PART XVI

DIABETIC NEPHROPATHY

Chairmen: L Migone
           S Giovannetti
URINARY EXCRETION OF ALBUMIN WITH AN ALTERED THREE-DIMENSIONAL CONFORMATION IN DIABETIC FUNCTIONAL NEPHROPATHY. EVIDENCE FOR A PATHOGENETIC ROLE

G M Ghiggeri, G Candiano, G Delfino, C Querirolo

Hospital of Lavagna, Lavagna, Italy

Summary

The free sulphydryl group content of serum and urinary albumin has been evaluated in eight normal and 23 diabetic patients with various grades of urinary albumin excretion rates. While in normal subjects and in diabetics with either normal albuminuria or functional nephropathy, urinary albumin showed a statistically higher content of free sulphydryl groups compared to homologous serum, diabetic patients with clinical nephropathy showed no difference. These results indicate that an increased urinary excretion of albumin altered in its conformational status is the main feature of diabetic functional nephropathy and suggest that a molecular mechanism determines the glomerular accumulation of albumin.

Introduction

Very early in the course of diabetes mellitus the urinary excretion of albumin increases to values detectable only by sophisticated techniques and characterises the so-called functional phase of diabetic nephropathy [1]. While a strict control of metabolic parameters can return this early dysfunction to normal [1], the chronic renal leakage of albumin leads to its accumulation in the mesangium, which is perhaps the decisive stimulus towards glomerulosclerosis and the consequent decline of the renal function [2]. Once urinary albumin has become detectable by common laboratory methods (0.25–0.5g/24hr), any intervention aimed at decreasing pressure values only succeeds in delaying but not reversing the deteriorating course of the renal disease [3]. On the basis of animal studies, it has been suggested that the lesions of diabetic nephropathy develop as a consequence of altered haemodynamics [4]; however an unequivocal proof of the influence of haemodynamic factors as unique determinants of diabetic nephropathy is still lacking. Furthermore other studies have shown that diabetes is a necessary prerequisite to the development of renal lesions in diabetic rats [5]. Recent evidence indicates that glycosyl albumin (a post-
translational derivate of albumin, the serum concentration of which is increased in diabetes mellitus) has an accelerated renal handling compared to normal homologous albumin, supporting the hypothesis of a molecular origin of diabetic microalbuminuria [6]. A clear understanding of the mechanisms governing renal filtration of albumin and glycosyl albumin in vivo could contribute to the development of a preventative strategy as a logical approach to an otherwise malignant disease.

Materials and methods

Eight normal controls (3 males, 5 females) ranging from 21 to 49 years (mean 32.7) and 23 patients with type 1 diabetes mellitus (14 males, 9 females) ranging in age from 6 to 60 years (mean 41.6) were studied. Diabetic patients were subdivided into three groups according to their urinary excretion rates of albumin (U_{alb}): (A) eight with U_{alb} <10 \mu g/min; (B) eight with U_{alb} from 10 to 100\mu g/min, and (C) seven with U_{alb} >100\mu g/min. All patients in groups A and B were normotensive, whereas four in group C had been previously hypertensive and were receiving therapy with diuretics and \beta-blockers to keep their blood pressure normal. Twenty-four hour urine collections were performed at home during a period of normal physical activity using thymol as preservative; only urines without signs of bacteruria were examined. Blood samples were obtained in the morning after an overnight fast and centrifuged at 3000g \times 30 min; cells were then discarded and sera kept at -20°C. Albumin was purified from other serum and urinary proteins by pseudo-ligand chromatography on Affi-Gel Blue [7] (Bio-Rad, Richmond, California, USA) previously regenerated according to the supplier’s instructions. One millilitre of serum and 300ml of urine were applied to 1.5 x 7cm columns washing out other proteins with 0.05M Tris-HCl, 0.1M KCl pH 7 with about 600ml of buffer until the concentration of protein in the effluent was less than 0.01 absorbance at 280nm. Albumin was eluted with 0.05M Tris-HCl, 1.5M KCl pH 7 with about 400ml of buffer. The fractions containing albumin were then dialysed and ultrafiltrated (Amicon PM-30 membranes). In all cases albumin purity was tested by rocket immunoelectrophoresis against a two layer gel containing either anti-albumin (first layer) or anti-total serum protein (second layer). Free sulfhydryl groups (SH) were measured by a modification of Ellman procedure as previously described [8] and results are given as nmol SH/nmol albumin, protein concentration was evaluated by the Coomassie Dye binding ass.-y. Serum and urinary albumin was quantitated by nephelometry (Beckman Immunochemistry Analyzer). \beta_2-microglobulin by a radioimmunoassay (Pharmacia, Uppsala, Sweden).

Statistical significance in Table I was calculated using Student’s ‘t’ test for paired groups. The least squares method was used for the calculation of the correlation coefficient.

Results

Urinary excretion rates of albumin and total proteins but not of \beta_2-microglobulin were higher in group B compared to group A and to normal controls, values
### TABLE I. Free sulphydryl groups of serum and urinary albumin in eight normal and 23 type I diabetic patients

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>$U_{alb}$ (µg/min)</th>
<th>SH albumin (mol SH/mol alb)</th>
<th>Serum</th>
<th>Urine</th>
<th>Urine Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normals</strong></td>
<td>8</td>
<td>3.8 (2.6–7.5)</td>
<td>0.49±0.02</td>
<td>1.67±0.37</td>
<td>2.95±0.54*</td>
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</tr>
<tr>
<td><strong>Group A</strong></td>
<td>8</td>
<td>4.7 (2.4–10)</td>
<td>0.43±0.02</td>
<td>2.03±0.52</td>
<td>4.65±1.17*</td>
<td></td>
</tr>
<tr>
<td>$U_{alb}&lt;10$µg/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Group B</strong></td>
<td>8</td>
<td>32.9 (15.1–99.5)</td>
<td>0.47±0.05</td>
<td>2.01±0.62</td>
<td>4.09±0.97*</td>
<td></td>
</tr>
<tr>
<td>$U_{alb} 10–100$µg/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Group C</strong></td>
<td>7</td>
<td>1648 (199–3800)</td>
<td>0.49±0.05</td>
<td>0.32±0.07</td>
<td>0.66±0.14†</td>
<td></td>
</tr>
<tr>
<td>$U_{alb}&gt;100$µg/min</td>
<td></td>
<td></td>
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</tbody>
</table>

* p<0.02 compared to serum  
† p<0.001 compared to serum concentration of other groups  
Abbreviations: $U_{alb}$=urinary excretion rate of albumin; SH=sulphydryl groups

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**URINARY SH**  
$-SH/MLM = \frac{SH}{mol ALB}$

![Graph](https://via.placeholder.com/150)

**Figure 1.** Relationship between the urinary/serum ratio of albumin SH and the clearance of albumin in 23 type I diabetic patients. $y=4.31x^{-0.33}$ is the curve giving the best correlation coefficient.
fitting the diagnosis of functional nephropathy. Five patients of group C had
proteinuria characteristic of the nephrotic syndrome, showing at the same time
a depressed creatinine clearance; by current criteria this last pattern is defined
as clinical nephropathy. SH content of serum albumin was the same in all
diabetic groups and in normal subjects with a mean value of 0.46±0.04nmol
SH/nmol albumin. Urinary albumin showed a statistically higher content of SH
compared to homologous serum albumin in normals in group A and group B,
but it was significantly depressed in diabetics with overt proteinuria (Group C).
Table I gives the detailed values for each group. The relationship between the
urinary/serum ratio of albumin SH and the clearance of albumin is depicted in
Figure 1.

Discussion

As for any other globular protein, the three-dimensional structure of albumin
is determined by the presence in its amino acid sequence of cysteines. In albumin
there are 35 molecules of cysteine, 34 of which are linked together by
means of the oxidative reduction of their SH to form 17 disulphide bridges.
The remaining one SH may be masked by a link with circulating cysteine or
glutathione in about 50 per cent of the molecules, so that each mole of human
serum albumin contains about 0.4–0.5 moles of SH.

The finding of a mean content of SH of two or more in urinary albumin
indicates that urine contains albumin whose conformational state has been
altered by the breakdown of at least one disulphide bridge. This important
difference between serum and urinary albumin may be explained on the basis
of a facilitated excretion into urine of those molecules which are less rigid
compared to their native homologous albumin owing to a decrease in their
interpeptide disulphide linkages. Glomerular selectivity towards macromolecules
depends on three major determinants which are size, charge and shape [9]. It
seems likely that for albumin, which is an anionic molecule (pl 4.7) the last
determinant plays the major role in determining urinary excretion in humans.
In addition to the glomerular basement membrane selectivity, renal handling of
macromolecules is also influenced by their tubular resorption which, for albumin,
is influenced by both its electrical charge and its conformational status
[10]. Hence, we cannot exclude that a more avid tubular resorption of albumin
with a normal conformation and the consequent increased excretion of deranged
molecules is the main mechanism in normal humans. However, the observation
in diabetic functional nephropathy of a concomitant increase in the urinary
concentration of albumin and of its SH content unequivocally indicates the
glomerular origin of these molecules. Diabetic functional nephropathy is thus
produced by an increased passage of albumin deranged in its conformational
status, being the most important mechanism in the excretion of these altered
molecules still unknown. In particular, it remains to be established whether in
diabetes mellitus there is an increased production of altered albumin or some
other mechanism, such as altered renal haemodynamics playing a central role.
It seems likely that the handling of these molecules may be influenced by high
pressure values in the renal microcirculation.

636
The chronic increase in filtration of altered albumin seems able to produce mesangial deposition and the induction of lesions characteristic of the late phase of the diabetic nephropathy. A knowledge of the intimate mechanisms of the increased production and/or excretion of altered albumin could lead to an understanding of the causes of diabetic nephropathy and eventually achieve specific prevention.

References

1 Viberti GC, McKintosh D, Bilous RW. Kidney Int 1982; 21: 714
3 Mogensen CE. Acta Endocrinol 1980 94 (Suppl 238): 103
10 Christensen E, Rennke HG, Carone FA. Am J Phys 1983; 244: F436

Open Discussion

MIGONE (Chairman) Can you comment on your finding of a serum sulphydryl albumin content even in your normal control groups?

GHIGGERI Yes, but I said that the mean content per mole of albumin is the same in normal and diabetic groups. The actual content, obtained by multiplying the excretion rates of the albumin by SH content per mole of albumin, is increased because of increased albuminuria. The actual content of the uraemia and diabetic groups is increased by three to four times.