Altered glycosylation and sialylation of serum proteins and lipid bound sialic acids in chronic renal failure

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Summary

Abnormalities in the metabolism of carbohydrates, lipids and proteins are well documented in chronic renal failure (CRF). We have studied the extent of glycosylation and sialylation of serum proteins and also the levels of lipid-bound sialic acids in non-diabetic patients with CRF maintained on haemodialysis. The total neutral sugar content (TNS) and the non-enzymatic glycosylation (NEG) of serum proteins, the protein-bound sialic acids (PSA) and the lipid-bound sialic acids (LSA) were found to be significantly elevated in the patients as compared to a group of healthy controls. A single dialysis therapy produced some alterations in these measurements. The TNS correlated significantly with the NEG and also with the PSA. No correlation existed between the PSA and LSA or between the LSA and serum total lipids. None of these measurements, however, correlated with the serum creatinine values. The established as well as putative pathophysiological consequences of these findings are discussed.

KEY WORDS: non-enzymatic glycosylation, N-glycosylation, protein-bound sialic acids, lipid-bound sialic acids, chronic renal failure.

Introduction

The glycosylation of proteins, which is generally controlled by enzymes precisely, increases the range of properties of the proteins. However, recently the non-enzymatic glycosylation of proteins (NEG) has received considerable attention with its possible contribution to the secondary complications of diabetes mellitus (Bailey, 1981). In this post-translational protein modification, glucose reacts non-enzymatically with the NH₂-terminal residues or forms keto-amine linkages with the ε-amino groups

of certain lysyl residues. Although first described for haemoglobin in diabetes mellitus, it is now clear that all classes of serum proteins can be non-enzymatically glycosylated both *in vitro* and *in vivo*, and the extent of such a glycosylation appears to be a function of the duration of exposure and the concentration of glucose (Day, Thorpe and Baynes, 1979; Baynes, Day and Thorpe, 1979).

Sialic acids are involved in the social behaviour of cells. These are also essential for the structural and functional integrity of hormones such as the pituitary gonadotropins and erythropoietin (Stevenson and Lorain, 1971; Erslev, 1977). It is well established that sialic acid residues determine the survival of the circulating glycoproteins and glycolipids, the desialylated proteins and lipids being cleared faster from the circulation (Ashwell and Morell, 1974; Barkai and Di Cesare, 1975).

Lipid-bound sialic acids (LSA) or acidic glycosphingolipids or gangliosides are membrane components and participate in the internalisation of signals elicited by glycoprotein hormones, cholera toxin, interferon among others (Fishman and Brady, 1976). They also restrict the undesirable amplification of the alternative complement pathway reaction especially on self cell membranes (Okada, Yasuda and Okada, 1982).

The present work was undertaken following preliminary observations in our laboratory on sera of patients with chronic renal failure (CRF), that showed significant alterations in the protein to sialic acid ratio as compared to the control sera. In view of the known pathophysiological consequences that can result from the alterations in the glycosylation and sialylation of proteins, we have measured the total neutral sugar content (TNS) and NEG of serum proteins, the protein-bound sialic acids (PSA) and

the lipid-bound sialic acids (LSA) in non-diabetic CRF patients maintained on regular haemodialysis.

Materials and methods

Subjects

CRF patients attending the dialysis units of the Niigata University and associated hospitals were selected randomly. The diagnosis was based on clinical approach, relevant laboratory investigations and renal biopsy. The pre-dialysis serum creatinine values were $1272 \pm 230 \mu\text{mol/l}$ (mean $\pm$ s.d., $n=68$). All the patients studied were free from diabetes mellitus and their fasting blood sugar values were within normal limits. Twenty-six healthy adults served as controls.

Collection of blood samples and assay methods

Blood was collected from each patient just before the start and at the end of haemodialysis therapy. The plasma or serum* separated was stored at -20°C until analysed. The TNS was measured by the anthrone reaction (Spiro, 1966), using pure dextrose (Wako Pure Chemical Ind., Japan) as standard. The PSA was assayed by the thiobarbituric acid method of Warren (1959) after acid hydrolysis with $0.1\text{N H}_2\text{SO}_4$ at 80°C for 1 hr. For these 2 assays, the serum proteins were precipitated by 5% trichloroacetic acid (TCA), centrifuged and the pellet was dissolved in an appropriate volume of 0.1N NaOH . Aliquots of this preparation were used to avoid the interference caused by free glucose and certain amino acids in the case of anthrone reaction, and the elevated non-proteinaceous thiobarbituric acid reac-

tive materials (Fillit *et al.*, 1981) in the case of Warren's method. The NEG was measured according to the method adapted by McFarland *et al.* (1979), using 5-hydroxymethylfurfural (Wako Chemicals, Japan) as standard. For this, the plasma samples were dialysed first to prevent spurious elevation of NEG due to the free glucose. The LSA were estimated by an improved Katopodis and Stokstad (1980) method. Protein in the dialysed serum samples and the reconstituted TCA precipitates was measured by the method of Lowry *et al.* (1951), using crystalline bovine serum albumin (Sigma Chemical Co., Missouri, U.S.A.) as the standard. Serum total lipids were assayed by the sulphophosphovanillin method (Zöllner and Kirsch, 1962), using a pooled human serum of known lipid content as the standard. N-acetylneuraminic acid used as a standard in the assay of PSA and LSA was purchased from the Fluka AG, Buchs, Switzerland. All other chemicals used were of the highest purity available in Japan, and a Hitachi Model 200-20 Spectrophotometer was employed for the absorbance readings.

Relationship between the variables was assessed from the correlation coefficients and the difference between the means by the Student's *t*-test (Snedocor and Cochran, 1978). Standard deviation (s.d.) was used to express dispersion.

Results

Table 1 gives the mean $\pm$ s.d. values for the 4 assays as well as the serum total lipid content in the controls and the patients with CRF. There was a statistically significant elevation of all the values in both pre- and post-dialysis samples as compared to the controls. However, the TNS and NEG were significantly reduced and the LSA elevated after a single dialysis therapy. There was no significant hyperlipidaemia in the patients. The scattergrams (Fig. 1, 2) depict the PSA and LSA values in the individual patients.

TABLE 1. The total neutral sugar content of serum proteins (TNS), the non-enzymatically glycosylated serum proteins (NEG), protein-bound sialic acids (PSA), lipid-bound sialic acids (LSA) and serum total lipids (TL) in controls and in patients with chronic renal failure [Results are expressed as mean $\pm$ s.d.(n)]

Group	TNS (μg dextrose/mg serum protein)	NEG (nmoles HMF/mg serum protein)	PSA (μg /mg of serum protein)	LSA (mg/dl)	TL (mg/dl)
Controls	5.46 ± 0.61 (26)	0.097 ± 0.021 (26)	7.59 ± 2.02 (26)	16.15 ± 1.21 (20)	586 ± 125 (20)
Chronic renal failure					
Pre-dialysis	* 11.24 ± 3.74 (68)*	† 0.163 ± 0.051 (30)*	§ 10.90 ± 3.84 (68)*	* 17.91 ± 2.57 (45)*	506 ± 229 (45)
Post-dialysis	* 8.25 ± 3.58 (68)*	† 0.123 ± 0.061 (30)‡	§ 9.73 ± 3.68 (68)*	* 20.51 ± 4.17 (45)*	623 ± 381 (45)

* $P < 0.001$; † $P < 0.01$; ‡ $P < 0.05$; § $0.1 < P < 0.05$; HMF: 5-hydroxymethylfurfural.

The statistical significance of pre- and post-dialysis values as compared to controls at different levels of '*P*' are earmarked on the upper right corner, whereas the symbol adjacent to the vertical bars on the left of the values shows the significance of difference between the pre- and post-dialysis samples.

FIG. 1. Scattergram of levels of PSA in controls and pre- and post-dialysis serum samples of CRF patients. Mean $\pm$ s.d. are indicated by vertical bars.

FIG. 2. Scattergram of levels of LSA in controls and pre- and post-dialysis serum samples of CRF patients. Mean $\pm$ s.d. are indicated by bars. The upper horizontal line demarcates the upper limit of normal (20 mg/dl) as adapted by other workers, while the lower one shows the upper limit of normal observed in our study (19 mg/dl).

Although there is a considerable overlap of values between the controls and the patients, there is a clear

evidence for a tendency towards higher values in the patients.

Table 2 gives the zero order correlation coefficients between the results of the assays. Statistically significant direct correlations were found between the TNS and NEG and also between the TNS and PSA in both the pre- and post-dialysis samples. There was no correlation either between the TNS and LSA or between the serum total lipids and the LSA ($r = -0.153$ in pre-dialysis samples and $r = -0.081$ in post-dialysis samples). Further, no correlation could be found between the serum creatinine levels and any of the parameters studied.

TABLE 2. Zero order correlation coefficients between the total neutral sugar content of serum proteins (TNS), non-enzymatically glycosylated serum protein (NEG), protein-bound sialic acids (PSA) and lipid-bound sialic acids (LSA) in the pre- and post-dialysis serum samples of the chronic renal failure patients

Variable	Variable		
	NEG	PSA	LSA
TNS	(a) 0.441† (b) 0.682* (n = 30)	(a) 0.464* (b) 0.524* (n = 67)	(a) -0.004 (b) -0.164 (n = 45)
NEG		(a) 0.293‡ (b) 0.458† (n = 30)	§
PSA			(a) 0.146 (b) 0.218 (n = 45)

(a) pre-dialysis samples; (b) post-dialysis samples; * $P < 0.001$; † $0.01 < P < 0.02$; ‡ $0.1 < P < 0.2$; §Could not be determined due to small sample size.

Discussion

Bailey (1981) has concisely reviewed the chemistry, mechanism of formation and the established, as well as the possible, consequences of NEG of proteins with reference to the pathophysiological changes seen in diabetes mellitus. Briefly, the secondary complications of diabetes mellitus, which accounts for most of the mortality and morbidity, involve organs such as the lens, peripheral nerves, kidney, retina and blood vessels. And interestingly, these organs are not dependent on insulin for glucose uptake and therefore are subjected to high intracellular glucose concentrations during periods of hyperglycaemia. The secondary complications are due to the modification of the properties of the proteins.

The abnormalities in the carbohydrate metabolism in CRF are to some extent similar to those of diabetes mellitus. Glucose intolerance occurs in about 70% of patients with CRF. Although the fasting blood sugar values of CRF patients are usually normal, the post-prandial levels are elevated. Plasma insulin levels in

response to intravenously administered glucose are normal or accentuated, suggesting that glucose intolerance is a result of peripheral resistance to insulin (Alfrey, 1980). The concentration of NEG observed in our patients is about 1.5 times, whereas that reported in diabetes is 2–3 times as high as in healthy controls (McFarland *et al.*, 1979; Bailey, 1981). Although at present it is not possible to correlate our observations on NEG with the pathophysiological changes seen in CRF, at least 2 of the organs listed above with reference to diabetes, namely peripheral nerves and blood vessels, are involved in long standing CRF, calling for further longitudinal studies to evaluate the contribution of NEG for CRF complications. Glycosylation can modify the physical, biochemical and immunological properties of proteins. Evidence is accumulating that NEG of low-density lipoproteins (LDL) can markedly change its properties. The lysyl residues primarily involved in the N-glycosylation of LDL are on or near the important residues of LDL that control the interaction of LDL with its receptors (Kim and Kurup, 1982). *In vitro* studies with cultured human fibroblasts have shown that glycosylated LDL was internalised and degraded significantly less than the control LDL, in proportion to the estimated degree of glycosylation (Gonen *et al.*, 1981). Glycosylated LDL is a poor stimulator of cholesteryl ester synthesis by cultured human fibroblasts. These preliminary *in vitro* observations on N-glycosylated LDL are significant in view of the accelerated atherosclerosis in diabetes and CRF.

Kirschbaum (1975) reported an increase in the activities of the enzymes glycoprotein:glycosyl transferase and glycoprotein:sialic acid transferase in CRF. This lends further support to our observations on PSA. Kirschbaum (1975) suggested that the increased activity of the enzymes in CRF might be due to altered intracellular environment or the reduced availability of nucleotide sugar precursors. However, we would like to speculate that there might be a possible derangement in the biosynthetic pathway for sialic acids, resulting in increased biosynthesis and incorporation of these into glycoproteins and glycolipids. Serum levels of pyruvate are elevated in uraemia with a possible block in the production of lactate and acetyl coenzyme A (Galloway and Morgan, 1964). The accumulating pyruvate, through oxaloacetate, can give rise to phosphoenolpyruvate, one of the precursors in the biosynthesis of N-acetylneuraminic acid, the principal sialic acid in man (Mayes, 1979). Hypersialylation of serum proteins in CRF can prolong the life-span of serum glycoproteins and this further enhances the NEG to occur, as one of the parameters that controls the rate of NEG, as mentioned in the introduction, is the duration of the exposure. In addition, it has been

shown that type V hyperlipoproteinemia is associated with an abnormal preponderance of sialylated to desialylated apolipoprotein C III in triglyceride-rich lipoproteins, presenting evidence for the possible involvement of sialic acids in hyperlipoproteinemia (Kashyap *et al.*, 1981).

LSA are reported to be elevated in a number of malignant conditions (Dnistrian and Schwartz, 1981) and it is speculated that they reflect the release and accumulation of tumour products like the cell surface antigens. It is hard to imagine such a mechanism operating in renal failure. Elevation of LSA in non-malignant conditions is very rare (Katopodis and Stock, 1980) and at present the cause for its elevation in CRF is not clear. The increased mean value of LSA in post-dialysis samples over that of pre-dialysis ones (Fig. 2) might be due to haemoconcentration that occurs during dialysis. The findings of serum total lipids within normal limits and the non-significant correlation between the serum total lipids and LSA rules out the possibility of hyperlipidaemia as a cause of elevated LSA in our patients.

In conclusion, we have shown that in CRF, the neutral sugar content and the non-enzymatic glycosylation of serum proteins are increased, probably as a result of glucose intolerance and other derangements in the metabolism of sugars. The cause for the hypersialylation of serum glycoproteins and glycolipids needs further investigation. The possible pathological physiological consequences of these findings are discussed.

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