Advanced glycation end-products in diabetic nephropathy

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Abstract. Throughout the industrialized (well-fed) world, diabetes mellitus is the most prevalent cause of end-stage renal disease (ESRD). Diabetic nephropathy is as likely to develop in long-duration non-insulin-dependent diabetes (type 2) as in insulin-dependent diabetes mellitus (type 1). Nephropathy in diabetes follows a well outlined course, starting with microalbuminuria through proteinuria, azotaemia and culminating in ESRD. Renal functional decline in diabetic nephropathy is slowed by establishment of euglycaemia and normalization of hypertensive blood pressure. Diabetic ESRD patients, compared with other causes of ESRD, sustain greater mortality and morbidity due to concomitant systemic disorders, especially coronary artery and cerebrovascular disease. A central role for glucose toxicity, especially the adverse impact of accumulated advanced glycosylated end-products (AGEs), appears likely from experimental data generated both in induced diabetic rodents and diabetic individuals. Treatment with aminoguanidine raises the possibility of blocking end-organ damage in diabetes without the necessity for correcting hyperglycaemia.

Background

Registries of end-stage renal disease (ESRD) in 1997, in the US, Japan and industrialized Europe, show that diabetes mellitus is the most prevalent cause of treated renal failure world-wide. More than a decade previously, Mauer and Chavers grasped the trend in ESRD demographics in stating that ‘Diabetes is the most important cause of ESRD in the Western world’ [1]. Both the incidence and prevalence of diabetic ESRD patients have increased yearly over the past decade. Statistics for 1995, listed in the 1997 report of the United States Renal Data System (USRDS), underscore this point. Of 257 266 US patients receiving either dialytic therapy or a kidney transplant in 1995, 80 667 had diabetes [2], a prevalence rate of 31.4%. During 1995, of 71 875 new (incident) cases of ESRD, 28 740 (40%) had diabetes (Figure 1).

Toxicity of glucose

Whereas initial formulations of how glucose might be toxic to tissues and organs were simplistic (Figure 2), subsequently, an enormous literature documents the injurious consequences of complex protein–kinin–enzyme interactions stimulated by a high ambient plasma glucose concentration (Figure 3). Based on observational studies tying glycaemic control to proteinuria and decline in glomerular filtration rate (GFR) plus more than half a dozen interventional studies predicting and confirming the DCCT, it is established that hyperglycaemia is the main metabolic perturbation causing irreversible kidney damage in diabetes. There are three candidate mechanisms to explain how hyperglycaemia damages tissues [7]: (i) Acceleration of the aldose reductase pathway leading to toxic accumulation of sorbitol in nerves. (ii) Activation of isoform(s) of protein kinase C (PKC) in vascular tissue initiating a cascade of events culminating in diabetic complications [8]. PKC activity is increased in renal glomeruli, retina, aorta and heart of diabetic animals, probably because of increased synthesis de novo of diacylglycerol (DAG), a major endogenous activator of PKC [9]. (iii) Accelerated non-enzymatic glycosylation with...
Fig. 1. Proportion of incident ESRD patients whose diagnoses was listed as diabetes mellitus was 40% in 1995: extracted from the 1997 report of the United States Renal Data System (USRDS) [2]. The USRDS does not distinguish between type 1 and type 2 diabetes in their reporting forms. Registries in Europe and Japan also list diabetes as the disease accounting for the greatest number of incident and prevalent ESRD cases.

Deposition of advanced glycosylated end-products (AGEs) [10] (discussed below).

Diabetes type in diabetic nephropathy

Dialysis patients in the US, Japan and Europe, whose ESRD is due to diabetes are predominantly type 2: <8% of diabetic Americans are insulinopenic, C-peptide-negative persons who have type 1 diabetes. ESRD in diabetic persons reflects the demographics of diabetes per se [11] in that: (i) incidence [12] is greater in women, blacks [13], hispanics [14] and native Americans [15]; and (ii) peak incidence of ESRD occurs from the fifth to the seventh decade. Consistent with these attack rates is the reality that blacks over

Fig. 2. A simplistic relationship between hyperglycaemia and diabetic nephropathy as understood in the 1970s.

Fig. 3. The complexity of multiple factors and variables that interrelate in the pathogenesis of diabetic protein injury.
the age of 65 years face a seven times greater risk of diabetes-related renal failure than do whites. In the US, it is not surprising, therefore, that ESRD associated with diabetes is largely a disease of elderly blacks [16]. Recent surveys of Brooklyn city and state hospital ambulatory haemodialysis units in October 1997 discovered 97% of prevalent patients to have type 2 diabetes.

Severity and duration of hyperglycaemia rather than diabetes type determine the extent of vasculopathic complications of diabetes [17,18]. Unfortunately, however, neither the USRDS nor the European Dialysis and Transplant Association reports distinguish between type 1 and type 2 diabetes in terms of dialysis morbidity and mortality or post-transplant patient and allograft survival. Imprecise diabetes classification provokes confusing terms such as ‘insulin requiring’ to explain treatment with insulin in persons thought to have resistant type 2 diabetes. Present criteria are unable to classify as many as one-half of diabetic persons as type 1 or type 2 diabetes [19,20].

Observations in carefully followed type 2 diabetic subjects contradict the common impression that type 2 diabetes induces nephropathy relatively infrequently [21]. Manifestations of nephropathy are remarkably similar in the two main diabetes types [22] despite differences in genetic predisposition [23]. Early nephromegaly, as well as both glomerular hyperfiltration and microalbuminuria, previously thought limited to type 1, are equally prevalent in type 2 [24].

Co-morbid risk factors

Management of a diabetic person through progressive renal insufficiency is more difficult than in age- and gender-matched non-diabetic persons because of extensive, often life-threatening extrarenal (co-morbid) disease. Diabetic patients manifesting ESRD suffer an increased death rate compared with non-diabetic ESRD patients due to greater attack rates for cardiac decompensation, stroke, sepsis and pulmonary disease (Figure 4). Co-morbid disease (Tables 1 and 2), especially blindness, limb amputations and cardiac disease, limits and may prevent rehabilitation. The difference between rehabilitation and invalidism in diabetic ESRD patients depends on attaining a renal transplant. Restricted to dialytic treatment, the diabetic ESRD patient experiences an unending sequence of complications, especially atherosclerotic heart disease and cerebrovascular catastrophes that induce a high rate of withdrawal from therapy (a form of suicide) [25] (Figure 4).

The threat of blindness, a dreaded complication at all times in every diabetic individual, is noted in the histories of laser treatment and/or vitrectomy for retinopathy elicited from > 90% of diabetic individuals who begin maintenance dialysis or receive a renal allograft. Comprehensive management [26] requires integration of laser and/or vitreous surgery. Repetitive heart evaluation, even in asymptomatic patients, including coronary angiography (if indicated), is vital to detect those individuals for whom prophylactic coronary artery angioplasty or bypass surgery is likely to extend life [27]. A key additional member of the patient’s protective team is a podiatrist who delivers routine foot care while maintaining surveillance for risk of major lower extremity disease, thereby reducing sharply the chance of toe, foot and limb amputations [28].

Orthostatic hypotension, gastropathy, cardiovascular arrhythmia [29] and cystopathy are frequently overlooked, highly prevalent co-morbid disorders that plague the long-duration diabetic patient restricting life quality in ESRD. Additional expressions of autonomic neuropathy, e.g. obstipation and explosive night diarrhoea, often co-exist with gastroparesis [30].

Pregnancy

Pregnancy in diabetes complicated by kidney disease (proteinuria and/or azotaemia), previously regarded as an unavoidable prelude to disaster—inordinate fetal loss and/or maternal morbidity and death—can be managed with a high probability of success. Detection of macroproteinuria, in the absence of urinary infection, in a type 1 pregnancy will be followed by pre-eclampsia in 30–50%, though perinatal survival should exceed 95% [31]. Once the serum creatinine concentration exceeds 2.0 mg/dl, diabetic women are counselled to avoid pregnancy. Facilitated by a satisfactory renal transplant, however, perinatal outcome can be ‘excellent’ despite a superimposed pre-eclampsia rate of 20–40%. In 182 pregnant women with type 1 diabetes, 46 of whom had overt nephropathy for a minimum of 3 years after delivery, pregnancy neither increases the risk of subsequent nephropathy nor accelerates progression of pre-existing renal disease [32]. An equally encouraging series from Finland reported the course of 28 diabetic women for 7 years after delivery compared with 17 nulliparous controls matched for age, duration of diabetes and severity of vasculopathy, advising: ‘pregnancy does not seem to affect development or progression of diabetic nephropathy’ [33].

The dilemma in selecting uraemia therapy

Suboptimal care is the rule in diabetic nephropathy in both Europe and the US, an inference discerned from defective attention to co-morbidity in symptomatic kidney patients. In Germany, for example: ‘preterminal care in diabetic patients with ESRD’ is deficient in amount and quality [34] with inadequate attention to control of hypertension, hyperlipidaemia or ophthalmological intervention [35]. In the UK, at variance with prior consensus, withdrawal from renal replacement therapy was not infrequent, accounting for 17% of all deaths, and was due mainly to co-morbid medical problems (89%) especially diabetic vasculopathy [36]. Likewise, in the US, Ifudu et al. found that because of excessive co-morbidity: ‘Few elderly, diabetic hemo-
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Fig. 4. Management of diabetic patients throughout the course of progressive diabetic nephropathy in actuality becomes the treatment of a series of worsening co-morbid conditions. As shown in selected data from the most recent report of the United States Renal Data System (USRDS) [2], deaths during treatment for ESRD are greater in diabetic than in non-diabetic patients for myocardial infarction, septicaemia and cerebrovascular disease.

Table 1. Diabetic complications which persist and/or progress during ESRD

| 1. Retinopathy, glaucoma, cataracts |
| 2. Coronary artery disease. Cardiomyopathy |
| 3. Cerebrovascular disease |
| 4. Peripheral vascular disease: limb amputation |
| 5. Motor neuropathy. Sensory neuropathy |
| 6. Autonomic dysfunction: diarrhoea, dysfunction, hypotension |
| 7. Myopathy |
| 8. Depression |

Table 2. Variables in morbidity in diabetic kidney transplant recipients: the co-morbidity index

| 1. Persistent angina or myocardial infarction |
| 2. Other cardiovascular problems, hypertension, congestive heart failure, cardiomyopathy |
| 3. Respiratory disease |
| 4. Autonomic neuropathy (gastroparesis, obstipation, diarrhoea, cystopathy, orthostatic hypotension) |
| 5. Neurological problems, cerebrovascular accident or stroke residual |
| 6. Musculoskeletal disorders, including all varieties of renal bone disease |
| 7. Infections including AIDS but excluding vascular access site or peritonitis |
| 8. Hepatitis, hepatic insufficiency, enzymatic pancreatic insufficiency |
| 9. Haematological problems other than anaemia |
| 10. Spinal abnormalities, lower back problems or arthritis |
| 11. Vision impairment (minor to severe—decreased acuity to blindness) loss |
| 12. Limb amputation (minor to severe—finger to lower extremity) |
| 13. Mental or emotional illness (neurosis, depression, psychosis) |

To obtain a numerical co-morbidity index for an individual patient, rate each variable from 0 to 3 (0 = absent, 1 = mild—of minor import will receive a kidney transplant. Maintenance haemodialysis is the only renal replacement regimen that will be employed for >80% of diabetic persons who develop ESRD in the US. Peritoneal dialysis is utilized for 12%, while only 8% will receive a kidney transplant. Maintenance haemodialysis fails to restore vigour to diabetic patients, as documented by Lowder et al. (1986), who found that of 232 diabetics on maintenance haemodialysis, only seven were employed while 64.9% were unable to conduct routine daily activities without assistance [38]. Approximately 50% of US diabetic patients begun on maintenance haemodialysis die within 2 years of their first dialysis due to co-morbid complications, mainly sepsis, heart and cerebrovascular disease. Figure 5 shows selected USRDS death rates by diagnosis in haemodialysis patients treated from 1993 to 1995 [2]. Peritoneal dialysis is hardly different from haemodialysis in its high mortality and dismal rehabilitation.

ESRD outcome

Maintenance haemodialysis is the only renal replacement regimen that will be employed for >80% of diabetic persons who develop ESRD in the US. Peritoneal dialysis is utilized for 12%, while only 8% will receive a kidney transplant. Maintenance haemodialysis fails to restore vigour to diabetic patients, as documented by Lowder et al. (1986), who found that of 232 diabetics on maintenance haemodialysis, only seven were employed while 64.9% were unable to conduct routine daily activities without assistance [38]. Approximately 50% of US diabetic patients begun on maintenance haemodialysis die within 2 years of their first dialysis due to co-morbid complications, mainly sepsis, heart and cerebrovascular disease. Figure 5 shows selected USRDS death rates by diagnosis in haemodialysis patients treated from 1993 to 1995 [2]. Peritoneal dialysis is hardly different from haemodialysis in its high mortality and dismal rehabilitation.

True, enthusiasts characterize continuous ambulatory peritoneal dialysis (CAPD) as ‘a first choice treatment’ for diabetic ESRD patients [39]. By contrast, Rubin et al., in a largely black diabetic population, reported that only 34% of their patients continued CAPD after 2 years and, at 3 years, only 18% remained on CAPD [40]. Limited by unequal cohorts and physician bias, there are no objective comparisons of either mortality or co-
morbidty in diabetic patients treated by haemodialysis vs peritoneal dialysis. Illustrating this problem are reports from the CANUSA study that attempted comparative survival and other variables in CAPD patients in the US and Canada [41]. All results were unable to account for a much increased relative risk of death (1.93) in the US, perhaps a function of a 2-fold greater acceptance rate for ESRD that may have enrolled sicker (greater co-morbidity) patients. Data subsets from the USRDS Report for 1997 [1] show that diabetic patients in all groups have a greater death risk on CAPD than on haemodialysis. Also, peritoneal dialysis patients in the US had a 14% greater risk of hospitalization than did patients undergoing hemodialysis [42]. At variance with these findings, Fenton et al., using data from the Canadian Organ Replacement Register for 11 970 patients who began ESRD therapy between 1990 and 1994, concluded that peritoneal dialysis ‘is not associated with increased mortality rates relative to hemodialysis’ [43]. The decision to treat a diabetic patient with CAPD, therefore, must be individual-specific.

Provision of a kidney transplant is unquestionably the best ESRD option in diabetes. In one centre, a retrospective review of all kidney transplants performed between 1987 and 1993 noted no significant difference in actuarial 5-year patient or kidney graft survival between diabetic and non-diabetic recipients overall or when analysed by donor source. Nor was any difference in mean serum creatinine at 5 years noted between diabetic and non-diabetic recipients [44]. The sharp superiority of survival following a kidney transplant when compared with peritoneal dialysis and haemodialysis is shown in Figure 6 extracted from the 1997 report of the USRDS [2]. Fewer than five in 100 diabetic ESRD patients treated by dialysis live for 10 years, while cadaver donor and living donor kidney allograft recipients fare far better. Rehabilitation is incomparably better following a renal transplant, compared with dialytic therapy. It is the enhanced life quality permitted by a kidney transplant that stimulates a strong preference for this ESRD option in newly evaluated diabetic persons with ESRD under the age of 60 years. Many 3-year survivors return to occupational, school and home responsibilities after a diabetic kidney transplant.

Applicable to no more than 9% of uraemic diabetic patients who have type 1 diabetes, pancreatic transplantation is growing in acceptability and technical success [45]. Global results in simultaneous kidney–pancreas transplants show that >90% of recipients were alive at 1 year, >80% had functioning kidney grafts, and >70% no longer required insulin [46]. However, inserting a functioning pancreas will not impede progression or recurrence of diabetic nephropathy. Unfortunately, pancreas transplantation in patients with extensive extrarenal disease has neither arrested nor reversed diabetic retinopathy, diabetic cardiomyopathy or extensive peripheral vascular disease [47,48]. Consensus thinking is that the ESRD patient with type 1 diabetes should consider a simultaneous kidney and pancreas transplant as at least a temporary cure of inexcusable disease [49].

For unexplained reasons, Europe attains better survival of diabetic ESRD patients [50] than does the US [51]. An example of European excellence is recorded from the UK: in seven large renal units between 1983 and 1985, patients starting CAPD or haemodialysis were monitored prospectively over 4 years. Of 610 diabetic and non-diabetic patients (median age 52 years, range 3–80 years) beginning CAPD and 329 patients (median age 48 years, range 5–77 years) starting haemodialysis, patient survival estimates at 4 years were 74% for haemodialysis and 62% for CAPD [52]. Only 8% of the study population had diabetes, however. Dialyser reuse and shortened treatment hours in the US have been incriminated as promoting fatal underdialysis [53]. On the other hand, rebutting the
allegation that US dialysis mortality is higher than that in Europe, it is argued that because the US has approximately twice the incidence of ESRD acceptance than Europe, older and sicker patients in the US obviously will experience greater mortality [54].

**Rehabilitation**

Pragmatic inferences from assessment of rehabilitation in diabetic ESRD patients include: (i) participation in structuring treatment is beneficial and (ii) renal transplantation permits markedly superior rehabilitation than the best attainable result employing either peritoneal dialysis or haemodialysis. In fairness to dialysis, however, unless patient cohorts (haemodialysis vs CAPD vs renal transplantation vs combined kidney and pancreas transplantation) have equivalent comorbidity, conclusions about modality effect on outcome are liable to be erroneous. We periodically inventory co-morbid problems as a co-morbid index utilizing the Karnofsky scoring system [55] to assess patient well being [56]. Gutman et al. in the 1970s who applied the Karnofsky scoring to 2481 dialysis patients found that diabetic patients achieved abysmal rehabilitation; only 23% of diabetic patients (vs 60% of non-diabetic patients) could perform physical activity beyond caring for themselves [58]. A decade later, Lowder et al. reported equally poor rehabilitation in diabetic haemodialysis patients [33]. Recent confirmation of this reality was presented by Ifudu et al. who documented pervasive failed rehabilitation in multicentre studies of diabetic and non-diabetic [58] and elderly inner-city [33] haemodialysis patients.

**What are AGEs**

In humans, ageing is associated with the Maillard reaction in which protein alteration results from a non-enzymatic reaction between ambient glucose and primary amino groups on proteins to form glycated residues called Amadori products. These Amadori products are then transformed to stable covalent adducts called advanced glycosylation end-products (AGEs) by a series of dehydration and fragmentation reactions. Hyperglycaemia in diabetes accelerates synthesis and tissue deposition of AGEs, an abnormality contributing to the pathogenesis of co-morbid complications [59]. Orally absorbed AGEs from common foods prepared routinely may impose an additional threat to the diabetic individual with reduced kidney function [60]. Dietary restriction of AGE food intake may be clinically applicable, though no trials have been performed.

AGEs are bound to a cell surface receptor (RAGE) inducing expression of vascular cell adhesion molecule-1 (VCAM-1), an endothelial cell surface cell–cell recognition protein that can prime diabetic vasculature for enhanced interaction with circulating monocytes, thereby initiating vascular injury [61]. Co-localization of AGE and RAGE was found in experimental diabetes in the intima, media and adventitia of the aorta and the inner limiting membrane of the retina and nerve bundles from mesenteric arteries [62], a finding suggesting that ligand–receptor interaction may be important in the genesis of diabetic complications.

**Why is renal transplantation superior to haemodialysis for the diabetic ESRD patient?**

AGEs are excreted by the kidney. With progressive loss of GFR, plasma AGEs increase in a curve similar to that of creatinine. Renal clearance of AGE-peptides is $0.72 \pm 0.23 \text{ ml/min}$ for normal subjects and $0.61 \pm 0.2 \text{ ml}$ for diabetics with normal glomerular filtration ($P$-value NS) [63]. A positive correlation
between mean serum AGEs and progression of diabetic nephropathy by kidney biopsy underscores the probable toxicity of AGEs [64]. Diabetic uremic patients accumulate AGEs in ‘toxic’ amounts that are not decreased to normal by haemodialysis or peritoneal dialysis [65]. Accumulation of AGEs in the human diabetic kidney begins in arterial walls and with deposition in nodular and exudative lesions in glomeruli [66]. After successful kidney transplantation, within 8 h of restoration of half-normal glomerular filtration, the plasma concentration of AGEs falls sharply [67]. We hypothesize from the foregoing, that the higher mortality of peritoneal dialysis and haemodialysis-treated diabetic patients compared with those given a renal transplant results from, at least in part, AGE toxicity. A multicentre trial of aminoguanidine in diabetic haemodialysis patients was initiated in 1996 to test this hypothesis.

Linkage between hyperglycaemia, hyperlipidaemia, nitric oxide activity and atherosclerosis in the pathogenesis of diabetic complications is highly probable. Diabetic individuals with renal insufficiency have elevated plasma apoprotein B (ApoB), very low density lipoprotein (VLDL) and low density lipoprotein (LDL). Circulating increased concentrations of AGEs react directly with plasma lipoproteins preventing their recognition by tissue LDL receptors, significantly increasing AGE-modified LDL in the plasma of diabetic or non-diabetic uremic patients compared with normal controls, possibly contributing to the accelerated atherosclerosis that is typical of diabetes and uremia [68]. It has been shown that LDL modified in vitro by AGE-peptides (to the level present in azotaemic diabetic patients) markedly impairs LDL clearance kinetics when injected into transgenic mice expressing the human LDL receptor, a finding indicating that AGE modification of LDL receptors promotes elevated LDL levels in azotaemic diabetic patients.

In the induced diabetic rat, AGEs exert toxicity by impairing nitric oxide-mediated vital processes including neurotransmission [69], wound healing [70] and blood flow in small vessels [71]. Thus, AGEs, by blocking the synthesis of nitric oxide, almost certainly interfere with maintenance of normal physiological processes such as autoregulation of blood flow [72]. Those actions of nitric oxide that may be pertinent to nephrologists have been reviewed [73].

Aminoguanidine

Pharmacological prevention of AGE formation is an attractive means of pre-empting diabetic microvascular complications because it bypasses the necessity of having to attain euglycaemia, an often unattainable goal. Aminoguanidine interferes with non-enzymatic glycosylation [74] and reduces AGE concentrations, leading to its investigation as a potential treatment. Brownlee et al. selected aminoguanidine because its structure is similar to that of o-hydrizinohistidine, a compound known to reduce diabetes-induced vascular leakage, while having opposite effects on histamine [75]. By blocking non-enzymatic glycosylation [76], aminoguanidine reduces AGE concentrations in the streptozotocin-induced diabetic rat. In rats made diabetic, aminoguanidine: (i) prevents development of cataracts, (ii) prevents AGE accumulation (measured by tissue fluorescence) in glomeruli and renal tubules and lessens glomerular basement membrane thickening, (iii) reduces severity of experimental diabetic retinopathy [77], (iv) blocks development of the ‘stiff myocardium’ that is a main component of diabetic cardiomyopathy [78]; and (v) stops diabetes-induced 24% impairment in maximal endothelium-dependent relaxation to acetylcholine for phenylephrine-precontracted aortas [79].

The strategic potential of blocking AGE formation to impede development of diabetic complications has been reviewed [80,81]. An attractive aspect to decreasing AGE production as a means of impeding diabetic complications is the elimination of the necessity for euglycaemia [82] a burden that impinges on every waking moment of the diabetic patient.

References

3. Friedman EA. Diabetic nephropathy is a hyperglycemic glomerulopathy. Arch Intern Med 1981; 141: 1269–1270
9. Inoguchi T, Battan R, Handler E, Sportsman JR, Heath W, King GL. Preferential elevation of protein kinase C isoform bII in diabetic kidney begins in arterial walls and with deposition in nodular and exudative lesions in glomeruli [66]. After successful kidney transplantation, within 8 h of restoration of half-normal glomerular filtration, the plasma concentration of AGEs falls sharply [67]. Those actions of nitric oxide that may be pertinent to nephrologists have been reviewed [73].
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