EDITORIAL

Cell biology of Diabetic Nephropathy

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Introduction

40 % of patients with juvenile insulin dependent diabetes mellitus (IDDM) develop nephropathy within 10-20 years of diagnosis [1], 20 % of middle aged, non-insulin dependent diabetes mellitus (NIDM) patients are found to have nephropathy only 5-10 years after the initial diagnosis [2]. Generally the patients with early proteinuria are those who have had poor glucose contrd [3] a slightly higher blood pressure (135: 85 mm Hg) and more marked retinopathy. They are those who soon have an increase of their GFR above normal and so they have hyperfiltration. They tend also to have a higher cholesterol, a higher LDL and VLDL, and elevation of their plasma fibrinogen [4]. After 5-10 years of diabetes we might detect microalbuminuria, which means an albumin excretion rate of 20-200 micrograms/minute in an overnight urine, or 30-300 mg/24 hrs in a complete collection. Then after another 5-10 years they will develop albumin stick positive proteinuria. The clinical sequence of the development of diabetic nephropathy was described in detail by Castiglioni and Savazzi [5].

When biopsies are obtained from diabetic patients, the changes that are typical are ' the hyalinosis of the afferent and efferent arterioles of the glomeruli [2], the thickening of the basement membranes of the glomerular capillaries [6], and[3] the increased volume of mesangial cells leading to an increase in volume of glomeruli. Once the expansion of the mesangium is more than 37 % of the glomerular volume, there is pressure on the capillaries with loss of filtration surface [7], so that thereafter there is functional renal impairment. Ultimately there is closure and obsolence of glomeruli, called glomerulosclerosis. Those patients with severe glomerular lesions have hypertension and reduced creatinine clearance, and there is renal interstitial fibrosis. There is evidente that this fibrosis develops concurrently with the increased fractional mesangial volume [8].

Clomerular Capillary Hipertension and Hyperfiltration

In rats that are made diabetic by means of streptorotocin and kept alive by injections of insulin there is a decreased resistance of the afferent arterioles, so that there will be increased pressure in the glomerular capillaries and there is hyper-filtration [9], which is determined by the increase in plasma flow. The factors that determine hyperfiltration have been discussed by Bank [10]. Hyperglycaemia is a first consideration [11] but so is a high protein intake [12]. A very high glucose (20 mM) inhibits cytosolic calcium signaling in cultured mesangial or vascular smooth muscle cells [11b]Hence there is vasodilatation. Sodium intake is also relevant. During hyperglycaemia induced osmotic flow, there is increased reabsorption of sodium in the proximal tubules [13]. In subjects who are using insulin, insulin is known to increase sodium reabsorption in the tubules [14]. The pituitary of diabetic patients secretes large pulses of growth hormone, possibly as a result of raised glucose in the CSF, and growth hormone increases GFR and renal blood flow [15] by the intermediary of insulin growth factor IGF-1 [16], In any case there is increased formation of nitric oxide vasodilator in the afferent arterioles in diabetes [17]. The hyperglycaemia also results in production of vasodilator prostaglandins in the afferent artérioles [18]. This production of prostaglandins seems to depend on polyol formation, because it is arrested when an aldose reductase inhibitor is used [19]. Hyperglycaemia determines also an increased production of thromboxanes at the vascular pole of diabetic kidneys and thus an increased tone in the efferent arterioles [10]. Indeed one knows that urinary thromboxane excretion is increased [10], and that proteinuria is ameliorated in animals given thromboxane synthetase inhibitor [20] Yet another consideration is that hyperglycaemia leads to formation of excess diacylglycerol withi cells and thus there is activation

of protein kinase C. Excess protein kinase C is known to mediate vascular permeability of the endothelium of blood vessels [21], In fact it mediates down-regulation of thromboxane receptors in diabetic glomeruli and mesangial cells [22], Angiotensin II receptors are likewise downregulated. In all this will explain the predominant vasodilatation in the afferent arterioles and so the hyperfiltration. The data in humans indicates some relation between early hyperfiltration and the development of diabetic renal disease. Thus in one study of children a GFR in excess of 125 ml/min 1.73 m^2 conferred a predictive value for nephropathy of 53 % [23]. Yet one must note that in that study half of the hyperfiltering subjects did not develop nephropathy during the period of follow-up. Thus hyperfiltration is not a highly sensitive predictive parameter. NIDDM patients also show hyperfiltration [24]. Itis common in Pima Indians [25].

It has been suggested that the patients who progress to diabetic nephropathy are those with a family predisposition to hypertension [26]. Hypertension will raise intracapillary pressures and worsen proteinuria. In some populations genetic susceptibility to essential hypertension can be linked to increased erythrocyte sodium-lithium counter-transport. There is a similar link with diabetic nephropathy [27]. However one must emphasize that this does not apply in all populations that have been studied [28].

Hypertrophy of the Kidneys

Soon after the onset of diabetes the size of the kidneys increase. All the glomeruli and their nephrons hypertrophy [29]. In the rat this hypertrophy precedes the increase of GFR [30]. The finding of increasedprotein kinase C activity in the glomeruli is relevant to growth [31]. Both hyperglycaemia and the growth factors work via pkC activation. It has been shown that the action of EGF, epidermal growth factor, works through the intermediary of increased protein kinase C activity [32].EGF is produced in the distal tubules and ascending loops of Henle [33], There is increased excretion of EGF in the urine of streptozotocin diabetic rats [34], The effects of growth hormone are known to be mediated by IGF-1 and this is anabolic and it is involved in hypertrophy of diabetic kidneys [35]. In short term studies the somatostatin analogue, octreotide, which suppresses growth hormone secretion, stops the early renal hypertrophy of diabetic rats [36]. Yetin the long-term it does not [37]. Hyperglycaemia stimulates production of TGFbeta in cultured proximal tubules [38].It is therefore possible that it could contribute to hypertrophy of the tubules, albeit how TGFbeta behaves depends on the phase of development.

There has to be a metabolic component to the hypertrophy that stems from the hyperglycaemia. Thus it has now been demonstrated that in the kidneys of diabetic rats there is an increase of glucosylceramide, a glycosphingolipid precursor. Furthermore an inhibitor of glucosyl-ceramide prevents the renal hypertrophy [39].

Vascular Changes in Diabetes

Hyalinosis of blood vessels is recognised as an adverse feature. It means that the vessel walls are permeable to molecules as large as fibrinogen. It implies that glomerulosclerosis will develop [40]. When it occurs leakiness of the postglomerular peritubular capillaries leads to protein accumulation in the interstitium of the kidneys [41] That will lead to renal interstitial fibrosis. The process is probably facilitated by iron that comes from transferrin in the urine of diabetics [42].

What causes increased endothelial cell permeability in diabetes? Firstly metabolism of glucose to diacylglycerol that mediates the formation of excess protein kinase C has been mentioned [21]. Secondly a role for conversion of glucose to sorbito1 via aldose reductase enzyme is probable, because the inhibitor sorbinil can reduce vascular permeability [43]. Thirdly there is a definite role for lipid peroxides, which arise when there is free radical formation linked to non-enzymic glucosylation of proteins [44-45]. The free-radicals arise from auto-oxidation reactions of sugars and sugar adducts to proteins and by auto-oxidation of unsaturated lipids adjacent to altered membrane proteins. Monocytes of diabetics often produce superoxide anions and thus hydrogen peroxide [46]. Polymorphonuclear leucocytes in serum that is high in cholesterol also form superoxide anions [47]. It is usual for the low density lipoproteins of diabetics to become oxidised [48]. Apart from that one can gauge free radical activity in diabetic rats by their expiration of pentane [49], and one canmeasure plasma lipid peroxides and lipid peroxidation of kidneys in diabetic rats [50],

In human studies several groups have now related lipid peroxides in plasma [51] (measured by thethiobarbituric acid reaction) to endothelial cell damage as shown by a raised plasma von Willebrand factor. The patients with raised plasma malonyldialdehyde (indicative of lipid peroxides) and raised plasma von Willebrand factor were also those who had microalbuminuria [52-54]. Furthermore patients with raised plasma MDA were those with tubular damage as shown by increased excretion of NAG, N-acetyl-glucosaminidase, in their urine [54]. When sought in the correct

manner, marked evidence of tubular damage will be found in many diabetics [55,56].

Oxidised LDL has altered properties [57] that are pertinent to atherogenesis [58]. Oxidised LDL is chemotactic for monocytes and it is taken up by macrophages in arterial walls to cause their cholesterol enrichment and foam cell formation. Oxidised LDL causes platelet aggregation. It stops the action of nitric oxide and so it can promote vasoconstriction in small arterioles. Oxidised LDL can be cytotoxic for endothelial cells. In lesser doses it causes the expression of «tissue factor» thromboplastin on endothelial cells [59], so that there is a thrombotic tendency in small arterioles. It also prevents activation of protein C and so it thwarts protective fibrinolysis. The severe atheroma that many diabetics develop has implications for kidney function. For example platelet aggregates forming in the aorta will be swept into the glomeruli to cause lesions like focal segmental sclerosis [60]. Also many diabetics do have renal artery stenoses. Recognition of this fact is necessary before the prescription of ACE inhibitors.

Non-enzymatic glucosylation of proteins produces ACEP, advanced glycosylation end-products [45]. They accumulate in the tissues of diabetics [61] and will undoubtedly play a role in nephropathy. Mesangial cells express AGEP receptors [62] and when stimulated by AGEP they form basement membrane proteins [63]. AGEP have oxidising potential [64], and when they accumulate in blood, as they do in terminal renal failu- [65] they encourage procoagulant change on the endothelial cells [66], both directly and via release of cytokines like Il-1 and TNFa [67].

Glycosaminoglycans and Collagen

There is poor synthesis of heparan sulphate proteoglycans by the renal glomeruli in diabetes mellitus [68]. There is a genetic factor involved in different strains of rats [71], and it has also been suggested that the varying liability to diabetic vascular disease in humans [69] might also depend on some factor like this. Hyperglycaemia also stops proteoglycan synthesis by mesangial cells [70]. When thereis poor diabetic control there is inhibition of the N-acetyl heparan deacetylase enzyme that is required for heparan sulphate proteoglycan synthesis. Furthermore there is loss of heparan sulphates in the urine at the onset of diabetes in rats [72]. When biopsies from human diabetic nephropathy were examined, a marked reduction in reactivity to anti-heparan sulphate proteoglycan antibodies was observed, but one has to acknowledge that these were cases of quite advanced disease [73].

The importance of the heparan sulphates is that the negative charges of their sulphate groupings on endothelial cells, and in the basement membranes of the glomeruli and on the mesangial cells, repel negatively charged albumin molecules, so that their filtration is prevented. So it would seem that loss of heparan sulphates at such an early stage in diabetes [74] could account for the onset of micro-albuminuria. Indeed Gambaro et al.[75] have show.n that, when streptozotocin diabetic rats are given injections of either low MW heparin or dermatan sulphate glycosaminoglycan, there was inhibition of mesangial cell expansion and thickening of the glomerular basement membranes was reduced. The heparan sulphates of the basement membrane are essential for the integrated binding of the other components like the type IV collagen and laminin.

Another factor that contributes to proteinuria must also be the poor synthesis in diabetes [76] of thenegatively charged sialoproteins that line the slit pores between the epithelial cells.

The basement membrane width expands by about 30 % during the first 5 years of diabetes and by the time of clinical nephropathy its width has doubled [6]. Not only is there loss of negative charges [73] but the closely woven structure must be disorganised, perhaps by the addition of glucoadducts in the process of non-enzymic glucosylation [61,77-79], surely by the effect of ACE products causing collagen browning [80], and surely as a result of the loss of heparan sulphate proteoglycans [81]. When in an experimental situation aminoguanidine is used to decrease AGE products [82], the proteinuria is reduced [83].

A high ambient glucose (30 mM) increases the synthesis of type IV collagen by cultured endothelial, mesangial and epithelial cells [84-85]. Undoubtedly the ability of high glucose to drive protein kinase C may explain this [86]. However one should also be aware that lipid peroxidation enhances the synthesis of type I V collagen Also it can be shown in vivo that thromboxanes play a role, because when thromboxane synthase inhibitors are used basement membrane thickening and mesangial matrix expansion is reduced [87,88]. The basic fact that high ambient glucose causes increases messenger RNA for type IV collagen [89,90] has now been verified by many groups. Likewise proximal tubules that are exposed to glucose will synthesise type IV collagen of tubular basement membranes [91], It is reported that sorbinil prevents the biosynthesis [91].

Clearly the altered structure of the GBM explains the albuminuria and the loss of charge selectivity that can now be measured by study of the clearance of IgG/IgG4 [92,93]. As can be shown by dextran clearances, there is initially no increase of pore size at a time when there is substantial loss of albumin and IgG,

but pore size is increased later. It is relevant to note that glycated albumin readily leaks through the glomeruli and it is not reabsorbed by the proximal tubules [94]. In any case.there is often proximal tubule dysfunction as shown by lysozymuria [95].

The Altered Mesangia of Glomeruli of Diabetics

The feature that is so typical of diabetes is the early mesangial cell expansion that is followed years later by mesangial sclerosis [5,7]. Thus the picture differs from the glomerulonephritides in which one often expects to see mesangial cell proliferation that is caused by growth factors [96]. By now one can list various experimental observations that explain why the mesangial cells do not proliferate. 1) A high ambient glucose (20 mM) inhibits mesangial cell proliferation, although it does promote fibronectin synthesis [97]. 2) Although a high glucose increases protein kinase C in mesangial cells [98] and thus formation of extracellular matrix fibronectin, laminin and type IV collagen, high glucose also inhibits cytosolic calcium signalling [99]. Hence the cells will tend to be relaxed and spread out rather than contractile. 3) Non-enzymatic glucosylation and formation of ACE products causes mesangial expansion and inhibition of mesangial cell proliferation 4) On account of the aldose reductase content of the mesangia, exposure to high glucose will result in formation of polyols [101] and there will be a reduction of the myoinositol of the cells [102]. 5) When there is a high glucose, prostaglandin production by mesangial Cells is increased [103] Prostaglandins inhibit cell oroliferation [96]. Potential high glucose induced mesangial cell proliferation is inhibced also by transforming growth factor beta [104]. 6) Highglucose can inhibit the cell proliferative effect of ICF-1 [105]. 7) Low density lipoproteins at a concentration of only 10 g/ml stimulate proliferation of mesangial cells and yet at a level of 100-500 g/ml, as would be the case in any hyperlipidaemia, there is inhibition [106].Such LDL will stimulate superoxide production by mesangial cells and thus may become oxidised [107]. 8) Oxidised LDL reduces release of growth factors from macrophage like cells [108]. Oxidised LDL bind very well to mesangial cells and inhibit their proliferation at concentrations as low as 10-25 g/ml [109].

Although these observations make good biochemical sense, they are mainly based on in vitro studies. When RNA messengers are looked at in the early stages of diabetes in Sprague-Dawley rats those for TNFalpha, basic fibroblast growth factor and PDGF-B chain and for transforming growth factor beta are increased [110]. Indeed their levels are reduced by insulin therapy [110]. Nevertheless rats are not as hyperlipaemic as man might be.

We know from histology that glomerulosclerosis (mesangial sclerosis) will ultimately develop and that follows increasing deposition of fibronectin and type IV collagen. High glucose leads to loss of proteoglycans [111] and it promotes formation of fibronectin and collagen [97-98], albeit one set of studies showed that over a long time high glucose suppresses collagen production [111]. That might be due to ascorbic acid depletion, since that is well recognised in diabetes.

Certainly it seems that hyperlipidaemia will mediate glomerulosclerosis, as in other situations [112]. Immunohistochemical studies of the localisation of apolipoproteins in glomeruli has shown that fixation of apolipoprotein B with apoE gives rise to more glomerulosclerosis and interstitial scarring [113].

What more does one need to know? Since mice transgenic for bovine growth hormone[114] develop mesangial cell proliferation by 4 weeks, mesangial sclerosis by 20 weeks and glomerulosclerosis by 36 weeks, what are the mediators of the response? Presumably PDGF autocrine production in glomeruli is involved at the early stage [110]. Since TGFbeta is the mediator of glomerulosclerosis in most situations, one has to assume that this is also the case in diabetic nephropathy [110], Indeed it does seem that high glucose stimulates autocrine production of TGFbeta by mesangial cells [104].

Furthermore the release of TGFbeta stops mesangial cell proliferation but increases the deposition of mesangial matrix [115].

Final Synopsis

By now it has been shown in rats with diabetic nephropathy and in humans that TGFbeta values are elevated in the glomeruli [115]. Thisis a clear indication that TGFbeta mediates glomerulosclerosis.

Secondly Cohen and Ziyadeh [117] have examined the effects of glycosylated proteins on glomerular mesangial cells. They have shown that glycosylated proteins (i) stop proliferation of mesangial cells, and (ii) stimulate the mesangial cells to transcribe the genes for type IV collagen. So growth of mesangial cells is inhibited, as was recorded by Crowley et al.[100], and type IV collagen production occurs in diabetic glomeruli. One should note that when glycated proteins are taken up by mesangial cells, there is intracellular production of hydrogen peroxide [64,118] We do know that lipid peroxidation is a stimulus to collagen production [66], Infact Cohen and Ziyadeh[117] used glycated serum proteins that had no cross-links and showed no AGE fluorescente, whereas Crowley et al. [100] specificallyused fluorescent cross-linked AGE products. Thus either product can influente the genes for collagen.

Thirdly there is more information on the contraversial topic of whether or not nitric oxide production by endothelium is increased o decreased in diabetes. The answer is that NO production can be increased or decreased depending on the vascular bed. Nitric oxide production is increased in the afferent arteriales of diabetic glomeruli and thus this is a factor that explains vasodilatation and hyperfiltration. On the other hand it has been shown that carbamvlcholine induced cyclic GMP is decreased within isolated diabetic glomeruli in parallel with hyperglycaemia induced increases of protein kinase C [119]. Furthermore in that situation thromboxane formation is increased[88] and this is another factor [120] that contributes to production of glomerular extracellular matrix proteins.

Hence hyperglycaemia causes non-enzymatic glycosylation of proteins and they reduce mesangial cell proliferation but cause mesangial expansion and type IV collagen production. Also hyperglycaemia induced protein kinase C, and hence thromboxanes, cause matrix deposition.

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