Alpha₂HS-glycoprotein in the serum and urine of patients with renal diseases

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Summary

The concentration of alpha₂HS-glycoprotein (α_2 HS-GP), as measured by the single radial immunodiffusion technique in the sera of 52 patients with various renal diseases and varying degrees of proteinuria, was found to be significantly reduced ($P < 0.001$) when compared to the control values. Out of the 52 patients examined, 34 were found to excrete α_2 HS-GP in urine with no correlation between the serum and urine levels of this protein. Although there is a statistically significant correlation between the clearances of albumin and α_2 HS-GP, in only six patients were the clearances of α_2 HS-GP within $\pm 25\%$ of albumin clearance. Twelve had higher, and 16 had lower, relative clearances of α_2 HS-GP. The relative clearance of α_2 HS-GP had no relation to the serum levels of α_2 HS-GP, but correlated with the degree of proteinuria and the type of histological lesion in the kidney. In conclusion, there is a quantitative reduction of serum α_2 HS-GP in patients with renal diseases. It appears that the degree of proteinuria and the type of renal lesion influences its selective handling by the kidneys.

KEY WORDS: Alpha₂HS-glycoprotein, renal diseases, proteinuria, renal clearance.

Introduction

Alpha₂HS-glycoprotein (α_2 HS-GP), a normal human plasma globulin, is a negative acute-phase reactant and its concentration is altered in many unrelated disease states (Putnam, 1975; Lebreton *et al.*, 1979; Schelp *et al.*, 1980) including malignancy (Baskies *et al.*, 1980). It has been reported that α_2 HS-GP has some opsonic properties (Van Oss *et al.*, 1974) and a role in the mineral phase of bone (Ashton, Höhling and Triffitt, 1976). Its physicochemical properties and micro-heterogeneity are well documented (Gejyo and Schmid, 1981).

In the present work, we made a preliminary investigation of the serum and urinary levels of α_2 HS-GP in various renal diseases with special reference to its clearance in relation to that of albumin. Urinary concentrations of total protein and fibrinogen degradation products (FDP) were also determined.

Materials and methods

Patients

Fifty-two patients with renal disease (28 males and 24 females) selected randomly from the nephrology unit of the Niigata University Hospital constituted the study material. The diagnosis was based on the clinical, biochemical and histopathological examinations. Forty-seven patients had mainly glomerular diseases. Two patients had polycystic kidneys, another 2 had interstitial nephritis and one had only postural proteinuria. The proteinuria in the patients varied from a 'trace' to 14 g/litre, but the majority of them excreted more than 1 g/litre. Patients having disorders or complications known to influence the serum levels of α_2 HS-GP were not included in the study and all the patients had normal serum alkaline phosphatase activity.

Methods

Serum and urine samples collected from the patients were stored at -20°C . An aliquot (5 ml) of each urine sample was dialysed, lyophilised and finally reconstituted with a small volume of buffered solution to give 10-times concentrated samples. The amounts of α_2 HS-GP and albumin in these samples were measured by the single radial immunodiffusion technique using commercial plates (Behringwerke AG, Marburg, FRG) according to the instructions of the manufacturer. The serum levels of α_2 HS-GP were also measured by the same technique. Standard sera

were purchased from the same company. Total protein and FDP in unconcentrated urine samples were quantitated by Esbach's method and latex test (Melliger, 1970) respectively. Serum total protein was measured by standard methods on an autoanalyser (Hitachi M400). Glomerular filtration rate was assessed by sodium thiosulphate clearance (C_{Thio}) according to the method described by Newman, Gilman and Philips (1946).

Results

Table 1 shows the serum and urine levels of α_2 HS-GP in the patients with various renal diseases. The serum α_2 HS-GP in all the patients studied, as well as in the patients with membranous nephropathy, lupus nephropathy and membranoproliferative glomerulonephritis (MPGN), was found to be significantly reduced when compared to the control values established in 52 healthy individuals in our laboratory by the same technique. Out of the 52 patients studied, 34 showed α_2 HS-GP in urine with a mean value of 1.27 mg/dl. The number of cases in each group which were positive for urinary α_2 HS-GP, together with their mean values, are also given in Table 1. Out of the 5 healthy individuals screened for α_2 HS-GP in urine, only one was found to be positive with a value of 0.15 mg/dl.

Serum total protein concentration did not correlate with serum α_2 HS-GP ($r=0.266$; $n=52$), but correlated significantly and inversely with α_2 HS-GP in urine ($r=-0.357$; $n=34$; $P<0.05$). There was a significant correlation between the serum total protein and serum albumin ($r=0.78$; $n=52$; $P<0.001$). A highly significant correlation was found between α_2 HS-GP in urine and urinary FDP on one hand ($r=0.75$; $n=32$; $P<0.001$) and with urinary total protein on the other ($r=0.75$; $n=34$; $P<0.001$). A significant correlation also existed between urinary total protein and FDP ($r=0.54$; $n=32$; $P<0.01$). No statistically significant relation could be found between the glomerular filtration rate and urinary α_2 HS-GP ($r=0.24$; $n=33$).

Though the urinary losses of albumin and α_2 HS-GP ($r=0.50$; $n=34$; $P<0.01$) and their clearances correlated significantly ($r=0.68$; $n=34$; $P<0.001$), of special interest in this study was the finding of no correlation between the serum and urine levels of α_2 HS-GP ($r=-0.04$; $n=34$), whereas a good correlation existed between the serum and urinary levels of albumin ($r=-0.37$; $n=32$; $P<0.05$). A closer examination of the relative clearance of α_2 HS-GP ($C_{\alpha_2\text{HS-GP}}$) (relative to that of albumin) revealed that out of the 34 patients found to excrete α_2 HS-GP in urine, only 18% showed comparable clearances (i.e., within $\pm 25\%$ of albumin clearance, C_{albumin}), while 35% had higher and 47% had lower clearances. The relative

clearance of α_2 HS-GP had no correlation with its serum levels ($r=-0.13$; $n=34$), but had a correlation with its urine levels ($r=-0.39$; $n=33$; $P<0.05$). A semilog plot of $C_{\text{albumin}}/C_{\alpha_2\text{HS-GP}} \times 100$ v. proteinuria had revealed some more salient features (Fig. 1). In Fig. 1, each type of renal lesion can also be identified by a specific symbol and an inset is drawn to enclose such of those cases deviating markedly from a possible regression line (interrupted line). If these patients are excluded, the rest will have a statistically significant regression line at the 1% confidence level. The possible relevance of these findings is discussed below.

FIG. 1. Semilog plot of proteinuria v. $C_{\text{alb}}/C_{\alpha_2\text{HS-GP}} \times 100$. Each kidney lesion is identified as follows: Membranoproliferative glomerulonephritis (●); membranous nephropathy (○); lupus nephropathy (▲); toxemia of pregnancy (△); chronic renal failure (■); chronic glomerulonephritis (□); non-glomerular lesions viz. polycystic kidney and interstitial nephritis (*); renal amyloidosis with Behçet's disease (☆); postural proteinuria (+); minimal change nephritis (!).

Discussion

The present work is a study of the variation in the serum and urine levels of α_2 HS-GP in renal diseases with emphasis on its relative clearance. From our data, it is clear that the serum concentration of α_2 HS-GP is reduced in renal diseases and the reduction is more prominent in certain types. The fact that the decrease in the α_2 HS-GP concentration in the sera of patients is not a consequence of such non-specific factors as haemodilution or decreased plasma protein synthesis is substantiated by a non-significant correlation between the serum total protein and serum α_2 HS-GP levels, whereas a highly significant correlation existed between serum albumin and serum total protein.

The lack of correlation between the serum and urinary levels of α_2 HS-GP prompted us to consider its possible tubular reabsorption. The results of

TABLE 1. The concentration of α_2 HS-glycoprotein in the serum and urine of patients with renal diseases (mean $\pm$ s.d.)

	<i>n</i>	α_2 HS-GP in serum (mg/dl)	<i>n</i>	α_2 HS-GP in urine (mg/dl)
Normal controls	(52)	65.65 $\pm$ 6.96		—
All the patients studied	(52)	53.01 $\pm$ 15.72*	(34)	1.27 $\pm$ 1.45
Membranous nephropathy	(9)	52.71 $\pm$ 14.13†	(7)	1.71 $\pm$ 2.49
Lupus nephropathy	(7)	44.60 $\pm$ 15.38*	(4)	0.65 $\pm$ 0.34
Toxaemia of pregnancy	(7)	63.18 $\pm$ 12.60	(5)	2.14 $\pm$ 1.88
MPGN	(6)	42.79 $\pm$ 9.42*	(5)	0.95 $\pm$ 1.26
Chronic GN	(5)	56.73 $\pm$ 14.73	(2)	0.63 $\pm$ 0.68
Chronic renal failure	(4)	58.99 $\pm$ 17.33	(4)	1.09 $\pm$ 0.62
Acute GN	(3)	62.21 $\pm$ 18.22		ND
Minimal change nephritis	(2)	41.29 $\pm$ 14.62	(1)	2.01
Interstitial nephritis	(2)	67.25 $\pm$ 28.07	(2)	0.83 $\pm$ 0.54
Polycystic kidneys	(2)	50.45 $\pm$ 2.62	(2)	0.90 $\pm$ 0
Postural proteinuria	(1)	75.12	(1)	0.78
DM with essential benign hypertension	(1)	58.12		ND
Mixed connective tissue disease with mesangio-proliferative GN	(1)	55.56		ND
Renal amyloidosis with Behçet's disease	(1)	33.91	(1)	1.30
Focal glomerulosclerosis	(1)	23.83		ND

Abbreviations: MPGN: Membranoproliferative glomerulonephritis; DM: Diabetes mellitus; GN: Glomerulonephritis; ND: Not detectable. * $P < 0.001$, † $P < 0.01$ (Student's *t*-test).

clearance studies also supported this notion. α_2 HS-glycoprotein with a molecular weight of 51~56000 is smaller in size than albumin and a clearance very close to, or a little higher than that of albumin would be expected if it were not further handled by the tubules. However, the clearance data show that a considerable degree of this protein is being reabsorbed by tubules. As many as 47% of the patients had lower clearances. Although it is not possible to identify all the factors affecting the relative clearance of α_2 HS-GP, Fig. 1 reveals that it is influenced by the degree of proteinuria and the type of renal lesion. It is evident from the figure that there is a possible correlation between the degree of proteinuria and the relative clearance of α_2 HS-GP, and that patients with only certain types of lesions deviated markedly from this regression line. These deviating lesions (inset in Fig. 1) included interstitial nephritis, polycystic kidney, where tubules are af-

ected, postural proteinuria, renal amyloidosis with Behçet's disease and a few cases of lupus nephritis. Although all of these patients excreted less than 2.5 g/litre of protein, their tubular reabsorptive capacity was obviously not coping with the urinary losses of α_2 HS-GP, even at a comparatively low proteinuria. The rest of the patients depicted in Fig. 1, despite some scattering, followed a regression line that significantly correlated the relative clearance of α_2 HS-GP with proteinuria.

It is possible to interpret Fig. 1 in another way. As expected, patients with tubular interstitial diseases and polycystic kidneys had high relative clearances even at comparatively low proteinuria indicating the failure of the damaged tubules to reabsorb α_2 HS-GP effectively. On the other hand, patients with glomerular lesions, in general, showed low relative clearances indicating effective tubular reabsorption, the exceptions being only those cases where there was

massive proteinuria (>11 g/litre). These cases of glomerular lesions with massive proteinuria and high relative clearances obviously point to the saturation of tubular reabsorptive capacity and consequent 'overflow', rather than a defect in the tubules. In this context, it is noteworthy that such a type of overflow occurred only in one or two cases of glomerular lesions, only when the proteinuria was massive (more than 11 g/litre). It is an approximate measure of the tubular reabsorptive capacity for this protein. Though how far such a pattern of relative clearance is related to the histological lesions in the kidney and to the degree of proteinuria can be debatable, it is interesting that a strong association does exist among these 3 variables in our study.

Many studies are available on proteinuria in health and renal diseases both in experimental animals and man (Weise, 1981). Yet our knowledge of renal handling of proteins, even in health, is not as advanced as that of the handling of low molecular weight solutes such as amino acids, sugars and electrolytes by the nephron, for which many transport systems are identified and even characterised. Our understanding of the mechanism of tubular reabsorption of proteins, even in health, is fragmentary. The situation is further complicated in diseases where glomeruli, tubules or both are affected to varying degrees. Biennenstock and Poortmans (1970) studied the renal clearances of 15 plasma proteins (excluding α_2 HS-GP) in 10 patients with renal diseases of mainly glomerular origin. Their results strongly suggest that in renal diseases, the handling of proteins by the nephron may be selective. They found that even proteins of similar molecular size and charge were differently handled by the kidneys in disease. They showed that 3 proteins with lower molecular weights, α_1 anti-trypsin (mol. wt. = 45000), α_2 Gc-globulin (mol. wt. = 50000) and pre-albumin (mol. wt. = 61000) consistently had clearances lower than that of albumin. However, in our study with α_2 HS-GP (mol. wt. 51~56000), we could not find such a consistent lower clearance, possibly due to the influence of the other 2 factors, namely the degree of proteinuria and the type of renal lesion as discussed above. It seems reasonable that, apart from these two factors, our observations are in accordance with the

findings of Biennenstock and Poortmans and lend credence to the theory of selective clearance of proteins.

In conclusion, our study shows that the concentration of serum α_2 HS-GP is reduced in patients with renal diseases and its relative clearance is apparently influenced by the degree of proteinuria and the type of renal lesion.

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