Characteristics of renal handling of human immunoglobulin light chain by the perfused rat kidney

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Summary

1. The renal handling of purified human immunoglobulin light chain has been studied with an isolated perfused rat kidney preparation.

2. Human immunoglobulin light chain was freely filtered and largely reabsorbed. Fractional reabsorption was characteristic for each of four light chains and varied between 56% and 86%. No renal tubular maximum for human light chain was obtained.

3. Light chains at concentrations up to 10 times those seen in human myeloma were without effect on glomerular filtration rate or sodium and potassium reabsorption in experiments lasting up to 2 h.

4. Filtered and reabsorbed light chain returned ultimately to the perfusion medium, indicating a unique property of the tubular handling of this protein. None of the inhibitors tested (ouabain, frusemide, acetazolamide, probenecid) influenced light chain reabsorption.

5. The results are taken to indicate that light chain reaches the site of the transport enzyme, Na⁺,K⁺-dependent ATPase, at concentrations which vary with the nature of the light chain. This may provide a mechanism for renal damage in patients with myeloma, after prolonged exposure.

Key words: Bence-Jones protein, diuretics, immunoglobulin, kidney perfusion, myeloma, ouabain, renal protein transport.

Abbreviation: GFR, glomerular filtration rate.

Introduction

One in seven patients with myeloma dies in renal failure (Galton & Peto, 1973). Uraemia at the time of presentation is commoner in those patients excreting significant amounts (2 g/l) of immunoglobulin light chain (Bence-Jones protein), and these patients have a significantly worse prognosis (Hobbs, 1969; Stone & Frenkel, 1975). However, it is not known whether light chain is nephrotoxic (Fine & Rees, 1977). Some authors (Costanza & Smoller, 1963; Preuss, Hammack & Murdaugh, 1967; Preuss, Weiss, Iammarino, Hammack & Murdaugh, 1974) have produced both clinical and experimental evidence for a nephrotoxic effect but other workers have failed to repeat these effects when using more rigorously purified material (Fine & Rees, 1977).

In order to examine the acute effect of purified human immunoglobulin light chain on renal function and to establish details of the renal handling of this material the isolated perfused rat kidney was used. This allowed measurement of inulin clearance (glomerular filtration rate, GFR), sodium and potassium excretion and light chain handling in the presence and absence of added light chain. After perfusion the kidney was fixed, sectioned and studied histologically.
Methods

Light chain

Human light chain was isolated from the urine of four patients with Bence-Jones proteinuria (urinary total protein range 0.7–16.0 g/l, 0.9–24 g/24 h). Fresh urine was brought rapidly to the laboratory and centrifuged (4°C, 3500 rev./min for 45 min) in a Mistral 4L centrifuge. The clear urine was then passed through a 0.4 μm Millipore filter and either frozen (−20°C) or immediately transferred to a 2 litres capacity concentrator (Amicon fitted with UM 10 filter). This was kept at 4°C and enabled up to 100-fold concentration of urinary protein to be achieved in approximately 15 h without change in salt concentrations. Final protein concentrations were kept below 70 g/l.

Concentrates were frozen at −20°C in portions. Portions were thawed as required (without warming above room temperature) and dialysed against ammonium bicarbonate solution (0.1 mol/l). Portions (2–3 ml) were gel filtered (glass column, 1 m long × 2.5 cm internal diameter, containing ACR 44 Ultragel) with ammonium bicarbonate solution (0–1 mol/l) as eluent. Fractions (10 ml) were collected with an LKB fraction collector after passing through a u.v.-absorbance detector.

Light chain formed the major component and was separate from whole immunoglobulin (which appeared with the void volume), albumin and various chromogens, which appeared in the later fractions. Fractions containing light chain were pooled, freeze-dried to a white powder and stored in a desiccator at −20°C.

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Light chain from individual patients was kept separately. Each batch was sampled and tested by both sodium dodecyl sulphate-gel electrophoresis and immunoelectrophoresis on acrylamide gel (Corning). All light chains obtained formed a single band on sodium dodecyl sulphate gels with a mobility between that of lysozyme (mol. wt. 14 300) and α-chymotrypsin (mol. wt. 25 700) corresponding to light chain monomer (mol. wt. 22 000).

Immunoelectrophoresis showed that each sample migrated as a single sharply bordered band and showed a precipitation arc only against one light chain antiserum. No precipitation was seen against the other light chain antiserum nor against antisera specific for the heavy chains of the immunoglobulins IgA, IgG, IgM or transferrin (antisera were from Dakopatts).

Before its use in perfusion experiments, freeze-dried light chain was dissolved in Krebs–Henseleit buffer and dialysed against the same for 24 h at 4°C before use.

Immunohistological demonstration of human light chain

This technique involves a ‘sandwich’ of rabbit anti-(human light chain), pig anti-(rabbit serum protein) and immune complexes of peroxidase-habbit anti-peroxidase as described for lysozyme by Mason & Taylor (1975). Separate sections were stained with anti-λ and anti-κ antisera. Staining occurred with the antiserum to the class of light chain used in the perfusion; the other antiserum was used as a control to exclude non-specific staining. Sections were also stained by a conventional haematoxylin and eosin method.

Assay of human light chain in perfusing medium and urine

Initially a radial immunodiffusion method was used, essentially the method of Mancini, Carbonara & Heremans (1965) with minor modifications established in this laboratory (Roberts-Thomson, 1975). This method relies on an antibody–antigen reaction and thus more precisely measures light chain antigen sites. All duplicate results fell within 3% of the original. The major source of error was due to the need to dilute unknowns into the relatively narrow linear range of the assay (approximately 20–100 μg/ml).

Subsequently light chain was labelled with 125I by the McFarlane iodine monochloride method, with modifications essentially as described by Helmkamp (1960). All material used had a minimum of 97% of the radioactivity of the protein bound. Portions were counted for radioactivity with a Wallac gamma counter. A number of unknowns were assayed by both techniques and this showed a close correlation between the two methods ($R^2 = 0.98; n = 47$).

Kidney perfusion

An isolated rat kidney-perfusion technique was used (Ross, Epstein & Leaf, 1973). The haemoglobin-free perfusion medium consisted of 70 ml of 6.7% dialysed bovine serum albumin, fraction V, in Krebs–Henseleit buffer, gassed with O₂/CO₂ (95:5). Glucose (5 mmol/l) was the only added substrate. In individual experiments light chain was added in a maximum volume of 0.5 ml,
after a control period of 30 min perfusion. No significant dilution of perfusate albumin followed this addition. [$^{14}$C]Inulin clearance (GFR) and urinary [$\text{Na}^+$] and [$\text{K}^+$] were determined in successive 10 min periods. Samples for the analysis of light chain content in urine and perfusion medium were taken at 10 min intervals after addition of light chain.

Each kidney thus served as a control for the determination of the urine/plasma ($U/P$) inulin ratio, GFR, sodium reabsorption and potassium excretion in the presence and absence of light chain.

The duration of perfusions varied from 40 to 100 min. Commonly the perfusion time was between 50 and 60 min. Finally the kidney was weighed and a portion fixed in 10% formalin in saline for histological examination. In some a further portion was homogenized and analysed for light chain content.

Statistics
Where appropriate, the paired Student's $t$-test was applied.

Results
Effect of purified light chain on function of the perfused kidney

Normal human free light chain plasma concentrations are not accurately known but are certainly less than 20 $\mu$g/ml (Epstein, Gulyassy, Tan & Rae, 1968). Plasma concentrations in light chain myeloma are usually less than 200 $\mu$g/ml before the onset of significant renal failure. Concentrations up to 15 mg/ml have been reported (Solomons & Fahey, 1964), but only in patients producing large quantities of polymer light chain and with significant renal impairment. In this study perfusion medium concentrations of light chain between 30 $\mu$g/ml and 2500 $\mu$g/ml were examined.

In the first series of experiments there was an initial control period before light chain was added. Results in this control period were then compared directly with those of the period when light chain was present. No significant effect on the urine/plasma inulin ratio, sodium reabsorption or potassium fractional excretion was observed with any of the four light chains tested. In subsequent experiments, in which the primary object was to ascertain the effect of drugs on the tubular reabsorption of light chain, the initial control period was one in which light chain was present and this was compared with a later period in which both light chain and drug were present.

Fig. 1 shows the time course of a single perfusion in which light chain ($\lambda$, patient W.E.) was added after 35 min, at a final concentration of 200 $\mu$g/ml in the perfusion medium. Fractional sodium reabsorption remained above 97% for 120 min of perfusion, a value which is within the normal range for this experimental model (Ross et al., 1973). Fractional potassium reabsorption varied between

![Graph showing reabsorption of Na+ and K+](image)
FIG. 2. Effect of purified human light chain on function in the isolated perfused rat kidney. Data are presented from five consecutive perfusions in which light chain was added after 30 min of perfusion, which then continued for a further 30 min. The final concentration of light chain was between 100 and 1500 μg/ml. Results refer to at least two 10 min clearance periods each before and after addition of light chain. In a similar series of control kidneys in which no light chain was added renal function was unchanged over the same period of perfusion. FE, Fractional excretion. n, Number of kidneys used for each experiment. N.S., Not significant. Values are ±SE.

66% and 81% after addition of light chain, and was not different from the control.

Results from five kidneys perfused with purified light chain from the same patient (Fig. 2) at concentrations between 100 and 1500 μg/ml showed no significant inhibition of GFR, which remained about 0.79 ml/min throughout, or of fractional sodium reabsorption. Fractional excretion of potassium increased from 0.39 to 0.47 on addition of light chain but this change was not statistically significant.

Filtration and reabsorption of light chain

The filtered load of light chain depends upon three parameters: the concentration in the perfusion medium, the GFR and the sieving coefficient of the protein, a measure of hindrance to filtration at the glomerulus. Evidence for free filtration of light chain was obtained in two types of experiment. First, an indirect method introduced by Maack (1975), which depends upon total inhibition of tubular reabsorption, and secondly, omission of albumin from the perfusion medium to exclude any possible effect of protein-binding of light chain.

Inhibition of tubular reabsorption. 125I-labelled light chain was added to the perfusion medium and fractional light chain excretion was measured under control conditions and under conditions of complete inhibition of tubular uptake of light chain by iodoacetate. Filtered load of light chain was calculated as perfusion medium concentration of light chain × GFR ([14C]inulin clearance). Under control conditions, the fractional excretion for the light chain studied (κ, patient E.B.) was 51% (Fig. 3). Addition of iodoacetate (6 mmol/l) inhibited light chain reabsorption and the fractional excretion rose such that the final excretion rate was 95 ± 6% of the calculated filtered load. The real rate of filtration of light chain did not therefore differ significantly from the rate calculated on the assumption of no hindrance to light chain filtration. There is therefore no significant hindrance of light chain filtration as determined by this indirect method.

Albumin-free perfusion. These experiments were repeated in kidneys perfused with albumin-free medium (Fig. 4). In control kidneys sodium reabsorption was reduced to 54%, an effect previously reported by Little & Cohen (1974). However, light chain reabsorption was unchanged; this suggests that light chain filtration was not hindered by the presence of albumin and that no significant albumin–light chain binding occurred.
In a single experiment the effect of iodoacetate (6 mmol/l) on light chain reabsorption in the albumin-free medium was similar to that seen in the presence of albumin, in that both light chain and sodium reabsorption were completely inhibited.

**Characteristics of renal handling of different light chains**

Four purified light chains were studied. Of these one was λ and three were κ. All four light chains were characterized on sodium dodecyl sulphate–gel under reduced (5% β-mercaptoethanol) and non-reduced conditions, and had molecular weights not significantly different from 22 000. The mobility on agarose gel (pH 8.5, barbital buffer) differed for each light chain, indicating a difference in net electrical charge.

The fractional reabsorption of the light chain was distinct and different for each one (Fig. 5). The highest rate observed was 86% for a λ light chain, and the lowest 54% for a κ light chain. Renal reabsorption was virtually independent of perfusate concentration over the range 20–2500 μg/ml, corresponding to a maximum filtered load of 980 μg/min per kidney. Thus no tubular maximum (Tm) for human light chain in rat kidney has been observed (Fig. 6).

The fate of filtered light chain was characterized further by determining the concentration of light chain in the medium and in urine at the end of the experiment. As shown in Table 1, the majority of light chain (83.8%) was recovered in the perfusion medium; 11.6% was recovered in the urine and 4.6% was unaccounted for. In separate experiments, light chain determination in kidney tissue accounted approximately for this amount. During the course of the experiment illustrated in Table 1 light chain contained in the perfusion medium had

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**Table 1**

<table>
<thead>
<tr>
<th>Light Chain</th>
<th>n</th>
<th>Recovery (%)</th>
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<tbody>
<tr>
<td>λ (E.B.)</td>
<td>5</td>
<td>79.0 ± 2.4</td>
</tr>
<tr>
<td>λ (V.W. E.)</td>
<td>6</td>
<td>46.8 ± 2.1</td>
</tr>
<tr>
<td>κ (E.B.)</td>
<td>6</td>
<td>58.0 ± 2.5</td>
</tr>
<tr>
<td>κ (E.G.)</td>
<td>6</td>
<td>63.8 ± 2.4</td>
</tr>
<tr>
<td>κ (G.T.)</td>
<td>2</td>
<td>68.2 ± 2.3</td>
</tr>
</tbody>
</table>

Fig. 4. Effect of albumin concentration on reabsorption of sodium and light chain. Kidneys were perfused with medium containing 200 μg of light chain (κ, patient E.B.)/ml in the presence or absence of 67 g of bovine albumin (fraction V)/l. Fractional sodium reabsorption and light chain reabsorption were calculated from [14C]inulin clearance as described in the Methods section. In a smaller number of perfusions sodium iodoacetate (6 mmol/l) was added after 30 min perfusion (compare Fig. 3). n, Number of kidneys used for each experiment. Values are ±SE.

Fig. 5. Renal handling of four different human light chains. Individual purified light chain was added at concentrations between 75 and 250 μg/ml. Clearance was determined during 10 min collections over periods of 30 min or more of perfusion. Light chain was determined immunologically in the majority of experiments. Identical results were obtained with 125I-labelled light chain for patients E.B. and W.E. n, Number of kidneys used for each experiment.
Effect of increasing concentration of light chain (κ, patient B.E.) on renal tubular reabsorption. Light chain concentration was increased from 20 to 2500 μg/ml, corresponding to a maximum filtered load of 980 μg/min. Data from six perfusions have been plotted to obtain the curve, which indicates no tubular maximum. Slope: 0.0556; $R^2 = 0.976$. The broken line corresponds to 100% reabsorption.

**TABLE 1. Balance study of human immunoglobulin light chain filtered by isolated perfused rat kidney**

Results are from a single representative experiment.

<table>
<thead>
<tr>
<th></th>
<th>Light chain μg (%)</th>
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<tbody>
<tr>
<td>In medium at 5 min</td>
<td>7435 (100)</td>
</tr>
<tr>
<td>In medium at 75 min</td>
<td>6230 (83.8)</td>
</tr>
<tr>
<td>In urine 5–75 min</td>
<td>863 (11.6)</td>
</tr>
<tr>
<td>Unaccounted for</td>
<td>342 (4.6)</td>
</tr>
<tr>
<td>Total light chain filtered (5–75 min)</td>
<td>9529 (128)</td>
</tr>
</tbody>
</table>

All been filtered at least once during the 75 min of perfusion; total light chain filtered was 128% of that added.

**Effect of diuretic agents on renal handling of light chain**

The following drugs added to the perfusion medium were without measurable effect on the renal tubular reabsorption of human light chain (patient E.B.): ouabain (2 mmol/l), acetazolamide (0.1 mmol/l), frusemide (0.1 mmol/l), probenecid (1 mmol/l) and the basic amino acid lysine (10 mmol/l). Each drug nevertheless exerted its expected effect on electrolyte transport by the perfused kidney, as reported from this and other laboratories (Fig. 7).

Microscopic localization of light chain

In all kidneys examined perfusion with light chain resulted in highly localized accumulation of light chain within renal tubular cells. There was no staining within glomeruli or blood vessels and the luminal staining occasionally seen was limited to the distal convoluted tubule. Immunoperoxidase staining was noted in proximal tubular cells, where it was localized to the luminal pole. In addition, diffuse staining was seen in distal convoluted tubular cells. These results were obtained from three kidneys perfused with a light chain, which was avidly reabsorbed (86%). In eight kidneys perfused with a light chain (κ) that was less completely reabsorbed (59%), staining of proximal tubular cells was very much less marked, but the diffuse staining of distal tubular cells remained.
Discussion

Renal handling of human light chain

By indirect methods involving removal of protein from the vascular medium or inhibiting tubular reabsorption of all filtered protein, it has been shown reasonably conclusively that light chains, molecular weight 22 000, are freely filtered by the perfused rat kidney. This permits standard clearance methods to be used in the study of their renal handling. As expected, tubular reabsorption occurred in every case but, somewhat surprisingly, the extent of the reabsorption varied from one light chain to another. The amount of light chain that appeared in the urine varied between 13 and 45% of the filtered load and depended upon the light chain used. Nevertheless, no T_m was observed for any of the light chains studied. No explanation is at present available for this variation in fractional reabsorption, but it may be of importance in that it determines the concentration of light chain within the proximal tubular cell.

Histological evidence provided supports the concept of proximal tubular reabsorption of light chain. Light chain was observed within the proximal tubular cell, localized to the luminal pole. This would be the localization expected to result from pinocytosis, demonstrated for reabsorption of another protein of similar molecular weight (lysozyme, mol. wt. 13 000; Mason, Howes, Taylor & Ross, 1975). An additional feature of the histological distribution of light chain was the diffuse staining observed in distal tubular cells. This contrasts with the exclusively proximal localization of lysozyme and may indicate some unique characteristic of the renal handling of light chain.

Evidence for recycling of light chain by the kidney

The usual pattern of protein reabsorption in the kidney is for the protein to remain in pinocytic vesicles within the proximal tubule until it is degraded (Maack, 1975). The present experiments in perfused rat kidney permit a detailed quantitative analysis of the fate of filtered light chain. All of the light chain contained in the perfusion medium was filtered within 1 h of perfusion but only 16% was recovered in urine and kidney. The remaining light chain was recovered in the perfusion medium. A reasonable interpretation of these findings is that filtered light chain, once reabsorbed, is returned to the perfusate rather than remaining within the kidney. This would indicate unusual properties for the renal handling of light chain.

Relationship between light chain and renal function

No effect of added human light chain was observed on any renal functions tested in this study. High concentrations were used but the time of exposure was short, usually less than 2 h. The possibility that light chain is nephrotoxic after more prolonged exposure cannot be excluded.

In an attempt to modify the renal handling of light chain, diuretic drugs with marked effects on reabsorption of sodium, potassium or bicarbonate, or the uricosuric agent probenecid, were added but were without effect on light chain reabsorption. Lysine, a basic amino acid which can modify the reabsorption of lysozyme by virtue of its negative charge (Baumann, Bode, Ottosen, Madsen & Maursbach 1977), was also without effect on the tubular reabsorption of light chain although it promoted a marked increase in potassium excretion.

Light chain transport therefore followed none of the tubular transport pathways so far tested.

Significance of reabsorption of light chain for human myeloma kidney disease

Light chain is implicated in the aetiology of myeloma kidney disease. Variations in the rate of tubular reabsorption of filtered light chain may be important in this respect. One possible mechanism for tubular cell damage, the inhibition of Na^+\text{-}K^+\text{-}dependent ATPase, has been reported in a preliminary communication from this laboratory (McGeoch, Falconer Smith, Ledingham & Ross, 1978).

However, these present acute experiments showed no inhibition of sodium reabsorption by purified human light chain. The reasons for this remain unclear. Na^+\text{-}K^+\text{-}dependent ATPase is localized at the basolateral pole of the tubular cell. To reach this site, light chain may traverse the proximal tubular cell from the site of its luminal reabsorption, or arrive by the vascular route. The latter is unlikely since in two perfusions (not reported), in which glomerular filtration was prevented (by increasing albumin concentration to 100 g/l, and lowering perfusion pressure to 90 mmHg; non-filtering kidney), there was no light chain found within the kidney.

Filtered light chain is concentrated in pinocytic vesicles, at a site distant from Na^+\text{-}K^+\text{-}dependent ATPase. But the free exit of light chain from the cell and return to the perfusion medium also
indicates that the protein must pass the basolateral membrane close to the site of Na⁺,K⁺-dependent ATPase. The failure to observe significant inhibition of Na⁺ reabsorption implies either that the concentration achieved at the site of the enzyme is too low for inhibition to occur, or that light chain is altered by enzymic degradation within the proximal tubule.

The present information is insufficient to separate these two possibilities clearly. Preliminary experiments show that the maximum concentration of light chain within the kidney at the end of 90 min perfusion is about 0-01 mmol/l (J. F. Falconer Smith, R. I. van Hegan, N. P. Esnouf & B. D. Ross, unpublished work). On histochemical evidence, this concentration would be accounted for largely by the light chain sequestered at the luminal pole. Evidence for degradation of light chain by kidney has been presented by Wochner, Strober & Waldmann (1967), and the present results do not completely exclude this possibility. Alternatively, this failure to observe the expected inhibition of renal function may be due to the relatively short exposure of kidney to light chain, and a toxic effect may occur in vivo only after longer exposure. In this case it may be expected that the different relative rates of light chain reabsorption observed could determine the speed with which critical intracellular concentrations are achieved. The isolated perfused kidney offers a method of studying agents capable of modifying the rate of light chain reabsorption and thereby altering the progress of renal damage in myeloma.

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References