Rapid Publications

Effect of Diabetes on the Glycosaminoglycan Component of the Human Glomerular Basement Membrane

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SUMMARY

The glycosaminoglycan (heparan sulfate) component of glomerular basement membranes from human kidneys of diabetic and nondiabetic subjects has been quantitated after isolation from protease digests of the membranes on DEAE-cellulose microcolumns. A significant decrease ($P < 0.005$) in the glycosaminoglycan content of diabetic membranes was observed. Heparan sulfate was identified as the predominant glycosaminoglycan in both diabetic and control subjects and the extent of its sulfation appeared to be similar. The reduced level of glycosaminoglycan in the diabetic glomerular basement membrane was accompanied by a significant elevation of hexoses, which are primarily associated with the collagen component, suggesting that a redistribution of basement membrane macromolecules occurs in the diabetic state. Since heparan sulfate has been implicated as a major component of the glomerular anionic filtration barrier, its decreased content in diabetic basement membranes may contribute to the proteinuria observed in this disease. DIABETES *31*:738–741, August 1982.

Although it has been recognized for some time that basement membrane thickening and mesangial expansion is a characteristic morphologic feature of diabetic glomerulopathy, the relationship between these ultrastructural alterations and the clinically observed proteinuria remains to be established.[1] As the major functional component of the renal glomerulus and the only continuous anatomic barrier between the blood and urine, the basement membrane would appear to be a logical site at which changes brought about by disease could lead to a filtration defect.

The primary factors determining glomerular basement membrane permeability appear to be pore size as well as fixed electrostatic charge.[2] While the former may be a function of packing of the collagen polypeptide chains, recent studies suggest that the latter may reside in a heparan sulfate glycosaminoglycan[3] that has been isolated, characterized, and shown to be an integral constituent of the basement membrane.[4,5] Since the glycosaminoglycan component was until lately unrecognized and is present in very small amounts[6] (<1% by weight), previous studies of diabetic basement membranes have not provided information on this component.

In the present investigation we have quantitated the heparan sulfate of glomerular basement membranes from human diabetics with the help of a recently developed DEAE-cellulose microcolumn fractionation procedure[6] and find that it is present in significantly decreased amounts.

MATERIALS AND METHODS

Human kidneys from diabetic and nondiabetic subjects were obtained at autopsy from the New England Deaconess and the Brigham and Women's Hospitals and stored at −70°C in airtight plastic bags until use. Glomerular basement membranes from individual kidneys were studied from 9 diabetic and 10 nondiabetic subjects, whose ages ranged from 43 to 66 yr (mean 60 yr) and from 37 to 68 yr (mean 59 yr), respectively. In both groups all individuals but one were above the age of 50. In the diabetics, the duration of the disease ranged from 7 to 32 yr (mean ± SDM, 16.9 ± 3.4 yr) and microscopic examination revealed evidence of diabetic glomerulopathy in all cases with varying degrees of severity; six had been on insulin therapy while the remainder were treated with diet or oral hypoglycemic agents. The nondiabetic subjects died of causes unrelated to kidney disease, primarily due to myocardial infarction and cerebrovascular accidents; microscopic examination indicated that their glomeruli were within normal limits.

Basement membranes were prepared as previously de-

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scribed[7] by sonication of glomeruli that were isolated from renal cortex by a sieving procedure. The sonicates were filtered through a 230-mesh sieve to remove any tissue fragments or undisrupted glomeruli. The glycosaminoglycans were isolated from the collagenase-pronase digests of approximately 10-mg samples of basement membranes by DEAE-cellulose chromatography in pyridine formate buffers at pH 5.0 on microcolumns as previously reported.[6] This procedure permits separation of glycosaminoglycans that elute with 2 M pyridine formate from glycopeptides containing the hydroxylysine- and asparagine-linked carbohydrate units that emerge in the 0.05 M pyridine formate wash and 0.5 M pyridine formate eluate. After column separation, the glycosaminoglycans were quantitated as before[6] by a microadaptation of the carbazole reaction[8] with glucuronic acid as a standard, while the hexose content of the other saccharide units was determined by a microadaptation of the anthrone reaction[9] with glucose-galactose (1:1) as a standard.

Cellulose acetate electrophoresis, nitrous acid treatment, and total sulfate analysis of the glycosaminoglycans were carried out by procedures employed in previous studies,[5,6] as were amino acid and phosphorous determinations of the unfractionated basement membranes.[5,7]

RESULTS

The yield of glomerular basement membranes isolated from the individual diabetic kidneys (mean ± SDM, 57 ± 10 mg/100 g wet weight of kidney cortex) was about twice that obtained from the nondiabetic tissues (27 ± 4 mg/100 g cortex). Average phosphorous analyses of the diabetic and nondiabetic basement membranes (0.096 ± 0.020 mg and 0.093 ± 0.016 mg/100 mg of dry membranes, respectively) were low, indicating that cellular materials had been effectively removed during their preparation.

Hexuronic acid analyses (Figure 1) indicated that the diabetic basement membranes had a significantly lower glycosaminoglycan content than membranes isolated from nondiabetic kidneys. No correlation of the glycosaminoglycan content with age or duration of diabetes in the time span examined was evident. In contrast to the decreased hexuronic acid, the hexose content of the basement membranes from diabetic subjects was significantly elevated above that of nondiabetic individuals (Table 1), so that the ratios of nondiabetic to diabetic values for hexuronic acids and hexoses had an inverse relationship (Table 1). Accordingly, the average molar ratios of hexose to hexuronic acids in the diabetic and nondiabetic samples (52 and 27, respectively) differed substantially.

Electrophoresis on cellulose acetate of the glycosaminoglycan fraction from several diabetic and nondiabetic basement membrane preparations revealed in each case only a single alcian blue reactive component (data not shown) that migrated to the same position as previously observed for the glycosaminoglycan from bovine glomerular basement membrane,[5,6] which has been characterized as a heparan sulfate polymer. After nitrous acid treatment, the glycosaminoglycan component from human diabetic and nondiabetic membranes was no longer demonstrable on electrophoresis.

The total glycosaminoglycan-associated sulfate content of the diabetic basement membranes was found to be sig-

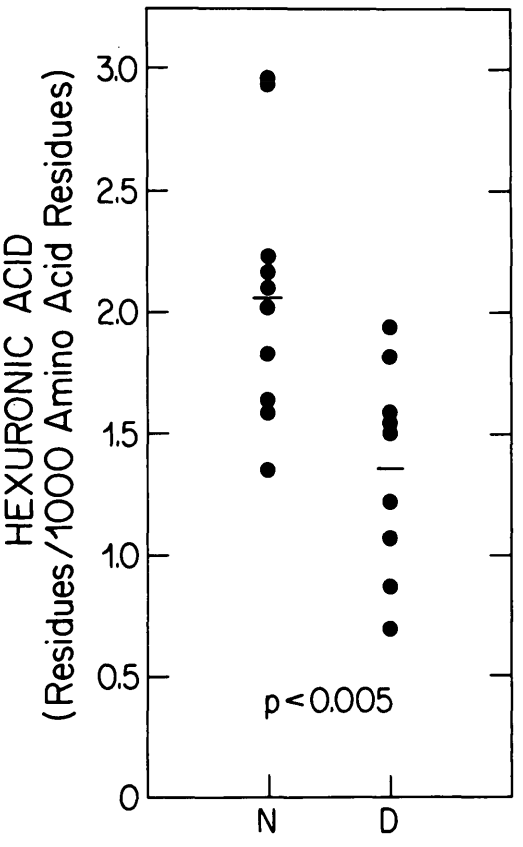

FIGURE 1. Comparison of the hexuronic acid content of glomerular basement membranes from individual nondiabetic (N) and diabetic (D) human subjects. Analyses were carried out on the glycosaminoglycan fraction obtained by DEAE-cellulose fractionation of collagenase-pronase digests of the basement membranes and are expressed per 1000 total amino acid residues of the unfractionated membranes. The mean is indicated for each group and the P value between nondiabetics and diabetics is shown.

TABLE 1
Glycoconjugate content of glomerular basement membranes from nondiabetic and diabetic human subjects*

	Residues/1000 amino acid residues†	
	Hexuronic acid	Hexose
Nondiabetic (10)	2.07 ± 0.17‡	56 ± 2
Diabetic (9)	1.36 ± 0.14‡	71 ± 5
Nondiabetic/diabetic	(1.52)	(0.79)
P value	< 0.005	< 0.025

* Analyses for hexuronic acid were performed on glycosaminoglycan fraction (2 M pyridine formate eluate) from DEAE-cellulose columns while hexose determinations were carried out on glycopeptide fraction (combined 0.05 M pyridine formate wash and 0.5 M pyridine formate eluate) from these columns.

† Values given as the means ± SDM are expressed per 1000 total amino acid residues of the unfractionated basement membranes; figures in parentheses indicate number of individual subjects analyzed.

‡ The glycosaminoglycan content calculated from hexuronic acid and sulfate analyses on the basis of a repeating heparan sulfate disaccharide unit (M_r = 460) and expressed as mg per 100 mg basement membrane peptide is 0.93 and 0.61 for the average nondiabetic and diabetic samples, respectively.

nificantly less ($P < 0.001$) than that of nondiabetic samples; the mean values ± SDM expressed per 1,000 total basement membrane amino acid residues were 1.66 ± 0.08 ($N = 5$) and 2.25 ± 0.06 ($N = 3$) for the diabetic and nondiabetic membranes, respectively. The decrease observed in the diabetic samples paralleled that of the hexuronic acid so that no significant difference in the sulfate to hexuronic ratios was apparent in the membranes from the two sources.

DISCUSSION

The present study indicates that the glycosaminoglycan content of the human glomerular basement membrane is significantly decreased in diabetes. Although glycosaminoglycan constitutes less than 1% of the basement membrane weight and a small portion of the total carbohydrate, accurate quantitative analyses for hexuronic acid could be carried out on samples from individual cases by means of a recently developed DEAE-cellulose microcolumn fractionation procedure that eliminates interference from the high content of neutral sugars associated with the collagen component.[6] As in basement membranes from other sources,[6] heparan sulfate was identified as the predominant glycosaminoglycan of both diabetic and nondiabetic human basement membranes, but the possible presence of a small amount of chondroitin sulfate cannot be excluded. While the extent of sulfation of the polysaccharide from diabetic and nondiabetic subjects appeared to be similar, further detailed investigations will be required to determine if any structural alterations in the heparan sulfate chains result from the diabetic state.

The decreased hexuronic acid content of the diabetic glomerular basement membrane contrasts to the significant elevation of the neutral sugar constituents of the diseased membrane observed in the present study and in previous reports from this laboratory.[7,10] Since the hexuronic acids are associated with the proteoglycan of basement membranes while the neutral hexoses of mature basement membranes, both normal and diabetic, are primarily (approximately 90%) found in the hydroxylysine-linked glucosylgalactose disaccharide of the collagen,[7,11] these results suggest that a redistribution of macromolecular components occurs in the diabetic state. The findings of in vivo investigations in the rat have suggested that the glomerular basement membrane turns over in a nonuniform manner[12] and indeed, in the case of the collagen and proteoglycan components, this might be anticipated from their location in distinctive portions of the basement membrane, namely, the lamina densa and lamina rara, respectively.[3] A collagen enrichment in the diabetic basement membrane has been indicated from amino acid and sugar analyses,[10] as well as from metabolic studies.[13,14] The reduced glycosaminoglycan content of the human diabetic basement membrane shown in the present investigation would appear to be consistent with recent reports that have observed a decreased incorporation of ^{35}S-sulfate into basement membrane glycosaminoglycan of glomeruli[15,16] and implanted EHS tumors[17] in diabetic animals.

Age-related changes in basement membrane composition, which appear to be similar to those observed in diabetes, have been reported. The glomerular basement membrane of the rat has been shown to take on a more collagen-like composition with increasing age[18,19] and a similar phenomenon has been noted in a comparison of the calf and cow lens capsule.[20] In the case of the lens capsule, an inverse quantitative relationship between the glycosaminoglycan and collagen content was observed, which has been attributed to a selective thickening of the lamina densa during growth.[6] Although it is not known whether similar mechanisms are responsible for the compositional changes observed during growth and diabetes, the relative overproduction of the collagen component in both situations could by itself result in a dilution of glycosaminoglycan; indeed, in diabetic glomeruli an increased amount of basement material is known to exist[7,21] and in the present study a higher yield of basement membrane was obtained from the diabetic kidneys.

Since heparan sulfate, with its strategic location at the cell-basement membrane interphase,[3] has been implicated as a component of the anionic filtration barrier, it is tempting to speculate that its reduced content in diabetic basement membranes might contribute to the increased glomerular permeability to proteins observed in this disease, even though other factors such as abnormal packing of collagen polypeptide chains and altered hemodynamic influences may also be involved.

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