Alteration of Collagen Metabolism in Hereditary Nephritis

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Summary

Hereditary nephritis (HN) is a progressive renal disease not uncommonly seen in children. Asymptomatic hematuria is an early and consistent finding with or without proteinuria. Distinctive abnormalities frequently associated with this condition involve basement membranes primarily (glomerular basement membrane, lens capsule, and possibly, tectorial membrane of the organ of Corti). These basement membranes contain collagen with similar biochemical composition suggesting that this association may be pathogenetically related. On the basis of this observation, we studied collagen metabolism in patients with HN by utilizing urinary hydroxylysine glycosides (hyl glyc) as an index of collagen metabolism. The hyl glyc hydroxylysine galactose glucose and hydroxylysine galactose are basic units in basement membrane as well as a large number of other collagen. We studied 25 normal children, seven patients with HN, and six HN siblings with hematuria. The mean excretion of total hyl glyc are 64 ± 5.5 , 93 ± 14 , and 123 ± 15.6 umoles/g creatinine, respectively. The difference in the mean excretion level of total hyl glyc between patients with HN and normal controls is statistically different ($P < 0.05$). The siblings of HN patients differed significantly from normal ($P > 0.005$), but was comparable to the HN patients. Two of 16 additional patients studied had the Nail Patella syndrome and showed increased urinary hyl glyc. The elevated urinary hyl glyc in HN patients and their siblings strongly suggests an altered rate of collagen degradation during the natural course of the disease. The origin of collagen is presently unknown because no data exists describing the biochemical composition of basement membrane collagen in HN. To elucidate further the pathogenesis of this important disease, studies should investigate the biosynthesis and metabolism of basement membrane collagen of involved structures.

Speculation

Collagen metabolism in HN suspect patients will raise the index of suspicion if found to be altered. This observation may provide increased understanding of the pathogenesis of this disorder.

HN is a progressive renal disease not uncommonly seen in children. Most frequently the renal disorder is a glomerulonephritis (2, 5, 8, 9, 17). Asymptomatic hematuria is an early and consistent finding with or without proteinuria. The association of familial nephritis and deafness is commonly referred to as Alport's syndrome (1). Deafness is neurosensory and seems to correlate with the severity of renal disease. Ocular abnormalities (cataracts and spherophakia) are also associated with this disorder, with or without deafness (18). In considering the pathogenesis of this disease, Spear *et al.* (19, 20, 21) discuss that HN may be a disorder of the physical and chemical structures or of the controls governing the production of the constituents of basement membranes. They point out that HN encompasses several distinct abnormalities which are related by the fact that they involve basement membranes (glomerular basement membrane, lens capsule, and, pos-

sibly, tectorial membrane of the organ of Corti). Collagen recently shown to be present in these basement membranes have very similar biochemical composition (10). Collagen changes have been reported in the organ of Corti in HN. (12).

On the basis of these observations we studied collagen metabolism in patients with HN by measuring urinary hydroxylysine glycosides. The hyl glyc are hydroxylysyl galactose glucose (HGG) and hydroxylysyl galactose (HG). These two glycosides are basic units in basement membrane collagen as well as a large number of other collagens. They are metabolized only to a minor degree before excretion and have a high threshold of clearance.

MATERIALS AND METHODS

Early morning specimens were collected and stored without preservative at -70°C pending analysis. Informed consent for the study was obtained from the parents. Seven patients (M-4, F-3) ages 6-13 with HN were studied. All patients had positive family and clinical history. Two of four affected males were brothers with neurosensory hearing loss and were both azotemic. One girl was uremic without hearing loss at the time of the study. No other abnormalities were observed. Pathologic studies of the kidney tissues were performed in all seven children and kidney tissues were examined by light, electron, and immunofluorescent microscopy. Of significance was the diffuse marked basement membrane alterations seen by electron microscopy in each patient. The glomerular basement membranes were thickened and split into multiple, thinned layers with clear spaces between the split layers. There were no deposits seen in the glomeruli. Siblings of HN patients with microscopic hematuria were also studied. Ages were 6-16 yr (M-3, F-3). All six siblings had normal renal function and were not biopsied. Twenty-five normal children (M-12, F-13) from 3-18 yr served as controls.

Sixteen additional patients were studied: nine with nephrotic syndrome (seven minimal change, two focal segmental glomerulosclerosis) one membranoproliferative glomerulonephritis, four benign familial hematuria, two Nail-Patella syndrome. The classic clinical criteria and standard morphologic picture for minimal change, focal glomerulosclerosis, and membranoproliferative glomerulonephritis were used. Four patients with the diagnosis of benign familial hematuria presented with asymptomatic recurrent hematuria without proteinuria. Usually, this condition has a hereditary pattern of transmission, however, progression to renal insufficiency does not occur. All four patients (M-2, F-2) ages from 8-14 yr have normal renal function. Renal tissues were examined from all patients under light, electron, and immunofluorescent microscopy. The biopsied tissue appeared normal on light microscopy. Electron microscopic studies showed marked degree of diffuse or segmental thinning of glomerular basement membrane with no deposits (8). The other two patients studied had the clinical diagnosis of Nail Patella syndrome, a family disorder of connective tissue which may be associated with renal dysfunction. Both patients had multiple osseous abnormalities with normal renal function.

PREPARATION OF SAMPLES FOR ANALYSIS

Urine specimen 500 μ l of each, was deproteinized by the addition of 100 μ l of 15% sulfosalicylic acid. The solution was allowed to stand 15 min before centrifugation at 13,500 rpm. Protein free filtrate, 200 μ l, was analyzed by an amino acid analyzer.

HYL GLYC ANALYSIS BY ION-EXCHANGE CHROMATOGRAPHY

A single column, ion-exchange chromatographic system of Hamilton (7) with a modified elution procedure (Sequence II) was employed for routine quantitation of urinary hyl glyc (14). Nin-hydrin reagent was prepared according to the method of Moore (15). The column with dimensions of 0.636×82 cm was packed

Fig. 1. Ion exchange chromatography of hydroxylysine glycosides. Leucine (Leu) 0.014 μ mole was added in every run as marker. A. Standard mixture: Hydroxylysine glucose galactose (HGG), 0.006 μ mole; Hydroxylysine galactose (HG), 0.005 μ mole; β -amino isobutyric acid (BAib), 0.002 μ mole. B. Urine chromatogram from a patient with hereditary nephritis: 100 μ l sulfosalicylic acid treated urine (see *Methods*) equivalent to 83.3 μ l of urine.

HYDROXYLYSINE GLYCOSIDE EXCRETION IN NORMAL CHILDREN, IN HEREDITARY NEPHRITIS AND H.N. SIBLINGS

Fig. 2. The mean excretion levels of total hydroxylysine glycosides in normal children, in patients with hereditary nephritis (HN), and HN siblings. Data was tabulated as mean $\pm$ SE.

with $10 \mu \pm 1$ micron sulfonated cation-exchange resin (Aminex A-7, BioRad Laboratories, Inc., Richmond, CA., Lot No. 3193). After conditioning with citrate buffer pH 3.04, 0.251 N with respect to sodium (0.05 M sodium citrate 0.065 M sodium chloride titrated with 6 N HCl) at 68° in the usual way, the column was developed with citrate buffer pH 3.55, 0.2 N with respect to sodium (0.05 M sodium citrate, 0.05 M sodium chloride titrated with 6 N HCl) at the same temperature. With these conditions, the acidic amino acids in the urine emerged sooner and were incompletely resolved from one another; HGG, however, was cleanly resolved after leucine and ahead of tyrosine while HG emerged as a single peak after β -alanine and ahead of β -aminoisobutyric acid (Fig. 1). The complete analysis took 15 hr.

Both HGG and HG in urine were expressed as $\mu\text{mole/g creatinine}$ (creat.). Urinary creatinine was determined by the Jaffe reaction (6). Data was tabulated as mean $\pm$ SE. Significance was assessed by using Student's *t* test.

RESULTS

The data shown in Figure 2 depicts the mean excretion level of hyl glyc in 25 normal children ($64 \pm 5.5 \mu\text{M/g creat.}$) and 6 HN siblings ($123 \pm 15.6 \mu\text{M/g creat.}$). The difference in the mean excretion level of total hyl glyc in the normal children and HN patients is statistically significant ($P < 0.05$). The mean excretion levels of hyl glyc in the siblings also differed significantly from normal ($P < 0.005$), but was comparable to the HN patients. The mean ratio of HGG/HG was < 1.0 for the three groups: 1) normal (0.94 ± 0.31), 2), HN (0.80 ± 0.09), 3), HN siblings (0.91 ± 0.25).

The mean excretion levels of hyl glyc in the patients with the nephrotic syndrome, benign familial hematuria and membranoproliferative glomerulonephritis were 60 ± 7 , 82 ± 17.4 , $58.3 \pm 12 \mu\text{M/g creat.}$, respectively. These values were comparable with the controls ($64 \pm 5.5 \mu\text{M/g creat.}$). The two patients with the Nail Patella syndrome excreted $123 \mu\text{M/g creat.}$ (child) and $43 \mu\text{M/g creat.}$ (mother), respectively. Both levels were 2 SD, higher than controls (children $64 \pm 5.5 \mu\text{M/g creat.}$, adult $26 \pm 3.8 \mu\text{M/g creat.}$). The HGG/HG ratio was comparable to normal controls. In normal adults, urinary HGG/HG ratio ranged from 0.83–2.0 with a mean of 1.2 (13).

DISCUSSION

Our findings demonstrate higher urinary hyl glyc in hereditary nephritis than comparable controls. Similar elevations are observed in two patients with the Nail Patella syndrome, a heritable connective tissue disorder. The majority of patients with connective tissue diseases have high levels of urinary hyl glyc reflective of increased collagen turnover (16). The use of urinary hyl glyc as a measure of collagen turnover has now received support from several studies (11, 16). The two glycosides HGG and HG occur in specific ratio in different tissues and degradation is very minimal before excretion. The ratio is 1:5 in skin and tendon, 0:15 in bone, and 19 in glomerular basement membrane (22). Two populations characterized by different urinary ratios of HGG to HG have been observed in pathologic states (3). Patients with bone disorders have increased bone collagen turnover and excreted high quantities of HG resulting in a low ratio (< 0.95). In conditions which involved skin, a high ratio (> 1.0) was obtained due to the higher skin HGG. In hyperthyroidism, a hypermetabolic state, urinary hydroxylysine glycosides were shown to be greatly in-

creased, however the HGG to HG ratio was comparable with normal adult controls (4). It was assumed that probably all collagen independent of its tissue localization may be involved. The mean ratio of HGG/HG in HN patients and their siblings was < 0.95 and did not differ from normal children.

The result of the present study supports the hypothesis of an altered rate of collagen degradation in HN. The origin of collagen is presently unknown because no data exists describing the biochemical composition of basement membrane collagen in HN. To elucidate further the pathogenesis of this important disease, studies should investigate the biosynthesis and metabolism of basement membrane collagen of involved structures.

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