in order to provide the patients with the best advice.

**Genetic aspects of diabetic nephropathy**

After 20 years of diabetes duration, 30% of type 1 diabetic patients develop diabetic nephropathy. It is remarkable that the occurrence of diabetic nephropathy gets less likely after more than 20 years of type 1 diabetes. Under good metabolic control (average HbA1c of 7.2%), <10% of type 1 diabetic patients who became diabetic before the age of 15 years develop diabetic nephropathy. Type 2 diabetic patients show a prevalence of 25–40% for diabetic nephropathy after 25 years of diabetes. This difference is based on the fact that type 2 diabetes can remain undiagnosed for a long period of time. Furthermore, the prevalence depends on ethnic background. Pima, Navajo, Winnebago and Omaha Indians show the highest prevalence [32], whereas Caucasians of European origin demonstrate the lowest prevalence [33]. In African Americans the prevalence of diabetic nephropathy is four times higher than in a population of non-hispanic whites [34].

**Genetic and non-genetic factors**

The pathogenesis of type 2 diabetes and diabetic nephropathy is influenced by an individual genetic predisposition (mono- and polygenic factors) that in turn is influenced by environmental factors (physical activity, diet, overweight, climate, metabolic control) in its phenotypic expression. Of note, a significant proportion of diabetic patients do not develop diabetic nephropathy despite long-standing severe hyperglycaemia. Genetic predisposition in type 1 and type 2 diabetic patients with nephropathy has been shown in several studies done in populations with different genetic background. Some families with several diabetic patients presented almost no clinical signs of diabetic nephropathy whereas 80% of diabetic patients from other families developed this complication. The
high ethnic variance points to geographic and dietary factors, but also reflects genetic heterogeneity. Siblings of type 1 diabetic patients with diabetic nephropathy have a higher risk of developing diabetic nephropathy [35]. The rate of relatives of type 2 diabetic dialysis patients who develop ESRD is five times higher (37%) than that of relatives of type 2 diabetic patients without nephropathy (7%; African Americans) [36]. If both parents presented with diabetic nephropathy the disease was observed in 46% of the offspring. In contrast, only 23% of Pima Indian offspring progressed to diabetic nephropathy if only one parent was proteinuric and 14% if neither parent had proteinuria [32]. Investigation of concordance rates in mono- and dizygotic twins would provide evidence for a genetic predisposition, but so far no twin studies have been performed in patients with diabetic nephropathy.

Basic problems of genetic analyses of complex diseases

The heterogeneous clinical picture of diabetes does not allow a clear-cut identification of subjects at risk for diabetic nephropathy among offspring, either biochemically or genetically. Children cannot provide any information about at-risk status. Therefore, it is difficult to perform traditional, LOD score-based pedigree analyses. There is also a good chance that diabetes runs in families independent of nephropathy. Because of the lack of extended pedigrees with a clear inheritance pattern researchers are focusing on association studies and special approaches to linkage analyses.

Association studies on diabetic nephropathy

The literature based on case–control studies contains contradictory observations. The best known polymorphisms are the ACE insertion(I)/deletion(D) polymorphism (DCP1), and the angiotensinogen polymorphism M235T [37–40]. Initial studies hinted to an association with diabetic nephropathy but subsequent reports failed to replicate those results. Similar data have been obtained for the AT II-receptor antagonist (RA), apolipoprotein E, interleukin-1-RA, ANP, aldose reductase, endothelial NO synthase, β3-adrenergic receptor, matrix metalloproteinase 9, thrombocyte glycoprotein IIIa, type 4 collagen, interleukin-1, renin and pronatriodilatin [41]. The discrepancies could be explained by genetic heterogeneity and relatively small gene effects in stratified populations with a relatively small number of cases (e.g. 200 individuals).

Linkage studies and genome scan

For an ideal linkage study extended pedigrees are required. Specific designs like the affected sib pair approach have been developed because large pedigrees have not been available for most ‘complex’ diseases. In principle, the focus is on the inheritance of a specific trait within families. Linkage studies are performed in two variants: (i) genome-wide scan of markers without a candidate gene hypothesis (positional cloning), and (ii) test of markers for candidate genes. On the other hand, as in most complex diseases, there are monogenic subforms for type 2 (but not for type 1) diabetes. The at-risk subjects come to clinical attention relatively early as in maturity-onset diabetes of the young (MODY). In most cases, there are several affected individuals in each generation. Larger pedigrees are available that make it easier to perform genetic studies. The idea of revealing the genetics of monogenic forms is based on easy identification of genes which could also explain the more common complex forms. For type 2 diabetes, that hope has not yet been fulfilled.

Monogenic, juvenile type 2 diabetes with non-diabetic renal failure (MODY5)

Until recently, six monogenic subforms had been identified for MODY. Of these six sub-forms, MODY5 (HNF-1β gene) presents type 2 diabetes together with chronic renal failure leading to dialysis. Hattersley’s group identified two other HNF-1β families in 2001 [42]. These families had been diagnosed as having glomerulocystic kidney disease before the detection of the mutations. The cystic kidneys were hypoplastic. Type 2 diabetes and renal failure can be expressed so variably that other diagnoses such as type 1 diabetes and diabetic nephropathy were suggested. Renal failure can be detected relatively early and at different progression steps, from a long history of proteinuria without hypertension to ESRD and kidney transplantation. Sometimes an association with Mullerian aplasia and hypoplastic cystic kidneys can be observed [43].

Polygenic diabetes with diabetic nephropathy

For the analysis of diabetic nephropathy a few genetic linkage studies had been reported, but the case numbers were always too small, so the sample size could not be considered as being representative [44–48]. A genome scan in affected type 2 diabetic sib pairs in Pima Indians with diabetic nephropathy revealed four linked regions: the regions on chromosomes 7 and 20 were only linked with diabetic nephropathy, whereas those on chromosomes 3 and 9 showed linkage with nephropathy and retinopathy. The sample size of 98 affected sib pairs in this study was also very small [47]. Further linkage studies in animal models and humans identified chromosomal regions containing the so-called renal failure genes (Rf1, Rf2). Rf1 and Rf2 predispose for renal failure in an animal model characterized by hypertension and nephrosclerosis (fawn-hooded rat). The homologous region in the human genome on chromosome 10 did not show linkage with the disease in African Americans [49]. Our knowledge of the genetic predisposition to developing diabetic nephropathy in type 2 diabetes mellitus has been improved by the recent study by Vardarli et al. [50], who performed a genetic linkage analysis, by means of genome scan, in 18 large Turkish
families (368 subjects examined) with recurrence of type 2 diabetes and diabetic nephropathy, and found a highly significant linkage of diabetic nephropathy with a locus on chromosome 18 (18q22.3–23, LOD score of 6.1). This chromosomal area was subsequently tested in an analysis of 101 affected sibling pairs of Pima Indians and its linkage with diabetic nephropathy was confirmed. The concordance between two totally different ethnic groups indicates that the 18q22.3–23 chromosomal tract contains a major locus that confers predisposition to diabetic nephropathy. Research is now focusing on the exact characterization of the gene. One obvious candidate gene is ZNF236, also called ‘Kruppel-like zinc-finger gene 236’, which shows a glucose-dependent expression in human mesangial cells [51].

Glycosaminoglycans (GAGs) and renal involvement in diabetes: from the Steno hypothesis to the clinical arena

The Steno hypothesis and its continuation during the last 10 years

In 1988, Deckert formulated the unifying proposal that albuminuria of diabetic nephropathy is a sign of global vascular dysfunction and reflects a defect in the sulphation pattern in the GAG side chains of heparan sulphate proteoglycans (HSPG) [52]. Defects in enzymes responsible for the sulphation of HSPG were considered as possible causes of a genetic predisposition for the development of nephropathy in patients with diabetes.

Although this concept has so far not been formally proven, it has greatly stimulated attempts to understand the molecular basis of diabetic nephropathy and its association with cardiovascular complications [53]. Three core proteins of HSPG, perlecan, agrin and collagen XVIII have been identified. Furthermore, following the development of new antibodies against GAG side chains significant changes have been identified in diabetic nephropathy. Changes in GAG synthesis by glomerular cells have also been reproduced in vitro under conditions simulating diabetes. Ang II was found to impair HS synthesis and this effect may add to its adverse haemodynamic effects on disease progression. In addition, genes for key enzymes involved in GAG side chain formation have been cloned: N-deacetylase/N-sulfotransferase, 3-O-sulfotransferase and 6-O-sulfotransferase [54]. It is as yet unclear, however, whether different allotypes of these enzymes exist, with different susceptibilities to high glucose concentrations.

At the same time, the concept of a broad-ranging function of the endothelium has been strengthened. This concept implies an important role for endothelial cells in the control of vascular permeability, angiogenic vascular remodelling, metabolic, inflammatory and pro- as well as anti-thrombogenic processes and vascular smooth muscle cell growth. Endothelial dysfunction, which initially becomes apparent from a defective vaso-relaxation, and eventually promotes atherosclerosis, has been proposed as an additional unifying cause of renal and vascular pathology in patients with diabetic nephropathy [55]. Many endothelial functions are mediated by NO and high levels of glucose can scavenge NO either directly or via the generation of AGES. Although many of these findings support the original concept of the Steno hypothesis or a broader concept of the original idea, some data have also questioned the specificity of a link between hyperglycaemia, glomerular changes and cardiovascular disease. For example, it has been demonstrated that reduced expression of HS is not confined to diabetic nephropathy and an increased transcapillary escape rate of albumin was also found in diabetic patients without nephropathy [56].

Notwithstanding these restrictions, and despite findings suggesting that changes in GAG composition are only one of several determinants of proteinuria, the therapeutic application of GAG has turned out to be a highly attractive option for the treatment of diabetic nephropathy.

GAGs in the treatment of diabetic nephropathy

Under experimental conditions heparin and more generally GAG can prevent and cure diabetic nephropathy. The underlying mechanisms remain incompletely understood. Heparin increases the biosynthesis of HS in endothelial cell cultures. However, the activity of GAGs is not solely explained by recovery of abnormalities in HSPG metabolism and restoration of anionic charges. GAGs also affect proteases and modulate extracellular membrane synthesis, in part through interference with TGF-β1 formation and signalling [57]. Approximately a dozen phase II trials with heparins/GAG published since 1994 have shown a reduction in proteinuria in micro- and macroalbuminuric type 1 patients and—less consistently—type 2 diabetic patients. While these results are highly encouraging, the trials are far too small and too short term to judge whether this intervention is capable of stabilizing renal function and reducing CV complications. The potential risks of this type of treatment include effects on bone metabolism and bleeding, in particular in the presence of proliferative diabetic retinopathy. However, one study even showed regression of hard retinal exudates, possibly related to effects on VEGF binding [58].

AGEs, oxidative stress, hyperhomocystinaemia and microvascular damage in diabetic nephropathy

Irrespective of the specific causes of diabetic nephropathy, the general mechanisms by which hyperglycaemia causes tissue injury are still not completely understood. Apart from unknown genetic determinants which account for differences in the resistance to and the pattern of hyperglycaemic damage, the formation of
AGEs and of reactive oxygen species (ROS) (i.e. oxidative stress) combined in carbonyl stress due to hyperglycaemia seem to be major factors for diabetic complications. AGE formation is part of the ageing process, but in diabetes mellitus it has been shown to be markedly increased due to hyperglycaemia, which is a promoter of glycation. The non-enzymatic and irreversible glycation of proteins and lipids by the Maillard reaction results in the generation of AGEs which can cross-link proteins and accumulate in cells and tissues. The rate of glycation is determined by the blood glucose concentration, renal function and protein turnover. Serum and tissue AGE levels are correlated with the time-averaged concentration of blood glucose and the severity of diabetic complications, including nephropathy and retinopathy. The important role of AGEs in diabetic micro- and macroangiopathy is confirmed by the fact that they have been immunohistologically localized in the vascular wall [59]. Moreover, in vitro studies revealed that AGEs interact via specific receptors with monocytes/macrophages and endothelial cells. The best-characterized receptor so far is RAGE. The way RAGE transduces intracellular signalling involves the generation of ROS, which then activates transcription factor NF-κB. The resulting cell activation leads to the release of cytokines and growth factors, promotes an enhanced formation of abnormal extracellular matrix and increases the rate of abnormal cell proliferation, e.g. of smooth muscle cells. In endothelial cells, the changes comprise pro-coagulatory effects as well as an increased expression of leucocyte-binding vascular adhesion molecule-1 and tissue factor, representing pathological changes in blood vessels in diabetes. The central role of AGEs and RAGE in diabetic complications has been confirmed in different in vivo studies. In several experimental animal studies, the AGE formation inhibitor aminoguanidine was capable of preventing diabetic microvascular glomerular, retinal and nerve lesions. However, clinical trials with aminoguanidine were stopped due to severe side effects. Furthermore, the administration of recombinant RAGE—which interferes with the AGE–RAGE interaction—to diabetic animals, prevented vascular lesions and hyperpermeability [60].

Glycation may also act together with oxidation to form AGEs. Glycoxidation products, a subclass of AGEs, represented by, for example, N-(carboxymethyl)lysine (CML) and pentosidine, require both glycation and oxidation for their formation. In diabetic nephropathy, CML and pentosidine accumulate in expanded mesangial matrix and nodular lesions in colocalization with malondialdehyde (MDA)-lysine, a lipoxidation product, while pyrroline, an AGE structure whose deposition is rather independent of oxidative stress, is not found within diabetic glomeruli [61]. Oxidative stress may lead to protein modification, either directly by ROS with eventual formation of oxidized amino acids, or indirectly by reactive carbonyl compounds formed by auto-oxidation of carbohydrates, lipids or amino acids ('carbonyl stress'). Both CML/pentosidine and MDA-lysine are formed by carbonyl amine chemistry between protein amino groups and carbonyl compounds derived from auto-oxidation of carbohydrates, such as glyoxal, methylglyoxal and glycolaldehyde as well as lipids, including MDA, 4-hydroxynonenal (HNE) and acrolein [62]. Co-localization of the glyoxidation products CML and pentosidine together with the lipoxidation products MDA-lysine, HNE–protein adduct and acrolein–protein adduct has been found in the glomerular lesions of diabetic nephropathy whereas this is only faintly expressed in IgA nephropathy. This implicates increased local oxidative stress and carbonyl modification of proteins, particularly in the presence of diabetic glomerular tissue damage [62].

Beyond its significance for the formation of AGEs, oxidative stress in the form of increased production of ROS may represent a pathophysiological link between hyperglycaemia and the other major pathways thought to be responsible for diabetic complications. Hyperglycaemia itself has been shown in an in vitro study on aortic endothelial cells to increase rapidly the intracellular generation of ROS and the lipid peroxidation product MDA [63].

Hyperhomocystinaemia is a recognized contributor to endothelial dysfunction in diabetic patients. Homocysteine, a sulphydryl-containing amino acid derived from the demethylation of dietary methionine, is a mediator for endothelial damage. Homocysteine promotes oxidant injury to the vascular endothelium, impairs endothelium-derived vasodilator NO production and also may alter the coagulant properties of the blood [64].

Experimental diabetes models indicate that the enzyme aldose reductase may be activated by ROS and may initiate a process favouring both glycoxidative and lipoxidative changes that can be damaging to renal microvascular as well as to glomerular and tubular cells [65]. Long-term aldose reductase inhibition in vivo has demonstrated the ability to prevent diabetes-induced decreases of nerve conduction velocity. However, other in vivo studies failed to show a beneficial influence of aldose reductase inhibitors on microalbuminuria, the earliest manifestation of diabetic nephropathy, in the long run [66].

ROS are also capable of activating PKC in vascular endothelial cells via enhanced diacylglycerol generation. Activated PKC increases the production of cytokines and extracellular matrix, the plasminogen activator inhibitor (PAI-1) and the vasoconstrictor endothelin-1. Furthermore, PKC mediates vascular endothelial growth factor activity. These alterations may lead to basement membrane thickening, vascular occlusion, increased vascular permeability, and the enhancement of angiogenesis.

Conclusion

Diabetic nephropathy is a significant health problem with increasing importance since its prevalence is expected to double within the next 10 years. Ongoing
The importance of diabetic nephropathy

Causes tissue injury are not yet completely understood. Although the mechanisms whereby hyperglycaemia, hypertension, and hyperglycaemia.

Pathogenesis of diabetic complications

Although the mechanisms whereby hyperglycaemia causes tissue injury are not yet completely understood, it is known that the formation of AGEs and ROS is involved.

The pathogenesis of hypertension accompanying both type 1 and type 2 diabetes involves sodium retention. Diabetic patients have an impaired ability to excrete a NaCl load.

Impaired GAG sulphation contributes to albuminuria, nephropathy and cardiovascular complications.

Treating diabetic nephropathy

Controlling chronic hyperglycaemia results in a lower incidence of diabetic nephropathy in both type 1 and type 2 diabetes mellitus.

The normalization of blood pressure (preferably by ACE inhibitors or angiotensin II-receptor antagonists) and an optimized control of hyperglycaemia are the two major tools to prevent microvascular damage in diabetes. Inhibitors of the renin-angiotensin system may have several benefits in addition to their blood pressure lowering effect.

Once diabetic nephropathy is present, it is important to control salt intake to help controlling salt-sensitive hypertension. Diuretics may be needed to normalize the sodium balance. ACE inhibitors again may help, as they seem to normalize the natriuretic response to a sodium load.

Treatment with heparin may prevent the albuminuria, nephropathy and cardiovascular complications caused by impaired GAG sulphation, but large clinical trials are needed to evaluate the risk benefit profile.

New treatment strategies under pre-clinical and, in part, clinical investigation include AGE inhibitors, PKC inhibitors, antioxidants, GAG therapy, COX-2 inhibitors, PPAR-γ agonists and AVP blockade.

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The importance of diabetic nephropathy


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