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Light chain effects on alanine and glucose uptake by renal brush border membranes

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Light chain effects on alanine and glucose uptake by renal brush border membranes. Effect of varying concentrations (0 to 800 μM) of three different light chains on sodium–dependent L-(^{14}C)alanine and D-(^{14}C)glucose uptake by rat renal brush border membrane vesicles were studied. One κ and two λ type light chains (λ-1 and λ-2) were isolated from urines of patients with multiple myeloma. At maximal inhibitory concentrations the κ chain reduced alanine uptake from 206 ± 18 to 77 ± 18 pmole/mg protein ($P < 0.005$) and glucose uptake from 357 ± 22 to 146 ± 8 pmole/mg protein ($P < 0.001$). λ-1 reduced alanine uptake from 136 ± 17 to 60 ± 8 pmole/mg protein ($P < 0.005$) and glucose uptake from 354 ± 17 to 77 ± 14 pmole/mg protein ($P < 0.001$). λ-2 reduced alanine uptake from 105 ± 9 to 28 ± 5 pmole/mg protein ($P < 0.001$) and glucose uptake from 194 ± 7 to 66 ± 7 pmole/mg protein ($P < 0.001$). The half maximal inhibitory concentrations (I_{50}) of κ, λ-1 and λ-2 light chains were 68, 76 and 140 μM for alanine uptake and 120, 70 and 105 μM for glucose uptake. Control experiments using bovine serum albumin and β-lactoglobulin showed no inhibitory effect on alanine and glucose uptake by either protein. These data reveal brush border membrane effects by myeloma light chains and confirm that direct Bence Jones protein nephrotoxicity may play an important role in the pathogenesis of kidney dysfunction associated with multiple myeloma.

Renal involvement ranging from Fanconi syndrome to frank renal failure is common in multiple myeloma [1–7]. Renal dysfunction associated with myeloma is usually accompanied by increased urinary excretion of light chains (Bence–Jones protein). Direct toxicity of light chains to renal tubular cells has, therefore, been implicated as a major mechanism of renal dysfunction in multiple myeloma [1, 5–7]. The presence of proximal and distal tubular dysfunction in myeloma patients [3–7] and demonstration of inhibitory effects of light chains on rat and rabbit kidney slice uptake of para-amino–hippuric acid (PAH), tetraethyl ammonium (TEA) and glucose and ammonia production [8, 9], the inhibition of Na-K ATPase in renal cortical tubular membranes [10] and induction of tight junctions in necturus gall bladder [11] leave little room to doubt direct nephrotoxicity of light chains. While these observations provide evidence of direct, toxic effects of myeloma light chains on metabolic and transport processes in the kidney, there are no studies on the effect of light chains on glucose and amino acid transport. Since proximal tubular dysfunction is common in myeloma, isolated brush border membranes would provide an appropriate system to investigate the effect of light chains on proximal tubular transport of glucose and amino acid at the cell membrane level. We, therefore, isolated and purified light chains from the urines of three patients with multiple myeloma. The uptake of L-(^{14}C)alanine and D-(^{14}C)glucose by rat renal brush border membrane vesicles was then measured with varying concentrations of purified light chains in the incubation medium.

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Methods

Three different light chains were obtained from urines of three patients with multiple myeloma. One patient had κ light chain and the other two had λ, which will be referred to as "λ-1" and "λ-2."

The patient with κ light chain was a 62-year–old black male who presented with bone pains and multiple lytic bone lesions. At his initial evaluation his BUN was 21 mg/dl, creatinine 3.2 mg/dl and calcium 10.2 mg/dl. His bone marrow aspirate revealed 36% sheet–forming plasma cells which contained only κ light chain and no other immunoglobulin. Quantitative serum immunoelectrophoresis showed that immunoglobulin G, A, M and D levels were all depressed; thus, he was diagnosed as κ chain myeloma. His initial urinary protein excretion was 7.6 g/day, 97% of which was β light chains. His renal function declined rapidly and serum calcium levels rose to 11.5 mg/dl, and he died one year later despite chemotherapy.

The patient with λ-1 light chain was a 56-year–old white male who had stage III myeloma when he was first diagnosed. He had IgG myeloma with 50% plasma cells in his bone marrow aspirate. His initial BUN was 19 mg/dl and creatinine 0.9 mg/dl. He died after one year of chemotherapy, and at the time of death his BUN was 92 mg/dl and creatinine was 8.6 mg/dl and his serum calcium 12.4 mg/dl. His urine contained 3.7 g protein, 93% of which was γ-globulin. He also developed mild glucose intolerance and at the time of his death his urinalysis revealed 3+ glucose by Multistix (Miles Laboratories, Elkhart, Indiana, USA) with a simultaneous serum glucose of 222 mg/dl.

The patient with λ-2 was a 59-year–old white male diagnosed as having IgG myeloma. His initial BUN was 30 mg/dl and creatinine 1.7 mg/dl. His urine contained 3.2 g/day protein, 92% of which was γ-globulin.

Light chains were initially precipitated with 70% saturated ammonium sulfate solution. The precipitate was dissolved in distilled water and desalted by dialyzing against distilled water

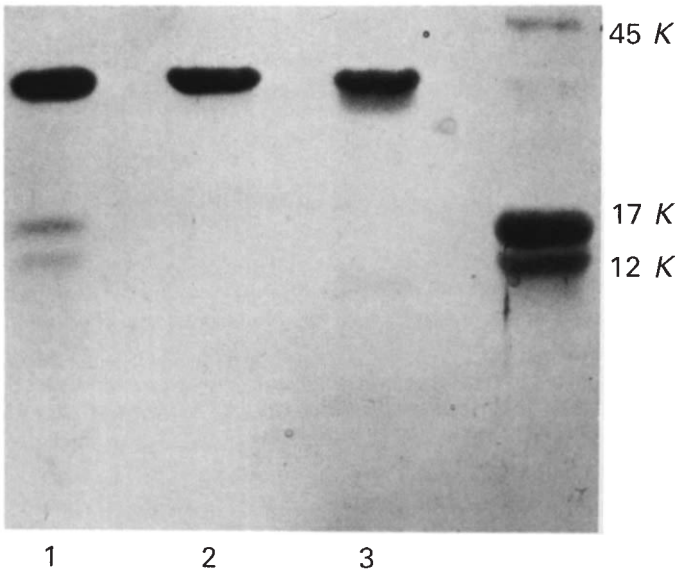

Fig. 1. *SDS-Polyacrylamide gel electrophoresis of the three light chains.* Column 1 is the κ chain; 2, λ-1 and column 3 is λ-2. The right–most column is the standards.

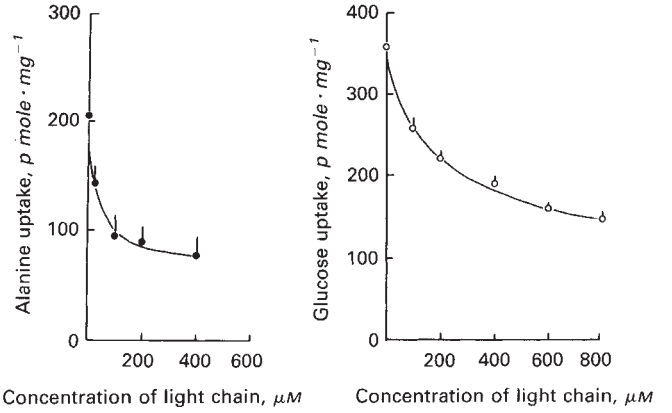

Fig. 2. *L-(^{14}C)alanine (●) and D-(^{14}C)glucose (○) uptake by brush border membrane vesicles at 30 seconds with varying concentrations of κ light chain in the medium.* The I_{50} is 68 μM for alanine uptake and 120 μM for glucose uptake.

for 24 hours. The concentrated protein solution was further purified in a Sephadex G-100 column. The eluate was then lyophilized and stored at −20°C. The identity and purity of the samples are confirmed by immunoelectrophoresis and polyacrylamide SDS gel electrophoresis. All three light chains appeared more than 95% pure, migrating as a single band in the 24 to 25,000 dalton range in the SDS gel electrophoresis (Fig. 1). Thus all three light chains studied here were monomeric.

Bovine serum albumin (BSA) with a molecular weight of approximately 67,000 daltons (Sigma Chemical, St. Louis, Missouri, USA) and β-lactoglobulin (BLG) with a molecular weight of about 35,000 daltons (Miles Scientific, Naperville, Illinois, USA) were used as controls.

Rat kidney brush border membrane vesicles were prepared from homogenized renal cortex by the calcium precipitation method as described previously [12]. Male Sprague–Dawley rats (Charles River Breeding, Massachusetts, USA) weighing 250 to 300 g were used for vesicle studies.

Effect of light chains on 30 seconds uptake rate of sodium–dependent L-(^{14}C)alanine and D-(^{14}C)glucose by brush border membrane vesicle was studied. Brush border membrane fraction of approximately 80 to 150 μg protein was incubated in 200 μl buffer containing either 20 μM L-(^{14}C)alanine or 20 μM D-(^{14}C)glucose (New England Nuclear, Boston, Massachusetts, USA) with varying concentrations of light chains (0, 50, 100, 200, 400, 800 μM) approximately from 1:10 to 1:200 weight to weight light chain to brush border protein ratio in the medium. These concentrations would be comparable to proximal tubular concentrations of light chains in a living person. For example, assuming that light chain was freely filtered, 1 g/dl light chain would yield 400 μM of monomeric light chain in the proximal tubular fluid. At the end of the incubation period, the membrane preparation was separated from the medium by rapid filtration technique using 0.45 μM pore size HAWP filters (Millipore Corp., Bedford, Massachusetts, USA). Nonspecific retention of radioactivity was estimated by using control filters treated identically by filtering 200 μl of incubation medium containing the same amount of radiolabelled alanine or glucose without the brush border fraction. The filters were dissolved in 8 ml Aquasol (Dupont-NEN Research Products, Boston, Massachusetts, USA) and counted in a liquid scintillation counter. Uptake of L-(^{14}C)alanine or D-(^{14}C)glucose was defined as total count less the nonspecific retention of radioactivity. Separate experiments were performed with BSA and BLG instead of light chains to rule out a nonspecific protein effect by light chains on the uptake of L-alanine and D-glucose by renal brush border membrane vesicles. The amount of alanine or glucose uptake by brush border membrane was determined in triplicate and expressed as picomole per mg protein. Protein concentrations were measured by Lowry technique using bovine serum albumin standards [12].

The half maximal inhibitory concentration (I_{50}) of each light chain was calculated from log dose response curves obtained with each light chain. Differences between means are evaluated using the unpaired Student's *t*-test.

Results

All three light chains inhibited both alanine and glucose uptake by renal brush border membrane at 30 seconds. At maximal inhibitory concentration the κ light chain reduced L-(^{14}C)alanine uptake from a control level of 206 ± 18 pmol/mg protein to 77 ± 18 pmol/mg protein ($P < 0.001$) and D-(^{14}C)glucose uptake from 357 ± 22 pmol/mg protein to 146 ± 8 pmol/mg protein ($P < 0.001$) (Fig. 2). The I_{50} of κ light chain was calculated as 68 M for alanine uptake and 120 M for glucose uptake. The λ-1 light chain decreased alanine uptake from 136 ± 17 to 60 ± 8 pmol/mg protein ($P < 0.005$) and glucose uptake from 354 ± 17 to 77 ± 14 pmol/mg protein ($P < 0.001$) (Fig. 3). The I_{50} of this λ light chain was 76 μM for alanine uptake and 70 μM for glucose uptake. Similarly, the λ-2 light chain reduced alanine uptake from 105 ± 9 to 28 ± 5 pmol/mg protein ($P < 0.001$) and glucose uptake from 194 ± 7 to 66 ± 7 pmol/mg protein ($P < 0.001$) (Fig. 4). The I_{50} for the λ-2 light chain was

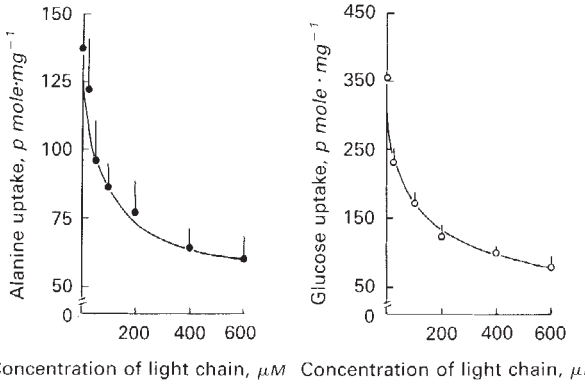

Fig. 3. *L-(^{14}C)alanine (●) and D-(^{14}C)glucose (○) uptake by brush border membrane vesicles at 30 seconds with varying concentrations of λ-1 light chain in the medium.* The I_{50} is 76 μM for alanine uptake and 70 μM for glucose uptake.

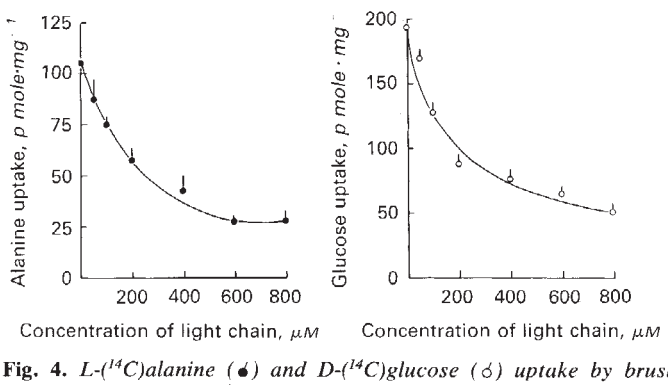

Fig. 4. *L-(^{14}C)alanine (●) and D-(^{14}C)glucose (○) uptake by brush border membrane vesicles at 30 seconds with varying concentrations of λ-2 light chain in the medium.* The I_{50} is 140 μM for alanine uptake and 105 μM for glucose uptake.

calculated as 140 μM for alanine uptake and 105 μM for glucose uptake.

Control experiments conducted using 0 to 800 μM concentrations of β-lactoglobulin or bovine serum albumin in the incubation medium revealed no inhibitory effect by either protein on the uptake of L-(^{14}C)alanine or D-(^{14}C)glucose. The results of these experiments are shown in Tables 1 and 2.

Discussion

Renal dysfunction is a major cause of morbidity and mortality in multiple myeloma and may be the first clinical manifestation of myeloma in as many as two–thirds of cases [1–7]. Renal involvement in myeloma includes diverse disorders such as adult Fanconi syndrome (manifested by glycosuria, aminoaciduria, phosphaturia, uricosuria, and bicarbonate wasting), urinary concentrating defects, decreased PAH clearance as well as frank renal failure [1–7]. In most cases, renal dysfunction is associated with light chain proteinuria and morphologically correlate with intratubular deposition of light chain containing casts and presence of intracytoplasmic protein crystals primarily of light chain origin [5, 6, 13–16]. The pathogenesis of renal disease in myeloma is, therefore, attributed to direct nephrotoxicity of light chain proteins [5–11, 17, 18]. On the other hand, the absence of renal dysfunction in some patients with large amounts of light chain proteinuria suggests variable toxicity of these proteins. Factors such as hypercalcemia, tumor mass, bone metastases, and volume depletion may contribute to renal disease in myeloma and may make it difficult to evaluate the nephrotoxicity of light chain alone.

Our experiments with the brush border membrane vesicle preparation permit in vitro evaluation of direct effects of light chains on renal tubular cell function at the cell membrane level. Our results revealed that three different light chains (one κ and two λ) obtained from three individuals with myeloma and varying degrees of renal insufficiency inhibited both alanine and glucose transport by rat renal brush border membrane vesicles. Control experiments using BSA and BLG had no inhibitory effect on either transport system, suggesting a unique toxic effect by light chains. These observations are in agreement with previous reports of direct light chain toxicity. Preuss et al observed that light chains in incubation media inhibited PAH and TEA uptake by rat and rabbit renal cortical slices, and suppressed glucose and ammonia production [8, 9]. Fine and Rees, in a letter to the editor, disputed the evidence that Bence Jones proteins had direct toxicity on the slice system and reported that purified light chains from two patients had no inhibitory effect on the slice uptake of TEA [19]. Reconciliation of these discrepant observations would require additional studies using the slice technique. The slice technique, however, yields access to the renal tubule only from the antiluminal side [20, 21]. Isolated brush border membrane system would closely resemble the conditions seen in a patient with light–chain proteinuria. Our observations are, therefore, different from the slice experiments in that we have found a specific effect of light chains on the brush border side.

Light chain toxicity has been observed in other systems as well. Alavi et al reported epithelial tight junction formation in the glomerulus of a patient with κ light chain proteinuria [11]. The light chain isolated from this patient's urine was further shown to increase transepithelial resistance and potential difference on the mucosal surface of the Necturus gall bladder. McGeoch et al, on the other hand, showed a direct inhibitory effect of light chains on ouabain sensitive Na-K ATPase in plasma membranes obtained from rat renal cortical tubules [10]. The presence of significant Na-K ATPase activity in this preparation indicates presence of significant quantities of antiluminal membrane fragments in this preparation [12]. Thus, this report pertains to toxicity of light chain on the antiluminal membrane and may account for the adverse metabolic effects of light chains in the slice system.

In conclusion, our studies reveal that light chains have direct toxic effects on the brush border membrane. We observed direct inhibitory effects of three different light chains obtained from the urine of patients with myeloma on the uptake of both alanine and glucose by isolated rat renal brush border membrane vesicles. Absence of inhibitory effects of BSA and BLG suggests that in our system the inhibitory effects are specific to myeloma proteins. A direct toxic action of light chains on renal tubular cell membranes may be an important mechanism in the pathogenesis of a variety of renal functional abnormalities as well as renal failure seen in multiple myeloma.

Table 1. L-(^{14}C)alanine and D-(^{14}C)glucose uptake by brush border membrane vesicles (mean ± SEM) with bovine serum albumin (BSA) in the medium[a]

BSA, μM	0	50	100	200	400	800
Alanine uptake, *pmole/mg protein*	259 ± 49	380 ± 42	340 ± 126	346 ± 124	387 ± 197	304 ± 90
Glucose uptake, *pmole/mg protein*	678 ± 180	728 ± 121	967 ± 189	883 ± 163	900 ± 220	879 ± 69

[a] None of the differences are statistically significant. $N = 4$ in each uptake.

Table 2. L-(^{14}C)alanine and D-(^{14}C)glucose uptake by brush border membrane vesicles (mean ± SEM) with β-lactoglobulin (BLG) in the medium[a]

BSA, μM	0	100	200	400	800
Alanine uptake, *pmole/mg protein* $N = 4$	378 ± 261	354 ± 125	456 ± 228	475 ± 303	354 ± 205
Glucose uptake, *pmole/mg protein* $N = 6$	198 ± 48	245 ± 39	246 ± 107	266 ± 66	202 ± 42

[a] None of the differences are statistically significant.

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