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Role of glycosaminoglycans in diabetic nephropathy

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Abstract. A number of abnormalities of glycosaminoglycan metabolism have been reported in diabetes mellitus. These include anomalous synthesis, catabolism and sulphation. In this review glycosaminoglycan metabolism in diabetes mellitus is discussed with reference to its possible impact on glomerular basement membrane permeability, and on the physiology and metabolism of glomerular resident cells, specifically of the mesangium. Experimental results demonstrating favourable therapeutic activities of some glycosaminoglycans in mesangioproliferative glomerulitis and diabetic nephropathy are reported. These pharmacological actions do not depend on the well-known anticoagulative activity of glycosaminoglycans, but most probably on their antiproliferative effect. A more complex role of glycosaminoglycans in the pathogenesis of diabetic nephropathy is suggested.

Key words: Diabetic nephropathy – Glomerular basement membrane – Growth factors – Glycosaminoglycans – Heparan sulphate

Introduction

As the basement membrane constitutes the major functional component of the renal glomerulus and the only continuous anatomical barrier between blood and urine, it also appears to be the logical site at which disease-induced changes could lead to a filtration defect.

Basement membranes are composed mainly of collagen IV, laminin, entactin and perlecan (a heparan sulphate proteoglycan) [1, 2]. Collagen IV provides the structural framework, while laminin, a large glycoprotein consisting of three chains, appears to mediate many cell–basement membrane interactions influencing cell attachment, movement, differentiation and polarization. Entactin binds tightly to laminin and also contributes to cell attachment. Perlecan has a large protein core to which three heparan sulphate (HS) glycosaminoglycan (GAG) chains are attached; it imparts a negative charge to the basement membrane and plays a role in molecular permselectivity. Perlecan and other proteoglycans in the different basement membranes also serve as structural organizers of other basement membrane components (collagen IV, laminin, entactin, fibronectin) by virtue of their ability to interact at specific sites within each of these molecules [3–5].

The primary factors determining glomerular basement membrane (GBM) permeability appear to be pore size and a fixed electrostatic charge [8]. Anionic sites on the GBM are arrayed in a periodic manner (60 nm) and composed mostly of a large, basement-membrane-specific HS proteoglycan [7]. This proteoglycan was postulated to be the main charge-selective exclusion barrier that restricts the passage of plasma proteins across the glomerular wall [8, 9]. The demonstration that removal of HS by in situ enzymic digestion led to a dramatic increase in GBM permeability to ferritin or ^{125}I-labelled Bovine serum albumin lends support to this hypothesis [8, 10]. This view is further corroborated by studies showing that GBM permeability increased following intrarenal or intravenous injection of cationic molecules due to neutralization of the HS-associated anionic sites of the glomerular capillary wall [11, 12], and that acute selective proteinuria was induced by intravenous injection of a monoclonal antibody against GBM HS [13]. In several clinical and experimental glomerulopathies, moreover, increased GBM permeability was associated with diminished GBM HS content [14], or a change in HS structure leading to an overall lower negative charge [9, 15–17].

Possible GAG abnormalities during diabetes mellitus have attracted the attention of many investigators because: (1) diabetic nephropathy appears early with albuminuria; (2) this phenomenon implies abnormal GBM permeability; and (3) GAGs, and HS in particular, have a crucial role in determining GBM charge permselectivity, as seen above. Here we will discuss GAG abnormalities in diabetes, essentially at the renal level, considering synthesis and content (Tables 1, 2), catabolism and sulphation (Table 3).

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Table 1. Glomerular glycosaminoglycan content in diabetes

Reduced	Normal
Parthasarathy and Spiro 1982 [19] Wu et al. 1987 [20] Shimomura and Spiro 1987 [21] Heuvel and Berden 1992 [22]	—

Table 2. Glycosaminoglycan synthesis in diabetes

Decreased	Normal
Brown et al. 1992 [24] Rohrbach et al. 1982 [26] Kanwar et al. 1983 [23] Rohrbach et al. 1983 [25] Kiellén et al. 1983 [27] Cohen and Surma 1984 [18] Levy et al. 1984 [28]	Spiro 1987 [21] Ledbetter et al. 1990 [29]

Table 3. Glycosaminoglycan sulphation in diabetes

Decreased	Normal
Kiellén et al. 1983 [27] Levy et al. 1984 [28]	Parthasarathy and Spiro 1982 [19] Kanwar et al. 1983 [23] Rohrbach et al. 1983 [25] Klein et al. 1989 [36] Deckert et al. 1991 [39]

Altered synthesis and content of glycosaminoglycans

In diabetes, glomerular GAG synthesis and content are reduced [18, 19]. In a study of human kidneys from normal individuals and diabetic subjects in which the exact level of clinical and histological renal involvement was not given, Parthasarathy and Spiro [19] found a decrease in the HS contents of the diabetic GBM; the extent of GAG sulphation appeared to be similar in both groups. With more accurate methods, Wu et al. [20] and Shimomura and Spiro [21] confirmed decreased HS levels in streptozotocin (STZ)-treated rats and diabetic humans, respectively. More recently, reduced GBM HS proteoglycan levels were also found using specific antibodies [22].

The decreased GAG contents of the human diabetic basement membrane would appear to be consistent with a decreased synthesis, as deduced by GBM GAG incorporation of ^{35}S [20, 23, 24]. In a similar manner, a reduced [^{35}S]heparan proteoglycan synthesis was observed in EHS (Engelbreth-Holm-Swarm) basement-membrane-producing tumour grown in STZ-treated mice [25], and in diabetic *db/db* mice [26], as well as in the aorta [24], liver [27] and intestinal epithelium [28] of STZ-induced diabetic rats, suggesting that the GAG metabolism disorder present in the glomeruli of experimentally diabetic animals might be a more generalized phenomenon.

Recent studies using cDNA probes for α1 chain of collagen IV and perlecan demonstrated a substantial increase in collagen IV mRNA levels relative to perlecan in KKAy diabetic mice [a model of (non-insulin-dependent) type 2 diabetes that demonstrates GBM thickening with age and diabetes duration], although average perlecan mRNA levels were unchanged in diabetic animals compared with controls [29]. Numerous biochemical studies in human and animal models of diabetic nephropathy demonstrated increased amounts of collagen IV, which probably account for the thickened diabetic basement membrane [30]. Interestingly, in KKAy diabetic mice a negative correlation existed between the HS proteoglycan/type IV collagen ratio and albuminuria [29]. Very similar results were obtained by Parthasarathy and Spiro in human diabetes, in which GBMs have a decreased hexuronic acid content, in contrast to the significant rise in the neutral sugar constituents [19]. Since hexuronic acid is associated with basement membrane proteoglycans, while the neutral hexoses are primarily found in the hydroxylysine-linked glucosylgalactose disaccharide of collagen, these workers' findings suggest that the macromolecular components undergo a redistribution in the diabetic state.

The above data indicate that the production of basement membrane proteins in response to diabetes is not regulated in a coordinated manner. The relative proteoglycan decrease demonstrated by mRNA assay, however, may not reflect the true perlecan contents of basement membrane. Proteoglycan contents in fact also depends on its catabolism; altered turnover rates of connective tissue components could be of particular importance in diabetes, because elevated blood glucose levels may accelerate non-enzymic glycation, and thereby modify protein-to-protein interactions affecting stability and turnover [30–32].

In STZ-induced diabetes, however, GAG turnover in the GBM does not seem to be altered [20], while collagen turnover was reported to be lowered [33, 34].

These phenomena, together with increased collagen synthesis, would translate into an expansion of the GBM/matrix and abnormal biochemical characteristics, consisting of relative depletion of sulphated GAGs and enhancement of collagen components.

Abnormal supramolecular complexation of glycosaminoglycans

Brown et al. [24] reported in increased proportion of ^{35}S-labelled material in the incubation medium of diabetic glomeruli. This phenomenon might have been due to either increased degradation or a decrease in the ability of normally highly negatively charged proteoglycans to interact with other connective tissue components in the diabetic glomerulus. In view of the very short experimental times, the first hypothesis seems unlikely. To explore the second hypothesis, this group used heparin treatment to release receptor-bound proteoglycans from glomerular cell surfaces or extracellular matrices; their findings suggested a diminished tissue affinity for heparin-releasable HS proteoglycan in diabetes [35, 36]. Data from Wu et al. support this observation [20]. It was held that the phenomenon might have been the consequence of a change either in GAG charge distribution/sulphation or

in other extracellular matrix macromolecules (i.e. fibronectin or laminin), possibly secondary to non-enzymic glycation, but since no difference in GAG charge was observed the latter possibility was favoured. The consequences of this phenomenon [20, 36] might be far-reaching; since cell-surface proteoglycans can be co-isolated with cytoskeletal elements, they may contribute to communication between the extracellular matrix and the cell [37, 38]. Changes in the interaction between cell-surface proteoglycan and extracellular matrices in diabetes, for instance, could signal the compensatory synthesis of other extracellular matrix components, such as type IV collagen.

Abnormal sulphation of glycosaminoglycans

Controversy surrounds the very interesting and recent issue of GAG sulphation. While most studies seem to deny the existence of any difference in the sulphation ratio of GBM GAGs [19, 23, 25, 36, 39], others, using perhaps more sensitive techniques, show a reduction in sulphation [27, 28]. These investigations, however, report very small differences in the degree of sulphation, and this might explain the contradictory results in the literature; nonetheless, even subtle differences might have a profound physiological impact [40].

The enzyme *N*-acetylheparosan deacetylase plays a key role in the biosynthesis of HS because *N*-deacetylation of the HS glucosamine units is a prerequisite for *N*-sulphation and further modification of the polymer [41–43]. Inhibition of *N*-deacetylase activity will therefore impair HS sulphation. In STZ-induced diabetic rats this enzyme's activity is significantly inhibited both in the liver and in the glomerulus [44]; insulin treatment reverses the inhibition. *N*-deacetylase expression depends on the concerted action of two protein components designated as E and F; component E shows *N*-sulphotransferase activity, and its reduced level in diabetes explains the low activity of *N*-deacetylase [45]. A genetic difference exists between different rat strains concerning *N*-deacetylase activity. It is lower in the Uppsala strain of Sprague-Dawley rats, which is characterized by diabetes-induced alterations in the synthesis of extracellular matrix components and a high rate of skeletal malformations among offspring [46–48]; moreover, despite excellent metabolic control, *N*-deacetylase activity cannot be normalized. It is noteworthy that after a follow-up of 8 weeks, diabetic Uppsala rats have a 10-fold higher albuminuria than diabetic control rats [49].

On the basis of these data, and the assumption that GAG sulphation is abnormal in diabetes, the Steno Memorial Hospital group postulated that albuminuria and the associated long-term diabetic complications are due to a genetic polymorphism of the enzymes involved in HS proteoglycan metabolism, and specifically *N*-deacetylase [50]. According to this hypothesis, patients who develop diabetic nephropathy would be characterized by iso-enzymes that are extremely vulnerable to poor diabetes control. Thus, polymorphism of the enzymes involved in the metabolism of HS proteoglycan might explain the varied prognoses in poorly regulated diabetic patients, as well as the fact that only 35% of type 1 diabetic patients develop albuminuria.

Deckert et al. studied this hypothesis in type 1 diabetic patients with or without diabetic nephropathy [39] by evaluating proteoglycan biosynthesis in fibroblast cultures established from skin biopsies. The relative distribution of synthesized GAGs was abnormal in diabetic patients with nephropathy; in fact, when [^{3}H]glucosamine incorporation was determined, HS was relatively decreased in relation to total GAG or hyaluronic acid; ^{35}S incorporation, however, showed no difference. The relative decrease in the ratio of [^{3}H]HS to GAG production, seemed in part therefore, a consequence of increased hyaluronic acid production. These findings were interpreted as evidence of a non-coordinated regulation of GAG biosynthesis in patients with diabetic nephropathy, a phenomenon that most probably has a genetic basis. No difference emerged when HS *N*-sulphation was evaluated in these fibroblast cultures, suggesting that no major genetic anomaly in *N*-sulphation exists in fibroblasts from diabetic patients.

Therefore, although the hypothesis of a genetic polymorphism of the enzyme involved in HS metabolism is fascinating, the evidence supporting it is still weak.

Other glycosaminoglycan abnormalities

The possibility of differences in mesangial matrix proteoglycans, as well as abnormalities in the protein core of proteoglycans, deserves attention, even though few studies have addressed these topics. Brown et al. [24] and Klein et al. [36] reported data supporting the view that there is no qualitative difference in mesangial matrix GAGs; in fact the percentage distribution of chondroitin sulphate, dermatan sulphate and HS was similar in control and diabetic rats. No gross difference seems to exist in diabetic glomeruli concerning the protein core of HS proteoglycans [21, 23, 36]. Other aspects of GAG biochemistry in diabetes that have not been sufficiently investigated are GAG anomalies other than HS, renal versus generalized GAG anomalies, and anomalies of matrix/basement membrane GAGs vs cell surface GAGs.

Glycosaminoglycan effect on cell biology and possible relation to diabetic nephropathy

We have seen how diabetes affects HS metabolism, and probably leads to generalized membrane alterations, specifically in the GBM and plasma membranes [51–55]. In diabetic patients with albuminuria these alterations are severe enough to induce changes in GBM permeability, and thus bring about an increased protein traffic through the mesangium, and possibly abnormal mesangial cell proliferative and/or metabolic behaviour. In this sense, abnormal GBM permeability and albuminuria might per se be the cause of glomerular sclerosis and renal failure.

However, several observations (Table 4) raise the possibility that abnormal GAG metabolism has a more

Table 4. Glycosaminoglycan effects on cell biology

Heparin/heparan sulphate:
Inhibits cell growth
Controls extracellular matrix composition
Controls assembly of matrix proteins
By several mechanisms:
Binding to cellular receptors
Being processed into potent antiproliferative metabolites for adjacent cells
Modifying the synthesis of individual extracellular matrix components
Modulating the availability of growth factors in the extracellular milieu
Interfering with specific events in the cell

intriguing role in the pathogenesis of long-term diabetic complications, namely nephropathy.

GAGs have been implicated in the inhibition of cell proliferation. HS-like molecules, derived from aortic and glomerular endothelial cells and glomerular epithelial cells, and heparin inhibit the proliferation of arterial smooth muscle cells, and similarly derived cells such as pericytes and mesangial cells [56–60]. Yet heparin inhibits rat glomerular epithelial growth [61]. Smooth muscle cells possess specific receptors for heparin/HS (these two GAGs have structural features in common), and can internalize this bound GAG [62]. Thus it is possible not only that these GAGs/GAG-fragments may suppress the growth of smooth muscle and derived cells at specific intracellular sites, but also that changes in the structures and amount of intracellular HS may alter the growth behaviour of these cells. Indirect evidence of such a phenomenon was found in hepatocytes [63].

At growth-inhibiting dosages, heparin alters the secretory phenotype of cultured arterial smooth muscle cells [64–67]; for instance, it can induce the synthesis of a 60000 molecular weight collagen-like protein [65], or of fibronectin and thrombospondin [67]. Moreover, it stimulates the synthesis of HS 2- to 3-fold and increases its degree of sulphation in cultured endothelial cells [68]. In this way, heparin was shown to influence the synthesis and composition of the extracellular matrix.

In vitro studies demonstrated that heparin might also affect the extracellular matrix composition by regulating the assembly of laminin and collagen IV [69].

Proteoglycans as well may act as receptors for growth factors. Cell-associated HS proteoglycans may serve as a receptor for thrombospondin [70], the importance of which in regulating smooth muscle cell growth [71] suggests that proteoglycans present on the smooth muscle cell surface are involved in growth regulation. The high molecular weight receptor for transforming growth factor-β (TGF-β) (a protein that influences growth and connective tissue synthesis in many cells) is a proteoglycan containing HS and chondroitin sulphate chains [72].

Proteoglycans may exert their activity on matrix composition and smooth-muscle-derived cells by influencing the availability of molecules necessary for cellular proliferation. Heparin's inhibitory effect on smooth muscle cells depends on the presence of TGF-β, which in the serum is bound in a biologically inactive form to α_2-macroglobulin. It is likely that heparin frees TGF-β from its binding site on α_2-macroglobulin, and forms a new stable complex in which TGF-β becomes biologically active in cell proliferation and matrix synthesis [73]. Heparan sulphate also exhibits affinity for other growth factors such as fibroblast growth factor (FGF) [74]. Furthermore, decorin, a dermatan sulphate proteoglycan synthesized by mesangial cells, forms a complex with its core protein and TGF-β [75]. HS binds basic FGF in the extracellular matrix and in the basement membrane [76–78]; if HS chains are destroyed by heparinase, basic FGF is released and easily degraded by proteases [74], and thus is no longer available for mitogen activity.

By binding growth factors to their core protein (i.e. decorin and TGF-β), or the carbohydrate chains (HS proteoglycan and basic FGF), it is possible that proteoglycans constitute a reservoir of growth factors in the mesangial matrix or in the GBM, very close to the target cells [79].

Therefore, by means of the mechanisms suggested above, we may speculate that the derangement in proteoglycan metabolism occurring in diabetes may have definite, important consequences on the growth and synthesis behaviour of resident cells in the kidney, and thus play an important role in the development of diabetic nephropathy. A few recent reports support this view. Border et al. showed that TGF-β is unique in regulating the matrix protein production in cultured mesangial cells [75]; moreover, in an experimental model of mesangioproliferative glomerulonephritis in the rat, matrix protein synthesis was increased, but following the administration of antibody against TGF-β matrix synthesis greatly diminished, glomerulosclerosis was prevented and proteinuria did not appear [80]. More recently these workers demonstrated that exactly the same results could be achieved by administering decorin [81].

Using STZ-induced diabetic rats, we verified that the administration of GAGs could prevent renal involvement [82]. A number of experimental studies in different models of glomerulosclerosis and mesangial proliferation demonstrated that heparin and heparin derivatives had favourable effects [83–87]. We studied male Sprague-Dawley rats in which diabetes was induced by STZ; one group was treated daily with a low molecular weight heparin, and another with dermatan sulphate. Animals were followed for 8 months. Morphometric analysis disclosed that GBM thickness was essentially normal in the animals treated with GAGs, while it was markedly increased in untreated diabetic animals; charge density in the former was in the normal range, and significantly reduced in the latter. Albuminuria was normal in treated rats, and markedly increased in the diabetic untreated rats. These results were obtained in the absence of a favourable GAG effect on metabolic control. Considering the different targets in the coagulation cascade of dermatan sulphate and low molecular weight heparin, we believe that the positive action exerted by the two GAGs does not depend on their effect on coagulation. Creatinine clearance showed no changes; moreover, using micropuncture in subtotally nephrectomized rats, Ichikawa demonstrated that heparin slowed down the progression to renal failure

without any haemodynamic effect [88]. The hypothesis that the protective effect exerted by GAGs is simply due to a mechanical restoration of glomerular charges by these polyanionic compounds [86] seems insufficient to explain the full picture in our experimental model. In fact, the favourable effect we observed was on the visceral side of the glomerular sieve, and not on the endothelium where such restoration of anionic charges had previously been demonstrated; moreover, the beneficial effect not only involved GBM charge density but, more interestingly, its thickness as well. This suggests a more complex GAG effect on glomerular cell synthesis of matrix/GBM constituents, leading to the correction of some biochemical or cell function abnormalities associated pathogenetically with diabetic nephropathy.

In conclusion, our data not only demonstrate that long-term GAG administration has a favourable effect on the morphological and functional renal abnormalities in diabetic rats, but also indirectly support the hypothesis that the derangement in GAG metabolism might have a pathogenetic role in the onset of diabetic nephropathy.

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