Proximal renal tubular function in myelomatosis: observations in the fourth Medical Research Council trial*

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SUMMARY Proximal renal tubular function was studied in 522 consecutive patients entered into the Medical Research Council's fourth myelomatosis trial. Assessment was made at presentation after a 48 h period of hydration but before administration of chemotherapy. The most common abnormalities in the urine other than light chain proteinuria were raised concentrations of the low molecular weight proteins α_1 -microglobulin and α_1 -acid glycoprotein. These were usually accompanied by increases in urinary β -N-acetyl-D-glucosaminidase concentrations. The concentration of these substances in the urine directly correlated with urinary free light chain output. This tubular proteinuria was seen whether or not patients had impaired glomerular function, as assessed by a rise in serum creatinine concentration. Urinary concentrations of retinol binding protein, however, were generally increased only when serum creatinine concentrations were raised. This applied even when there were high concentrations of light chains, α_1 -microglobulin, α_1 -acid glycoprotein, and β -N-acetyl-D-glucosaminidase in the urine.

There is therefore a selective tubular proteinuria in myelomatosis which is seen in almost all patients with urinary light chain values > 1 u/l. This proteinuria is generally reversible, when light chains no longer appear in the urine. Patients whose serum creatinine was > 200 μ mol/l, however, had increased urinary output of retinol binding protein in addition to increased excretion of α_1 -microglobulin, α_1 -acid glycoprotein, and β -N-acetyl-D-glucosaminidase. Tubular proteinuria in many of these patients presenting in renal failure persisted even when light chain output was reduced after chemotherapy.

The aetiology of the renal failure in myelomatosis is not fully understood, but the evidence points towards multifactorial causes. In most cases the excretion of light chains in the urine appears to be an essential factor. Nevertheless, many patients excrete large amounts of light chains without any impairment of glomerular filtration rate.^{1,2} There are several reports of varying degrees of defective reabsorption of low molecular weight proteins by the proximal tubular cells in myelomatosis, as detected by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis of urine^{3,4} or measurement of one or more low molecular weight proteins in the

urine.^{5,6} There are accounts of rare cases of Fanconi's syndrome occurring in myelomatosis, which indicate that occasionally severe irreversible global tubular damage can occur.^{7,8} It is difficult to infer from these studies the probable extent of proximal tubular dysfunction in myelomatosis and its relation to glomerular function for the estimates are influenced by the analytes chosen as indices of disease. Also there are technical problems in determining the total protein load applied to SDS polyacrylamide gels.

This paper analyses the ability of the kidney to reabsorb low molecular weight proteins from the glomerular filtrate in 522 untreated patients admitted to the Medical Research Council's fourth myelomatosis trial. This aspect of tubular function was correlated with the excretion of free light chains and impairment of glomerular filtration. A further

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aspect of proximal tubular state was assessed by measuring the excretion of the tubular lysosomal enzyme β -N-acetyl-D-glucosaminidase (E.C.3.2.1.30).

Patients and methods

PATIENTS

Between 1 March 1979 and 28 February 1982 522 patients with myelomatosis admitted to the fourth MRC myelomatosis trial were assessed at presentation after a 48 h period of hydration. The criteria for entry were as for the previous MRC trial.¹⁰ Only patients below the age of 75 were included.

CHEMOTHERAPY

The chemotherapy trial compared the use of melphalan and prednisone with melphalan, vincristine, and prednisone. Patients presenting in renal failure were also managed on a high fluid intake. Details of these treatments are given elsewhere.⁹

The controls were a group of normal subjects from a survey of industry aged 35–70.¹¹

SAMPLE COLLECTION

Samples of serum and 24 h urine specimens preserved with 0.1% sodium azide were sent to the trials laboratory, Birmingham, by first class mail. In general, samples arrived within 24 h of despatch. The material was stored at -20°C until analysis. The sodium azide in the concentration used does not inhibit the β -N-acetyl-D-glucosaminidase assay.

LABORATORY ASSAYS

A centrifugal analyser (Instrumentation Laboratories UK Ltd) was used for the following assays: serum and urine creatinine (Jaffé reaction); total serum protein (biuret reaction). Total trichloroacetic acid precipitable protein was assessed in urine samples by the biuret reaction.

SPECIFIC PROTEIN MEASUREMENTS

Urine free κ and λ light chains were assessed using specific sheep antisera which did not react with light chain bound to heavy chain. These were prepared by AR Bradwell and RL Drew in the Department of Immunology, University of Birmingham. They were used in radial immunodiffusion assays¹² with 3% (wt/vol) polyethylene glycol 6000 in agarose. κ and λ standards were prepared from the urine of several patients with myelomatosis without high molecular weight proteinuria by means of gel permeation and ion exchange chromatography. Each standard was made of a mixture of equal amounts (as assessed by optical density) of purified κ and λ light chains from several patients. The amount of protein in the κ and

λ standards was assessed by the Kjeldahl method.

Results are expressed as units, where one unit is equivalent to 1g of standard. Units are used because free light chains from individual patients vary in their degree of polymerisation and antigenicity.

The following proteins were measured by radial immunodiffusion using antisera and standards from various sources: serum and urine α_1 -microglobulin, urine retinol binding protein, and albumin (Behringwerke, Marburg, FDR); α_1 -acid glycoprotein (University of Birmingham). The proteins were measured in unconcentrated urine.

The urine proteins were corrected for urine concentration by presenting all results as milligrams or units of protein per gram of creatinine (g Cr).

SDS polyacrylamide gel electrophoresis was performed using a discontinuous system after Laemmli¹³ incorporating 0.1% (wt/vol) sodium dodecyl sulphate in a vertical slab apparatus. The running gels contained 12% wt/vol acrylamide. Unconcentrated urine containing 10–20 μg of protein was applied to the gel. The gels were stained with PAGE blue 83 (BDH).

Urine β -N-acetyl-D-glucosaminidase was measured by a fluorimetric method using a 4-methylumbelliferone substrate as described elsewhere^{14,15}; 1 unit of β -N-acetyl-D-glucosaminidase activity is that which will release 1 nmol of substrate/h/mmol urine creatinine. Twelve units was taken as the upper limit of normal. The 4-methylumbelliferone substrate was bought from Koch-Light Laboratories, Colnbrook, Berks.

All measurements of urinary α_1 -microglobulin, α_1 -acid glycoprotein, β -N-acetyl-D-glucosaminidase, retinol binding protein and creatinine were made in the Unit for Cancer Research, University of Leeds; all other analyses were made in the Department of Immunology, University of Birmingham.

PICTORIAL PRESENTATION

For simplicity the data points in the Figures have been restricted to about the first 30 patients taken sequentially who fell into the various class intervals of serum creatinine and light chain excretion subsets.

Results

Throughout the study arbitrary groups were used for convenience when comparing different degrees of renal involvement in myelomatosis. Three arbitrarily defined subsets were created with respect to serum creatinine to take into account the effects of impaired glomerular filtration: (1) patients with a serum creatinine $<130 \mu\text{mol/l}$; (2) those with a

Table 1 Urinary light chain concentration and serum creatinine at presentation

Urinary light chain concentration (u/l)	No of patients with serum creatinine concentration ($\mu\text{mol/l}$):			
	<130	130-199	>200	>400
>1	83	53	75	(38)
0.04-1	124	47	19	(7)
<0.04	102	16	3	(1)
Total	309	116	97	(46)

Assessment was made before chemotherapy but after rehydration.

small rise of serum creatinine 130-199 $\mu\text{mol/l}$; (3) those with a serum creatinine $\geq 200 \mu\text{mol/l}$. A serum creatinine concentration of 200 $\mu\text{mol/l}$ was chosen as a cut off value because survival rates of patients with values above and below this value differed considerably. A detailed analysis of the level of serum creatinine and light chain excretion in patients with creatinine >200 $\mu\text{mol/l}$ has been presented elsewhere.⁹ Three subsets were also made for light chain excretion: (a) <0.04 u/g Cr (this is the 95th percentile for polyclonal κ light chain excretion in healthy subjects for this laboratory); (b) 0.04-1.0 u/g Cr, and (c) >1.0 u/g Cr. The division between (b) and (c) was chosen arbitrarily after inspection of the distribution of the data.

RELATION OF URINARY LIGHT CHAIN CONCENTRATION AND TYPE TO RENAL FAILURE
The distribution of serum creatinine and light chain excretion after hydration but before chemotherapy in 522 patients with myelomatosis is shown in Table 1. The probability of the patients presenting with

Table 2 Distribution of light chain proteinuria according to paraprotein type and serum creatinine

Amount of free light chain in urine (u/g Cr)	Paraprotein type	Serum creatinine ($\mu\text{mol/l}$)		
		<130	130-199	>200
0.04-1	λ	1	1	0
	$\lambda+$	28	9	6
	κ	5	4	3
	$\kappa+$	88	28	10
1-10	λ	6	2	0
	$\lambda+$	23	8	17
	κ	6	6	5
	$\kappa+$	27	18	24
>10	λ	4	9	9
	$\lambda+$	9	2	7
	κ	1	2	10
	$\kappa+$	9	5	3
Total		210	95	94

λ = pure λ light chain disease.

κ = pure κ light chain disease.

$\lambda+$ = paraprotein with intact immunoglobulin containing λ light chain.

$\kappa+$ = paraprotein with intact immunoglobulin containing κ light chain.

Table 3 Frequency of raised urinary α_1 -microglobulin (>15 mg/g Cr) at presentation according to paraprotein type and serum creatinine

Paraprotein type	Serum creatinine ($\mu\text{mol/l}$)		
	<130	130-199	>200
IgA κ	30 (59)	15 (20)	12 (12)
IgA λ	5 (5)	12 (13)	11 (11)
IgG κ	64 (121)	20 (30)	20 (21)
IgG λ	39 (57)	15 (15)	11 (11)
IgD κ	1 (1)	—	—
IgD λ	4 (4)	—	4 (4)
κ only	10 (12)	12 (12)	19 (19)
λ only	10 (11)	12 (12)	9 (9)
None	5 (13)	2 (2)	—

Numbers of patients with α_1 -microglobulin >15 mg/g Cr are shown, with the total number of patients in each group in parentheses.

renal impairment (serum creatinine >200 $\mu\text{mol/l}$ after correction of possible dehydration) was 0.34 when the urinary light chain excretion was >1 u/g Cr. By contrast, the probability was only 0.023 when the excretion was <0.04 u/g Cr.

A raised serum creatinine concentration occurred as frequently in patients with κ light chain proteinuria as in those with λ light chain proteinuria (Table 2).

ANALYSIS OF TUBULAR PROTEINURIA

α_1 -microglobulin (MW 27 kDa, peptide content 80%, pI 4.3-4.8)

The frequency of a raised α_1 -microglobulin in the urine (>15 mg/g Cr) in the main myelomatosis immunoglobulin types is shown in Table 3. This is subdivided according to light and heavy chain class in the serum and serum creatinine concentration. From 522 patients studied at presentation 357 (68.4%) had raised α_1 -microglobulin concentrations (>15 mg/g Cr).

The relation between the concentrations of free light chain and α_1 -microglobulin in urine of patients with both normal and raised serum creatinine concentrations is shown in Fig. 1. Urinary α_1 -microglobulin increased as the amounts of free light chain increased. The Spearman correlation coefficient for the urinary α_1 -microglobulin and free light chain concentration was $r = 0.64$ in 247 patients with normal serum creatinine concentrations (<130 $\mu\text{mol/l}$) (Table 4). There was no significant difference in the range of urinary α_1 -microglobulin excretion between patients with free κ and those with free λ light chain proteinuria. Crossed immunoelectrophoresis showed that α_1 -microglobulin was not bound to free κ or λ light chains. Renal failure, as assessed by a raised serum creatinine concentration, resulted in a significant increase in urinary α_1 -microglobulin concentration

Fig. 1 Relations of urinary α_1 -microglobulin (α_1m) and light chain excretion (uLC) and serum creatinine (sCr) in myelomatosis. Median values indicated by horizontal bars.

compared with patients with equivalent levels of light chain output with normal serum creatinine (Table 5).

α_1 -acid glycoprotein (MW 41 kda, peptide content 58%, pI 2.7)

The excretion of α_1 -acid glycoprotein in the urine (Fig. 2) showed a similar correlation with the concentration of free light chains to that seen with α_1 -microglobulin (Table 3). In patients excreting light chains at a concentration of $>1 \mu\text{g/g Cr}$ no secondary effect of renal failure was seen (Fig. 2 and Table 5). Patients with high serum concentrations of α_1 -acid glycoprotein ($>2.0 \text{ g/l}$), however, tended to show higher urinary α_1 -acid glycoprotein excretions (Fig. 2).

Table 5 Effect of renal failure on the excretion of urinary proteins in patients with light chain output $>1 \text{ u/g Cr}$. χ^2 for trend of rise in urinary output with increase in serum creatinine

	χ^2	p	n
α_1m (mg/g Cr)	14.8	0.0002	106
AGP (mg/g Cr)	0.2	0.63	106
RBP (mg/g Cr)	14.1	0.0017	19
NAG (u/mmol Cr)	0.118	0.73	103

For abbreviations see Table 4.

Fig. 2 Relations of urinary α_1 -acid glycoprotein (AGP) and light chain excretion (uLC) and serum creatinine (sCr) in myelomatosis. Δ = patients with a serum AGP $>2 \text{ g/l}$. Median values indicated by horizontal bars.

Retinol binding protein (MW 21 kda, peptide content 100%, pI 4.7-4.8)

The distribution of retinol binding protein excretion in the various subsets of the patients in the study is shown in Fig. 3. The normal values of urinary retinol binding protein were below the detection limits of the assay in the presence of normal serum creatinine concentrations. Retinol binding protein excretion in the urine, unlike that of α_1 -microglobulin and α_1 -acid glycoprotein, only occasionally reached detect-

Table 4 Correlation between urinary light chain output and the concentrations of other urinary proteins in patients with serum creatinine $<130 \mu\text{mol/l}$

	Light chain v			
	α_1m	AGP	RBP	NAG
Spearman correlation coefficient (r)	0.64	0.64	0.16	0.39
n	247	248	96	245
Significance (p)	<0.0001	<0.0001	>0.1	<0.0001

α_1m = α_1 -microglobulin.

AGP = α_1 -acid glycoprotein.

NAG = β -N-acetyl-D-glucosaminidase.

RBP = retinol binding protein.

Fig. 3 Relations of urinary retinol binding protein (RBP) and light chain excretion (uLC) and serum creatinine in myelomatosis. Median values indicated by horizontal bars.

able concentrations, even where there was increased free light chain excretion, provided that the glomerular filtration was normal (Fig. 3, Table 3). There was, however, a pronounced accentuation of retinol binding protein excretion in patients with raised serum creatinine concentrations (Fig. 3, Table 4).

Albumin (MW 66 kda, peptide content 100%, pI 4.1)

The concentration of urine albumin was >1 g/g Cr in 117 of 118 patients taken at random from the various subgroups; moderate to severe increases of serum creatinine were present in 25 of these patients at the time of measurement. There was no significant relation between albumin excretion and that of light chain or other low molecular mass proteins.

SDS polyacrylamide gel electrophoresis of the urine
SDS polyacrylamide gel assessment of the urine from patients with a total proteinuria of >1 g/g Cr showed that the predominant pattern was that of a monomer or dimer light chain with a low molecular weight (tubular) proteinuria. A mixed tubular and glomerular pattern with protein bands >70 kda molecular weight was present in 54 of 522 patients (10.3%). A similar proportion (10 of 106 patients)

Fig. 4 Relations of urinary β -N-acetyl-D-glucosaminidase (NAG) activity and light chain excretion (uLC) and serum creatinine in myelomatosis. Median values indicated by horizontal bars.

with serum creatinine concentrations >200 μ mol/l at presentation had a mixed glomerular and tubular pattern.

β -N-acetyl-D-glucosaminidase

β -N-acetyl-D-glucosaminidase activity tended to rise when light chains were present in the urine (Fig. 4, Table 4). Urinary values were not augmented further in those patients with increased serum creatinine concentrations (Table 5).

Discussion

The relation of light chain excretion and renal failure is discussed elsewhere¹⁶⁻¹⁹ and in a separate paper relating to 80 patients who presented with serum creatinine values above 200 μ mol/l in this trial.⁹ Here we describe renal tubular dysfunction in myelomatosis. The clinical tests of tubular function such as urine concentration and acidification and the presence of aminoaciduria reflect the behaviour of distal tubules. In this study we used indicators of proximal tubular function, which are more sensitive

to change than tests of distal tubular function. In 60 cases of myelomatosis investigated by Virella *et al*⁴ using SDS polyacrylamide gel electrophoresis all patients with predominantly tubular proteinuria were also excreting light chains.

In this study almost every patient with monoclonal free light chain excretion (392/399; 98.2%) had impaired reabsorption of α_1 -microglobulin and α_1 -acid glycoprotein, and this was generally accompanied by increased β -N-acetyl-D-glucosaminidase activity in the urine.

There was some correlation between the amount of α_1 -microglobulin and light chain excreted by patients with serum creatinines $<130 \mu\text{mol/l}$ (1.47 mg/100 ml), but the extent of the blockade of α_1 -microglobulin reabsorption varied between patients with similar amounts of light chain in the urine. Free κ and λ light chains appeared to exert a similar effect on the reabsorption of α_1 -microglobulin. In patients with normal serum creatinine values the serum concentration of α_1 -microglobulin was independent of the concentration of IgG paraprotein. By contrast, in IgA myelomatosis the blood concentrations of α_1 -microglobulin tend to be raised due to the binding of this protein to IgA.¹¹ Crossed immunoelectrophoresis shows that the plasma contains both bound and free α_1 -microglobulin. Urine contains only free α_1 -microglobulin. As with other low molecular mass proteins α_1 -microglobulin is removed from the blood by glomerular filtration followed by reabsorption and catabolism by the proximal renal tubules.²⁰ α_1 -acid glycoprotein is not normally detected by radial immunodiffusion in unconcentrated urine, under the conditions of our assay. Its presence in the urine is influenced by two main factors: proximal tubular dysfunction and the concentration in blood. High concentrations of α_1 -acid glycoprotein in the blood are part of an acute phase reaction to infection, other inflammatory disease, or extensive neoplasia.²¹ A rise of the excretion of β -N-acetyl-D-glucosaminidase, a lysosomal enzyme originating in the proximal tubular cells,¹⁵ is a second characteristic feature of free light chain proteinuria. The filtration of free light chains by the glomeruli appears to cause little disturbance of the filtration and reabsorption of retinol binding protein. Similarly, in a study of 21 patients with myelomatosis and free light chains in the urine only four of them had slight increases of β_2 -microglobulin excretion.²²

The selective character of the tubular proteinuria as described above is found in patients with normal serum creatinine concentrations. A change in the pattern of tubular proteinuria usually occurs in patients with myelomatosis in whom there has been a fall of glomerular filtration rate. Retinol binding

protein then appears at detectable concentrations in the urine and the increased β -N-acetyl-D-glucosaminidase excretion persists. Apart from being of lower molecular mass than α_1 -microglobulin and α_1 -acid glycoprotein, retinol binding protein is a pure peptide. However, Scarpioni *et al*⁵ have reported that the reabsorption of lysozyme, a basic non-glycosylated protein, is inhibited by light chains. This suggests the relative positive charge of α_1 -microglobulin and α_1 -acid glycoprotein is not the reason for impairment of their reabsorption. The almost universal association of impaired α_1 -acid glycoprotein and α_1 -microglobulin reabsorption with light chain proteinuria makes it unlikely that this is a physicochemical effect produced by light chains of a particular range of pI. Also this effect is seen with cationic and anionic light chains²³ and is common to κ and λ chains. Hence the effect of the dimer formation, frequent in λ chains, does not appear to be an important factor. Non-selective tubular proteinuria is a common phenomenon and can be produced by drugs,²⁴ upper urinary tract infection, or in response to burn injury or acute shock syndromes^{25 26} without any increase of serum creatinine above the normal range. During the recovery phase from these lesions the non-selective tubular dysfunction changes to a selective tubular dysfunction, exhibiting selectivity similar to that caused by the light chains. But light chains are not usually detectable in high concentrations ($>0.04 \text{ u/g}$ creatinine) during the recovery phase of these diseases. The reabsorption of protein by proximal tubules is temporarily blocked by infusion of the basic amino acids lysine, and arginine.²⁷ In rats lysozyme and cytochrome C compete for endocytic reabsorption by the tubular cells²⁸; both these proteins are strongly basic. Permanent proximal tubular dysfunction is a feature of cadmium poisoning,²⁹ chronic post-infective interstitial nephritis, and various rare disorders such as Fanconi's syndrome. Partial loss of tubular resorption for low molecular weight proteins can accompany the glomerular lesion in diabetes and hypertensive glomerulosclerosis.³

This large series of patients has not confirmed the impression that λ light chains are more nephrotoxic than κ light chains.² However, high values of free light chain excretion in the urine are most frequent in pure λ light chain disease. This may explain the findings of Scarpioni *et al*,⁵ who found the greatest inhibition of reabsorption of low molecular mass when the light chain was of the λ type.

The raised urinary β -N-acetyl-D-glucosaminidase activity reflects an increased turnover and excretion of lysosomal enzymes in the proximal tubular cells. Atrophy of the proximal tubules is the prob-

explanation of the decreasing β -N-acetyl-D-glucosaminidase concentrations in persistent renal failure in myelomatosis.

The factors which precipitate renal failure in myelomatosis have been discussed in detail and include: dehydration; intravenous pyelography; precipitation of Tamm Horsfall protein, light chains, and other material to form casts; hypercalcaemia; pyogenic infection; and amyloid deposition.^{7,9} Our current studies indicate that most if not all light chains are capable of inducing selective tubular dysfunction. We have little evidence that patients progress to renal failure because their light chains are more toxic than those of patients in whom the serum creatinine remains normal. The selective tubular dysfunction may facilitate development of dehydration and other factors listed above and so predispose to the development of renal failure. Our previous report⁹ has shown that continued high fluid intake can control and in many cases improve glomerular function and reduce the serum creatinine concentrations to normal limits in patients with persistent light chain proteinuria. This underlines the multifactorial nature of events leading to renal failure. Further studies are required to show the precise way in which these factors interact.

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