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Original article

Reversible low-molecular-weight proteinuria in patients with distal renal tubular acidosis

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Abstract. Four patients with untreated renal tubular acidosis had a urinary excretion of low-molecular-weight (LMW) proteins which was restored to normal by alkali therapy. Hypokalaemic proximal tubular damage in untreated patients with distal renal tubular acidosis is believed to be the cause of LMW proteinuria. An examination of urinary excretion of LMW proteins is useful for determining hypokalaemic proximal tubular dysfunction, as well as the efficiency of alkali therapy.

Key words: Alpha$_1$-microglobulin – Beta$_2$-microglobulin – Distal renal tubular acidosis – Hypokalaemia

Introduction

Distal renal tubular acidosis (dRTA) is characterized functionally by the inability of the cells in the distal tubules to produce a sufficient hydrogen ion gradient between the blood and the tubular fluid, regardless of the degree of systemic acidosis [1]. Urinary potassium ion (K^+) wasting and hypokalaemia are often found in dRTA without the use of alkali therapy [2]. The disease is not usually transient, and life-long alkali therapy is necessary to correct metabolic acidosis and secondary hypokalaemia [1, 2]. In this paper, we describe reversible low-molecular-weight (LMW) proteinuria probably resulting from hypokalaemic proximal tubulopathy in hypokalaemic and acidaemic patients with dRTA.

Patients and methods

Four unrelated patients, two males and two females, each having been clinically diagnosed as having primary dRTA, were followed for time periods ranging from 5 to 25 years (Table 1). All patients presented with spontaneous hyperchloraemic acidosis and alkaline urine at the time of clinical onset. The diagnosis of primary dRTA was established by eliminating the possibility that the symptoms were caused by other renal and non-renal disorders, and by evidence of defective urinary acidification after ammonium chloride loading; none of the patients was able to decrease urinary pH below a value of 6.6, despite severe degrees of systemic acidosis. All patients showed low levels of fractional excretion of bicarbonate (HCO_3^-) of less than 5% when the metabolic acidosis was corrected with a sufficient amount of an alkalating agent. Bilateral nephrocalcinosis was detected on the X-rays of each patient. Neither glycosuria nor hyperamino-aciduria was detected. The glomerular filtration rate was within normal limits. Patient 1 was diagnosed as having dRTA on examination. The other three patients had been treated with a sufficient amount of sodium citrate and potassium citrate (60–120 mg sodium citrate and 70–140 mg potassium citrate/kg body weight, as HCO_3^- 1.5–2.5 mEq/kg body weight), although they had ceased taking sodium citrate and potassium citrate for 3 months prior to the examination. Fourteen controls aged between 14 and 27 years were also examined.

Blood samples were drawn from a peripheral vein before and during therapy in order to measure serum K^+, creatinine, alpha$_1$-microglobulin (a_1-m), and beta$_2$-microglobulin (b_2-m). Serum K^+ and creatinine were measured by standard techniques on a Hitachi 736 multichannel analyser (Tokyo, Japan). Arterial blood samples were drawn for pH, base excess, and HCO_3^- measurements from a peripheral artery, and analysed by the AVL 945 automatic blood gas system (Schaffhausen, Switzerland).

Urine was collected for 24 h before and after therapy. After urine pH was measured, urine samples were centrifuged at 3,000 rpm for 10 min and stored at –20° C.

Radio-immunoassay kits were used to measure a_1-m and b_2-m in the serum and urine (Shionogi, Tokyo, Japan) [3–5], and N-acetylglucosaminidase (NAG) was measured in urine using a fluorimetric technique with 4-methylumbelliferyl-2-acetamido-2-deoxy-beta-D-glucopy-

Table 1. Summary of patients with distal renal tubular acidosis

Patient no.	Sex	Age (years)	Age at onset	Follow-up period (years)	Nephrocalcinosis	$FEHCO_3^-$ (%)
1	M	13	8 years	5	+	3.0
2	F	16	3 months	16	+	1.2
3	M	26	9 years	17	+	0.2
4	F	28	3 years	25	+	0.8

M, Male; F, female; $FEHCO_3^-$, fractional excretion of bicarbonate

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Table 2. Data obtained before and during alkali and potassium supplementation

	Serum				Arterial blood		
Patient no.	K (mEq/l)	b_2-m (mg/l)	a_1-m (mg/l)	pH	HCO_3^- (mmol/l)	BE (mmol/l)	C_{cr} (ml/min per 1.73 m^2)
1							
Before	3.0	1.2	15.4	7.302	17.4	–6.6	123
During	4.2	1.2	14.7	7.381	24.0	–0.8	139
2							
Before	3.0	2.0	12.9	7.320	17.3	–5.0	88
During	3.8	1.7	13.4	7.418	25.0	2.6	111
3							
Before	3.2	1.2	13.6	7.312	20.3	–4.2	86
During	4.0	1.2	12.1	7.437	22.9	0.8	98
4							
Before	3.1	2.0	16.1	7.301	19.4	–4.5	98
During	4.4	1.6	15.7	7.430	23.5	0.4	102
Control (Mean ±1SD)	4.2±0.3	1.5±0.3	16.6±3.7	7.41±0.03	24.2±1.8	2.1±1.7	118±21

	Urine								
Patient no.	pH	NAG (units/ml)	a_1-m (mg/l)	FEa_1-m (%)	b_2-m (µg/l)	FLb_2-m (mg/day)	UEb_2-m (mg/day)	ARb_2-m (mg/day)	FRb_2-m (%)
1									
Before	6.5	5.4	30.4	2.50	135.294	213.0	57.5	155.5	73.0
During	7.2	2.5	1.7	0.08	412	240.2	0.2	240.0	99.9
2									
Before	6.8	4.5	17.4	1.05	15.882	253.4	8.8	244.6	96.5
During	7.4	2.2	1.5	0.07	276	319.6	0.2	319.4	99.9
3									
Before	6.9	7.9	15.7	1.10	8.334	148.6	6.4	142.2	95.7
During	7.3	2.1	1.6	0.08	432	152.9	0.4	152.5	99.7
4									
Before	6.9	2.8	18.3	1.08	48.780	282.2	44.0	238.2	84.4
During	7.3	1.7	1.3	0.06	60	235.0	0.4	234.6	99.8
Control (Mean ±SD)	6.9±1.0	3.9±1.9	1.9±0.8	0.12±0.04	370±147	242±88	0.5±0.2	242.3±76.5	99.8±0.01

K, Potassium; b_2-m, beta$_2$-microglobulin; a_1-m, alpha$_1$-microglobulin; BE, base excess; C_{cr}, creatinine clearance; NAG, *N*-acetyl-β-glucosaminidase; FEa_1-m, fractional excretion of a_1-m = (urine a_1-m × serum creatinine/serum a_1-m × urine creatinine) × 100; FLb_2-m, filtered load of b_2-m = C_{cr} × serum b_2-m × 60 × 24; UEb_2-m, urinary excretion of b_2-m = urine b_2-m × urine volume; ARb_2-m, absolute reabsorption of b_2-m = FLb_2-m–UEb_2-m; FRb_2-m, fractional reabsorption of b_2-m = ($ARBb_2$-m/FLb_2-m) × 100

ranoside as a substrate (Shionogi) [3]. NAG was measured as millimoles 4-methylumbelliferyl liberated at 37° C/h per millilitre of urine. Each sample was measured twice in all assays. The assays were sensitive enough to accurately detect 10 ng/ml a_1-m, 1 ng/ml b_2-m, and 0.01 units/ml of NAG. Controls and standards were run on each analysis day to ensure assay reproducibility. Intra-assay and interassay CVs were below 10%.

Results

All patients showed metabolic acidosis and hypokalaemia at the start of the study (Table 2). Mean urine concentrations of a_1-m and b_2-m before therapy were 20.5 mg/l and 52,672 µg/l respectively. Mean fractional excretion of a_1-m (FEa_1-m) and mean fractional reabsorption of urinary b_2-m (FRb_2-m) were 1.43% and 87.4% respectively before therapy. Although the hypokalaemic symptoms were corrected after 4 weeks of alkali and K^+ supplementation, urinary excretion of a_1-m and b_2-m did not decrease to within the normal range in patient 1 (Fig. 1). An additional 4 weeks of supplementation were needed to normalize the urinary excretion of a_1-m and b_2-m in this patient. In the other three patients, hypokalaemia was corrected after 2 weeks of therapy and urinary excretion of a_1-m and b_2-m decreased to within the normal range after 4 weeks of therapy. Mean urine concentrations of a_1-m and b_2-m after therapy were 1.5 mg/l and 295 µg/l respectively. Mean FEa_1-m and FRb_2-m were 0.07% and 99.8% respectively after therapy. The pH of the collected urine was between 6.5 and 7.6.

Urinary excretion of NAG decreased in every patient after therapy; with a mean value of 5.2 units/ml before therapy and 2.1 units/ml after therapy. However, they remained within normal ranges.

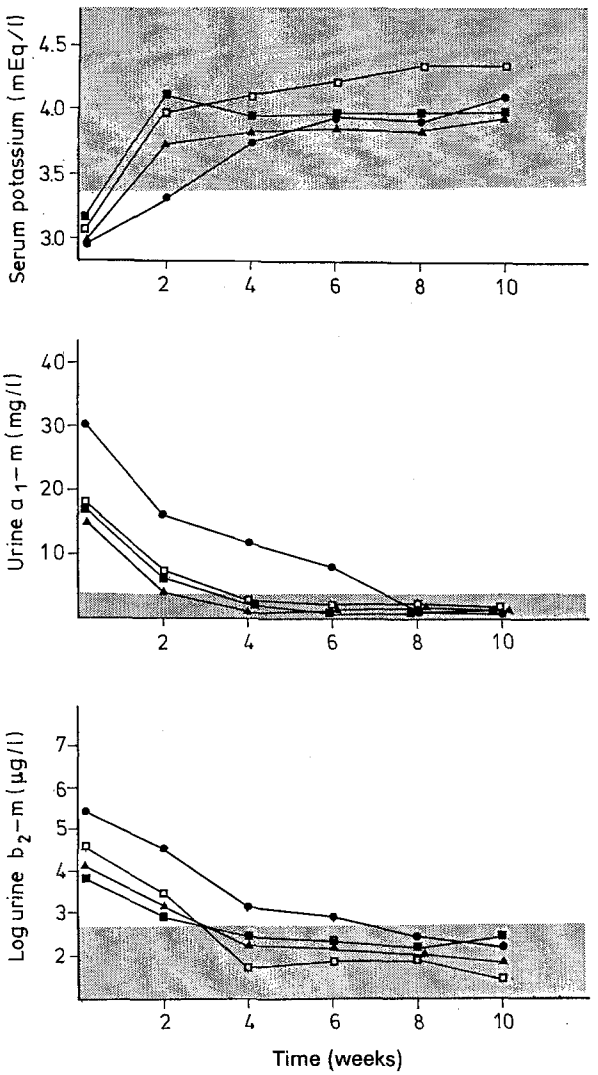

Fig. 1. Changes of serum potassium and urine low molecular weight proteins before and during therapy. The shaded areas indicate normal ranges; ●, patient 1; ▲, patient 2; ■, patient 3; □, patient 4; a_1-m, alpha$_1$-microglobulin; b_2-m, beta$_2$-microglobulin

Discussion

a_1-m and b_2-m are LMW proteins (33,000 daltons and 11,800 daltons respectively) which are filtered through normal glomeruli [4, 5]. It is known that the glomerular sieving coefficient (GSC) of a_1-m is low compared with the GSC of b_2-m [4, 6]. Most of the filtered a_1-m and b_2-m are absorbed and catabolized by the proximal renal tubules [7]. It has been shown that urinary excretion of a_1-m and b_2-m are increased in proximal renal tubular disorders such as Fanconi's syndrome, Lowe's syndrome, Wilson's disease, pyelonephritis, proximal renal tubular acidosis, Balkan nephropathy, cystinosis, and cadmium poisoning [8–11]. Urine levels of a_1-m and b_2-m are therefore good indicators of proximal tubular function.

Hypokalaemia is occasionally associated with gross disturbances of renal tubular function, and damage to the tubular cells [12–14]. Histological analysis revealed proximal tubular dilatation and atrophy, intratubular cast formation and interstitial infiltrate of lymphocytes in hypokalaemic kidneys [15]. Moreover, long-standing hypokalaemia has been seen to produce progressive renal insufficiency due to interstitial fibrosis and secondary glomerular sclerosis [15, 16]. Electron microscopy can be used to disclose multiple discrete vacuoles, or the formation of dense, periodic-acid-Schiff-positive lysosomes primarily in the proximal tubules [17, 18].

Emery et al. [19] demonstrated that 45% of patients with hypokalaemia showed an increased urinary excretion of b_2-m. Kjellberg et al. [20] indicated that starvation induced a reversible tubular proteinuria, probably secondary to K^+ deficits. Tubular proteinuria disappeared following K^+ treatment but not after treatment with HCO_3^-. Tolins et al. [21] also reported an increase in the excretion of the LMW proteins in hypokalaemic rats; alkali supplementation reduced the excretion of LMW proteins and chronic tubulo-interstitial disease in the rat model of hypokalaemic nephropathy. Additionally, we have seen two patients with Bartter's syndrome who also displayed hypokalaemia, metabolic alkalosis, and an increased urinary excretion of b_2-m (unpublished data). These results suggest that hypokalaemia in untreated patients with dRTA could disturb the proximal tubular function, resulting in LMW proteinuria.

Three patients (nos. 2–4) showed hypokalaemia and increased urinary excretion of a_1-m and b_2-m after they were off therapy for 3 months. However, patient 1 who was diagnosed as having dRTA when he was 8 years old, required a longer time for correction of his urinary abnormality, possibly due to the prolonged nature of the hypokalaemic proximal injury.

The examination of urinary excretion of LMW proteins in dRTA patients is very useful as an estimation of secondary hypokalaemic proximal tubular dysfunction and the efficiency of alkali therapy in the treatment of this disease.

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Ask the expert

What should be the modification in the dosage of prednisolone (or prednisone) for a child with minimal change nephrotic syndrome receiving this drug at a dose of: (1) 2 mg/kg per day, or (2) 1.5 mg/kg on alternate days, who develops (a) a serious infection (peritonitis, pneumonia, pyogenic meningitis) or (b) a minor infection (urinary tract infection, pharyngitis, pyoderma)?

Key word: Prednisolone

Treatment with steroids in the above-mentioned dosages is associated with an immunosuppression leading to increased susceptibility to serious life-threatening complications of infections [1], and with suppression of the hypothalamopituitary-adrenocortical axis, which may cause acute adrenal insufficiency with circulatory collapse [2]. Therefore, in the case of a severe infection it is necessary to reduce the immunosuppression in order to lower the risk of overwhelming infection, but to maintain a certain steroid level which allows the patient to combat the infection. Prednisone medication should not be stopped completely at the onset of severe infection [3]. Instead, it is our policy in the case of a high prednisone dosage [1], to reduce the daily prednisone dosage to 1 mg/kg (in 2–3 doses) until the infection subsides, and in the case of alternate-day treatment [2] to switch to daily administration of prednisone with the same dosage of 1 mg/kg per day (in 2–3 doses). If the infection is minor, e. g. viral pharyngitis or a bacterial urinary tract infection, it is usually not necessary to change the prednisone dosage. However the patient needs close observation to detect early signs of superinfections.

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