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Amyloid Urinary-Tract Calculi in Patients on Chronic Dialysis

Kenji Watanabe[a], *Ryuichi Nakamura*[b], *Shojiro Kano*[c], *Teruko Ohtake*[c], *Yasuhiro Hosoda*[d], *Takao Saruta*[e]

[a]Department of Internal Medicine, Ashikaga Red Cross Hospital, and [b]Department of Internal Medicine, Municipal Ida Hospital, Kawasaki; [c]Department of Laboratory Medicine, Keio University Hospital, [d]Department of Pathology, Keio University School of Medicine, and [e]Department of Internal Medicine, Keio University Hospital, Japan

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Abstract. Urinary calculi found in 4 patients on chronic hemodialysis or continuous ambulatory peritoneal dialysis (CAPD) were identified as protein calculi by infrared spectroscopic analysis. Positive Congo red staining and immunological assessment revealed that the calculi were composed of amyloid protein derived from β_2-microglobulin. A comparison of the patients who excreted calculi with 10 patients on chronic dialysis without urinary calculi showed no significant differences in the urinary and serum levels of β_2-microglobulin. The mechanism of amyloid calculus formation may involve factors independent of the concentration of β_2-microglobulin in urine or serum. Urinary calculi found in patients on chronic hemodialysis or CAPD were composed of amyloid protein derived from β_2-microglobulin.

Introduction

Urinary calculi in dialysis patients have conventionally been considered to be rare, but it has recently been reported that renal calculi are observed in 5–51% [1–5] of patients undergoing hemodialysis or CAPD. However, the pathogenesis and composition of urinary calculi have not been studied extensively. We analyzed the calculi from 4 patients on chronic dialysis. The calculi were composed of amyloid protein derived from β_2-microglobulin.

Subjects

Four patients with chronic renal insufficiency had spontaneous excretion of soft and brown urinary calculi during hemodialysis or CAPD at our hospital from 1985 to 1987. In all the patients, repeated spontaneous excretion of urinary calculi occurred without severe pain. Clinical data are shown in table 1.

Methods

Infrared Spectroscopic Analysis

A Nihon Bunkou IR 810 was used for infrared spectroscopic analysis (Mitsubishi Petrochemical Co., Japan).

Morphological Examination

For light microscopy, the calculi were fixed with neutral buffered formalin and decalcified with 5% EDTA (pH 6.5), then they were dehydrated and embedded in paraffin.

The sections were stained with hematoxylin-eosin and Congo red and then subjected to the periodic acid-Schiff reaction. For electron microscopy, the calculi were fixed with glutaraldehyde and osmium tetroxide, and embedded in Epon 812. Ultrathin sections were observed with an electron microscope (JEOL type 100C).

Immunochemical Assessemnt

Calculi were stored at –70°C. They were homogenized with 300 µl of destilled water and sedimented at 8,000 rpm for 10 min at 4°C. The precipitates were mixed with 300 µl of 50 m*M* glycine-NaOH buffer (pH 10.4). The calculi were nearly all dissolved. The solution was adjusted to pH 7.0 with 50 m*M* glycine-HCl buffer (pH 3.6) and sedimented again. The supernatant was assessed by the Ouchterlony test [6]. Antibodies against β_2-microglobulin, amyloid A and component P were obtained from Dakopatts, and antihuman whole serum and immunoglobulin light chains were purchased from Silenus Laboratory.

Analysis of Serum and Urine of Patients with Calculi

Serum total protein, calcium, inorganic phosphate, magnesium, uric acid, Vitamin C, calcitonin, $1\alpha,25(OH)_2D_3$, parathyroid hormone (PTH) and β_2-microglobulin were determined. $1\alpha,25(OH)_2D_3$ was determined by radioreceptorassay. Calcitonin, PTH (C-terminal) and β_2-microglobulin were determined by radioimmunoassay. The urine was analyzed for pH, protein, calcium, inorganic phosphate, uric acid, oxalate and β_2-microglobulin. Oxalate was deter-

Table 1. Clinical data

			Patient 1		Patient 2		Patient 3		Patient 4		Controls	
Age (years), sex			48, M		43, F		58, M		53, F		52±12	
Dialysis method			HD		CAPD		CAPD		CAPD		4 CAPD, 6 HD	
Renal disease			CGN		CGN		CGN		CGN		–	
Period of dialysis, years			3.2		2.7		0.8		1.6		2.2±1.7	
Serum chemistry												
Total protein,	g/dl	(g/l)	4.9	(49)	7.1	(71)	4.4	(44)	6.3	(63)	6.3±0.6	(63±6)
Calcium,	mg/dl	(mmol/l)	7.6	(1.9)	9.0	(2.2)	8.4	(2.1)	10.0	(2.5)	9.3±0.9	(2.3±0.2)
Inorganic phosphate,	mg/dl	(mmol/l)	7.7	(2.5)	3.6	(1.2)	3.7	(1.2)	3.3	(1.1)	5.3±1.4	(1.7±0.5)
Magnesium,	mg/dl	(mmol/l)	3.0	(1.2)	3.3	(1.4)	3.8	(1.6)	2.3	(0.9)	4.0±2.0	(1.6±0.8)
Uric acid,	mg/dl	(µmol/l)	12.4	(740)	5.8	(350)	7.3	(430)	7.6	(450)	7.6±2.7	(450±160)
Vitamin C,	mg/dl	µmol/l)	5.1	(290)	2.3	(131)	3.4	(193)	5.8	(329)	2.1±0.9	(119±51)
Calcitonin,	pg/ml	(pmol/l)	171	(50)	110	(32)	551	(162)	50	(15)	201±154	(59±45)
$1\alpha,25(OH)_2D_3$,	pg/ml	(nmol/l	11	(29)	10.5	(27)	22.7	(59)	24	(62)	15±6	(39±16)
PTH,	ng/ml	(pmol/l)	1.9	(200)	2.7	(280)	1.6	(170)	1.4	(150)	2.6±3.5	(270±370)
β_2-Microglobulin,	µg/l	(nmol/l)	19	(1.6)	31	(2.7)	32	(2.8)	10	(0.9)	24±8	(2.1±0.7)
Urinary findings												
Volume,	ml/day		400		520		1,400		670		570±370	
pH			6.0		6.0		6.0		5.5		5.5±0.5	
Protein,	mg/l	(g/l)	48	(0.05)	280	(0.28)	250	(0.25)	37	(0.04)	160±94	(0.02±0.1)
Calcium,	mg/l	(mmol/l)	58	(1.4)	32	(0.8)	36	(0.9)	10	(0.3)	47±22	(1.5±0.7)
Inorganic phosphate,	mg/l	(mmol/l)	460	(15)	140	(4.5)	160	(5.2)	180	(5.8)	224±138	(7.4±4.5)
Uric acid,	mg/l	(µmol/l)	180	(1,070)	60	(360)	134	(800)	160	(950)	140±85	(830±510)
Oxalate,	mg/l	(µmol/l)	25	(280)	37	(410)	61	(670)	31	(340)	49±12	(540±130)
β_2-Microglobulin	µg/l	(nmol/l)	97	(8.4)	3,520	(300)	38	(3.0)	>9,600	(>780)	5,240±4,250	(450±370)

HD = Hemodialysis; CAPD = continuous ambulatory peritoneal dialysis; CGN = chronic glomerulonephritis. Controls were patients on maintenance dialysis without nephrolithiasis (n = 10). Data of the controls are given as means ± SD.

mined by gas chromatography and β_2-microglobulin by radioimmunoassay. Because degradation occurs at pH levels of 5.5 or less [7], we added NaOH to the urine before collecting for 1 day and adjusted the urinary pH between 6.0 and 7.0. The levels of these compounds were compared with those from 10 chronic dialysis patients without urinary calculi.

Statistical Analysis

All values were stated as means ± SD. Data were analyzed by the non-paired t test.

Results

Infrared Spectroscopic Analysis

In the infrared spectra of all the calculi tested, there were no absorption patterns attributable to the inorganic or organic compounds usually detected in urinary stones, such as calcium, magnesium, inorganic phosphate or oxalate. The calculi were composed of protein, and the absorption pattern was similar to that of dried serum protein.

Morphological Examinations

Macroscopically, the protein calculi were small, 1–3 mm in diameter, blackish brown and moderately soft. As shown in figure 1, the calculi had a concentric lamellar structure. They were positive to Congo red staining, suggesting amyloid protein, but the material was different from typical amyloid since it showed no polarization. Electron microscopy revealed irregularly arranged interwoven nonbranching microfibrils. They were composed of two or more filamented subunits which ran in parallel and twisted. The diameter of the fibrils ranges between 120 and 160 Å. The average diameter was 140 Å (fig. 2).

Immunochemical Assessment

The Ouchterlony test (fig. 3) showed a marked precipitation line between the extracts of the calculi and the anti-β_2-microglobulin antibodies, but there was no precipitation line with anti-human whole serum or with any of the anti-sera against the other components of amyloid such as amyloid A, λ light chain, κ light chain or component P.

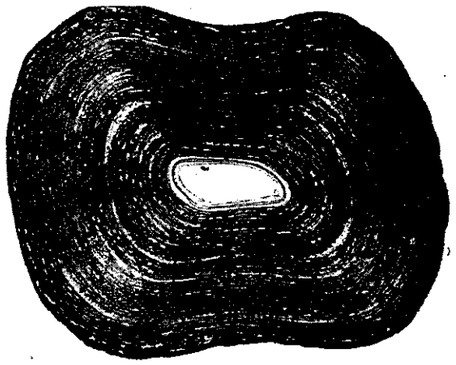

Fig. 1. Semithin section of protein calculus (patient 2). Note the lamellar composition of stone. HE. × 400.

Fig. 2. Electron micrograph of urinary calculus (patient 2). Irregularly arranged interwoven nonbranching microfibrils in diameter between 120 and 160 Å: × 120,000.

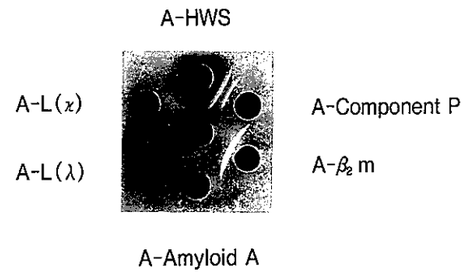

Fig. 3. Ouchterlony test schowing a marked precipitation line between the extract of calculi and anti-β_2-microglobulin antibody (patient 2). A-HWS = Anti-human whole serum antibody; A-Component P = anti-component P antibody; A-β_2m = Anti-β_2-microglobulin antibody; A-Amyloid A = anti-amyloid A antibody; A-L(κ) = anti-κ-light-chain antibody; A-L (λ) = anti-λ-light-chain antibody.

Analysis of Serum and Urine in Patients with Calculi

Serum vitamin C and uric acid levels in the patients with calculi were slightly elevated, and serum total protein, calcium and inorganic phosphate levels were slightly reduced compared to those in the control subjects, but there were no significant differences between the two groups in the levels of the biochemical constituents ($p > 0.05$).

In urinalysis, the oxalate and calcium concentrations were slightly lower than those in the control subjects, but the differences were not statistically significant ($p > 0.05$). The concentration of urinary protein was not high in the patients with calculi, and neither serum nor urinary β_2-microglobulin levels were higher than those in the control subjects ($p > 0.05$, table 1).

Discussion

The occurrence of calculi has been considered rare in dialysis patients, since their urinary concentrations of calcium are low. However, in recent years it has been reported that renal calculi occurred in 5–51% of hemodialysis of CAPD patients with chronic renal insufficiency [1–5]. There have been two kinds of components of calculi: some calculi consisted of calcium oxalate [1–3] and others of protein matrices [4].

Caralps et al. [1] observed spontaneous excretion of urinary calculi in 12 of 160 hemodialysis patients. These calculi consisted of calcium oxalate which might be associated with hyperoxaluria. Oren et al. [2] reported that the occurrence of calcium oxalate calculi in CAPD patients was related to high levels of urinary calcium concentration, due to the administration of $1\alpha,25(OH)_2D_3$. On the other hand, Bommer et al. [5] reported 7 hemodialysis patients associated with matrix calculi consisting of protein. According to them, all these patients had chronic nephritis and showed persistent proteinuria.

Nakayama et al. [8] reported 7 patients with hemodialysis-associated renal calculi: the calculi consisted of matrices in 5 and calcium oxalate in 2. They concluded that a difference between the two types of calculi was due to quantitative differences in the inorganic components in the urine at the various stages of calculus development. Cheng et al. [9] reported similar findings: urinary calculi consisting of various components, although the rate of calcium oxalate or matrix formation varied, making it impossible to distinctly divide the two types of calculi.

Infrared spectroscopic analysis revealed that the renal calculi in our patients consisted of protein. The infrared spectroscopic analysis was semiquantitative. It is therefore necessary to study further the possibility that the components of the calculi were pure protein or contained calcium oxalate.

The amyloid protein calculi we examined consisted of β_2-microglobulin. Linke et al. [10] reported the same components of the calculi they examined. They obtained protein calculi showing polarization characteristic of amyloid protein from patients on chronic dialysis and demonstrated that they consisted of β_2-microglobulin-derived amyloid by Congo red staining, immunohistochemical assessment and identification of the amino acid sequence at the N-terminal. The β_2-microglobulin of amyloid calculi was 7 kilodaltons, less then the 11.7 kilodaltons for the usual β_2-microglobulin. Our morphological assessment revealed a laminar structure, in agreement with conventional reports [5, 8]. The positive Congo red staining indicated amyloid protein, but no polarization was observed in the microscopic examination of any of the calculi. This might be due to irregular packing of fibrils. Further, the components of amyloid were revealed to be β_2-microglobulin by the Ouchterlony test.

Linke et al. [10] reported that the amount of urinary protein was increased in patients who excreted calculi. Gejyo et al. [11] reported that the blood level of β_2-microglobulin in patients on chronic dialysis with the carpal tunnel syndrome was no higher than the level in patients without carpal tunnel syndrome, indicating the existence of other factors independent of blood β_2-microglobulin concentration. In our patients with protein calculi, the amount of urinary protein was not always increased, and the urine and blood levels of β_2-microglobulin were rather lower than those in the control group. Amyloid calculus formation as a result of the binding of β_2-microglobulin may involve factors independent of β_2-microglobulin concentration in urine and serum. The mechanism of formation will be the subject of future studies.

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Ryuichi Nakamura, MD
Department of Medicine
Municipal Ida Hospital
City of Kawasaki
1272 Ida Nakahara
Kawasaki, Kanagawa (Japan)