Acquired Fanconi syndrome due to long-term adefovir administration in a patient with IgG-kappa monoclonal gammopathy and kappa Bence-Jones protein

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Abstract A 77-year-old man was admitted to our hospital for a right femoral neck fracture. He had been prescribed lamivudine for chronic hepatitis B infection for 11 years, and adefovir was added 5 years ago. After hospitalization, a right femoral head prosthesis was performed successfully, but an unknown hypokalemia was revealed. Hypophosphatemia, hypouricemia, glucosuria, and panaminoaciduria were also revealed, and multiple microfractures were detected by bone scintigraphy. We diagnosed him as ‘osteomalacia associated with Fanconi syndrome,’ which was likely due to the adefovir. Moreover, a monoclonal IgG-kappa and a kappa Bence-Jones protein were detected in his serum and urine, respectively. We switched from adefovir plus lamivudine to entecavir and started calcitriol. His excessive urinary β_2 -microglobulin excretion and glucosuria had decreased dramatically at 10 weeks after the modification of drugs; those of the phosphate, uric acid and total protein, however, continued. Renal biopsy specimens obtained at 10 weeks after discontinuation of adefovir revealed focal tubular atrophic changes with/without inflammatory cells, which were predominantly observed next to glomeruli. Kappa-dominant staining was not observed in either glomeruli or tubules with immunostaining by the enzyme-labeled antibody method. Electron microscopy revealed neither crystalline structures in the cytoplasm of proximal tubules nor electron-dense deposits. Because of the remarkable proportional reduction of other urinary protein fractions, urinary M-peak appeared 26 weeks after discontinuation of adefovir, but the net amounts of the fraction decreased gradually.

Keywords Fanconi syndrome · Adefovir · Monoclonal gammopathy

Introduction

Fanconi syndrome (FS) is a disease of the renal proximal tubules in which sodium, glucose, amino acids, uric acid, phosphate, and bicarbonate are lost into the urine instead of being reabsorbed. The clinical manifestation of the disease mainly consists of hypophosphatemic osteomalacia, metabolic acidosis, and some symptoms of hypokalemia and/or volume depletion ascribable to the overflow of sodium at distal nephron sites. FS may be inherited, or ‘acquired’ by drugs, heavy metals, and occasionally, overload of immunoglobulin light chains accompanied by plasma cell dyscrasia.

Adefovir is one of the nucleotide analog reverse transcriptase inhibitors used for hepatitis B infection, and its long-term use may cause FS. It has been estimated that low doses of adefovir (i.e., 10 mg per day) are clinically safe, even though high doses (i.e., 60 or 120 mg per day) can cause concerns about the severity and frequency of kidney toxicity [1–3]. In recent years, cases of acquired FS due to low-dose adefovir have been reported sparsely. We herein describe the first case of adefovir-induced FS in a patient with monoclonal IgG-kappa (mIgG- κ) and kappa type of Bence-Jones Protein (BJP- κ), which may become another origin to induce and/or deteriorate the disease.

Case report

A 77-year-old man visited orthopedists at our hospital for right coxalgia. He had fallen down 2 days previously. He

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Fig. 1 Glomeruli and tubules are shown by light microscopy. Periodic acid-Schiff staining (**a**, **b**), immunostaining by enzyme-labeled antibody method [anti- κ (**c**), - λ (**d**)]. **a** Tubular atrophic changes with inflammatory cells located next to a glomerulus are shown. Atrophic tubules are recognized as proximal tubules accompanied by thickening of tubular basement membrane, while distal tubules (located at two o'clock) are kept intact. Glomerulus shows

mild mesangial expansion; **b** Tubular atrophic changes located next to a glomerulus are also shown. In the picture, infiltrations of inflammatory cells are inconspicuous. Distal tubules and connecting tubules are kept intact. The glomerulus is intact; both anti- κ (**c**) and - λ (**d**) were nonspecifically stained in both the glomerulus and tubules, but there was hardly any difference between them (**c**) and (**d**). Original magnification; a, b, c, d: $\times 200$

was diagnosed with a right femoral neck fracture, and was admitted. Although a right femoral head prosthesis was successfully performed, an unknown hypokalemia was revealed in the process, and he was referred to our department 2 months after admission. He had been prescribed 100 mg per day of lamivudine by a hepatologist for chronic hepatitis B infection for 11 years, and 10 mg per day of adefovir for 5 years. His physical and laboratory findings were as follows: height 170 cm, weight 58 kg, blood pressure 94/56 mmHg, and pulse rate 90 per min. No abnormalities were observed except for slight muscle

weakness both of upper and lower extremities. A blood panel revealed that total protein and albumin were low at 6.4 and 2.8 g/dL, respectively. Blood urea nitrogen was 15.9 mg/dL, and creatinine was slightly elevated at 1.24 mg/dL. Levels of uric acid, K, and inorganic P (iP) were low at 2.4 mg/dL, 3.0 mEq/L, 2.3 mg/dL, respectively. Alkaline phosphatase was elevated at 421 U/L, and these levels had begun to exceed the reference range 10 months prior to admission. His complete blood count—white blood cell, red blood cell, hemoglobin concentration, and platelet—was almost normal at $7500/\mu\text{L}$, $358 \times 10^4/\mu\text{L}$,

Fig. 2 Glomeruli studied by immunofluorescence microscopy are shown (a–e). Stainings were for IgG, IgA, IgM, C3, and C1q, respectively. No staining was observed

12 g/dl, and 23.9/ μ L, respectively. Levels of IgG, IgA and IgM were almost normal at 1830, 341, and 26 mg/dL, respectively.

Complements were all within normal limits, and he tested negative for the antinuclear antibody. HBV-DNA was not detected in his serum. Spot urinalysis revealed persistent glucosuria, despite blood sugar levels within normal range, and he did not have diabetes mellitus. Urinary *N*-acetyl- β -D-glucosaminidase (NAG) was elevated at 27.4 U/L (normal: <11.9 U/L), and urinary β 2-microglobulin was extremely elevated at 136000 μ g/L (normal: <360 μ g/L). The values of fraction excretion of K were relative high at 20 % (normal: 10–20 %) even in hypokalemic states, and fraction excretion of uric acid (FE_{UA}) was also elevated at 37 % (normal: 4–11 %).

Despite his hypophosphatemic state, transtubular reabsorption of iP (TRP) and tubular maximal reabsorption rate of iP to glomerular filtration rate (GFR) (TmP/GFR) were quite low at 46.5 % (normal: 80–90 %) and 1.02 mg/dl (normal: 2.8–4.2 mg/dl), respectively. The urinary protein excretion rate was 2.6 g/g creatinine, and panaminoaciduria was detected. On endocrinological blood test, plasma renin activity was elevated at 8.4 ng/ml/hr (normal: 0.1–2),

while a plasma aldosterone concentration was 195.6 pg/ml (normal: 35.7–240). Levels of intact-PTH and vitaminD25 (OH) were 55 pg/ml (normal: 10–65) and 6.0 ng/ml (normal: 6–41), respectively; the latter was relatively low given his hypophosphatemic state. Multiple microfractures were detected by bone scintigraphy.

We diagnosed the patient with osteomalacia associated with FS, likely due to adefovir. Furthermore, we worked extensively to rule out other origins, and found that he had a mIgG- κ and a kappa BJP- κ in his serum and urine, respectively. We switched from adefovir plus lamivudine to entecavir, and started 0.25 μ g per day of calcitriol. The patient was then transferred to a rehabilitation center. He was re-admitted to our hospital to undergo renal biopsy and bone marrow aspiration because of persistent hypophosphatemia and hypouricemia 10 weeks after the suspension of adefovir. Bone marrow aspiration showed 1.2 % plasma cells with no atypia. No tumors, including extramedullary plasmacytoma, were detected in computed tomography of neck and head. His renal biopsy was studied by light microscopy; three of 25 total glomeruli were obsoleted, while other glomeruli were almost normal. In 10 % of the total cortical tubulointerstitial area, focal tubular atrophic

Fig. 3 A portion of both proximal tubules (**a**, **b**) and the glomerulus (**c**) are shown by electron micrograph. **a** In the picture, brush borders of proximal tubular cells are well kept in normal form, but basal infoldings are absent, which led to the disappearance of the regular mitochondrial arrangement. There were no crystalline-like structures detected in the cytoplasm of any proximal tubules checked by

electron microscopy; **b** no morphological abnormalities of mitochondria were observed; **c** no deposit of any type (including electron-dense-, granular-, organized-, and amyloid fibril) was observed in the subendothelial area, subepithelial area, mesangial area, or glomerular basement membrane. Original magnification; **a** $\times 800$, **b** $\times 6,000$, **c** $\times 3,000$

changes with or without inflammatory cells were observed, predominantly located next to glomeruli (Fig. 1a, b). Kappa-dominant staining was not observed in either the glomeruli or tubules in immunostaining by the enzyme-labeled antibody method (Fig. 1c, d).

Immunofluorescence staining revealed no significant staining for IgG, IgA, IgM, C3, or C1q (Fig. 2). (Immunofluorescence staining for κ and λ was not performed). No deposit of any type (including granular-, organized-, and amyloid fibril) was detected in glomeruli or tubular basement membranes by electron microscopy. In proximal convoluted tubules, loss of basal infoldings with scattered rounded mitochondria was seen, while brush borders were almost intact. Crystalline structures were not observed in the cytoplasm of proximal tubules (Fig. 3a–c). At 10 weeks after modification of drug protocol, excessive urinary $\beta 2$ -microglobulin excretion and glucosuria had decreased dramatically. However, both hypophosphatemia accompanied by high ALP and hypouricemia had persisted over 8 months, despite slight and slow improvement of FE_{UA} and TmP/GFR . GFR was almost stable (serum creatinine value: 1.17–1.30 mg/dl) during his clinical course.

The patient's clinical course is shown in Fig. 4. In the examination of urinary protein fractions, M-peak protein between the beta and gamma zones appeared at 26 and 32 weeks after discontinuation of adefovir; however, amounts of total proteinuria and albuminuria, as well as those of the beta plus gamma zones, had decreased over time (Fig. 5).

Discussion

Tests of high-dose adefovir (i.e., 60 or 120 mg per day) to treat HIV infection raised concerns about the severity and frequency of renal toxicity (i.e., decline of GFR, hypophosphatemia, and FS). Currently, 10 mg per day of adefovir is a common treatment for HBV infection, and is judged to be clinically safe [1–3]. However, there have been a few reports of cases of renal toxicity, including acquired FS, due to 'low-dose' adefovir. We researched a total of 21 detailed cases of 'acquired FS due to low-dose adefovir in HBV patients'; interestingly, most of patients (19/21) were East Asian. Sex ratio was 3F:19 M, and

Fig. 4 Clinical course of the present case is shown. Administration of adefovir 10 mg per day was started 5 years ago, and the elevation of ALP had begun to manifest 10 months prior to hospitalization. Hypokalemia had been corrected within normal range with supplement of potassium aspartate. At 10 weeks after switching from adefovir plus lamivudine to entecavir, urinary β 2-microglobulin excretion and glucosuria had decreased dramatically, and urinary NAG was also reduced. Afterwards, both urinary β 2-microglobulin

and NAG had gradually decreased a bit, but had not reached a normal range. Both hypophosphatemia accompanied by high ALP and hypouricemia had lasted over 8 months, despite slight and slow improvement of FE_{UA} and TmP/GFR over time. To intensify the treatment for hypophosphatemic osteomalacia, the dosage of both the calcitriol and supplemental phosphorus was stepped up (from 0.25 μ g per day to 0.5 μ g per day for the calcitriol, and to 600 mg per day for the phosphorus)

length of time from start of drug to outbreak of disease was quite long in almost all cases (average $\pm$ standard deviation was 45.5 ± 16.8 months). Patients in all but two cases (which were not described) recovered (i.e., had improved renal function, reduced abnormalities of serum electrolytes, bone pain, bone mineral density, etc.) after discontinuing adefovir, both with and without vitamin D and/or phosphorus supplementation. Duration of recovery period varied from several weeks to 8 months.

In the present case, the clinical course from the start of drug treatment to the outbreak of the disease was quite similar to that of past cases. A recent, retrospective, single-center study from Japan showed that 27 % (73 of 292) chronic HBV patients treated with low-dose adefovir plus lamivudine for more than 6 months developed hypophosphatemia; this included 14 patients with persistent hypophosphatemia. Their multivariate analysis identified male sex, hepatocellular carcinoma, low baseline serum phosphate as determinants of hypophosphatemia [4]. The researchers also reported that cumulative incidence of both hypophosphatemia and renal impairment abruptly increased 3 years after treatment, and that three of the 14 patients with persistent hypophosphatemia developed FS.

The mechanism of adefovir-induced FS is not clearly understood, but several hypotheses have been put forward. It has been verified that adefovir enters proximal tubular epithelial cells mainly through human organic anion transporter1 (hOAT1), located on the basolateral membrane [5, 6]. It is then secreted into urinary lumen by multidrug resistance protein 4 (MRP4; a member of the ATP-binding cassette transporter family) located on the luminal membrane [7]. The relationship between gene polymorphism of MRP4 and adefovir-induced renal toxicity has been reported [8]. Some drugs that are pharmacologically competitive for these transporters are known, but the clinical influence of interaction with adefovir has not been discussed. Furthermore, accumulation of adefovir may be harmful for mitochondrial DNA replication and the ATP-generating system in proximal tubular epithelium because of its inhibitory effect against mitochondrial DNA polymerase γ [9–11]. Differences at some point among the excretory pathway and/or in the accumulative properties of the proximal tubular epithelium may potentially lead particular individuals or ethnicities to develop FS.

However, no previous report has discussed clinical recovery periods for tubular reabsorption function after

Fig. 5 The longitudinal changes in the amount of net urinary excretion of total protein, albumin, and β plus γ zones (*figure top*), and in the pattern of urinary protein fraction (albumin, $\alpha 1$, $\alpha 2$, β , γ) (*figure bottom*) after discontinuation of adefovir are shown. Data points at 10, 26, and 32 W after discontinuation of adefovir are shown from left to right, respectively. The *black, gray, and white bars* (*figure top*) show the amounts of net urinary excretion (g/g Cr) of each

fraction of total protein, albumin, and β plus γ zones, respectively. There was a steady reduction of each fraction over time. Manifestation of M-peak at 26 W, followed by enhancements at 32 W, is shown in the lower portion of the figure. The amplitudes of urinary protein fraction are adjusted to amounts of excretion based on that of albumin

discontinuation of adefovir, or differences among electrolytes, uric acid, glucose, and proteins. We continued to evaluate follow-up data, including urine, even after the drug was suspended because the patient had mIgG- κ and BJP- κ . Rapid recovery of reabsorption function for glucose and $\beta 2$ -MG, and slight and slow recovery for phosphate and uric acid were observed in this patient (Fig. 4). In a recent Japanese case report, clinical signs manifested in the following order—hypouricemia, serum ALP elevation, and glucosuria/proteinuria—at 11, 36, and 48 months, respectively, after the start of adefovir administration [12]. The order reported in this previous case seems to be the reverse of the tubular reabsorption recovery observed in the present case. This may possibly explain the differences of standby capacity among each transporter/molecule (i.e., SGLT, GLUT, URAT1, URATv1, Na-Pi, etc. or megalin/cubilin) in the proximal tubular segment.

For our patient, the recovery of reabsorption function for phosphate, uric acid and total protein fraction was quite insufficient at the time of renal biopsy, while that of glucose had almost been achieved. We at first assumed that monoclonal BJP- κ was the real offender in inducing FS, but careful pathohistological observations did not reveal any evidence of light chain renal involvement. In fact,

‘crystalline-like structures’ in cytoplasm of proximal tubules are not always detected in cases of monoclonal gammopathy accompanied by ‘light chain-induced tubulopathy (including FS)’ [13, 14], conversely, FS is not always occurred even ‘crystalline-like structures’ were detected [15], therefore, we cannot conclude simply relying on only pathohistological information. However, steady and gradual reduction of both total urinary protein (including albumin) and M-peak fraction were observed, which strongly support the notion that FS might have been maintained by adefovir administration. Interestingly, we could observe urinary M-peak manifestation in accordance with the recovery of proximal tubular reabsorption function for other proteins, but those amounts were reduced over time. This finding might be explained by either or both of the following interpretations: (1) the gradual reduction of BJP- κ production; or (2) the increase of BJP- κ uptake in proximal tubules due to the recovery of reabsorption capacity. The latter seems to be the more reasonable explanation, judging from the parallel reduction in the excretion of other urinary proteins, and natural course of monoclonal gammopathy (even transient paraproteinemia has been reported in healthy populations, or in association with autoimmune disorders such as SLE, autoimmune

hepatitis, rheumatoid arthritis, and infections) [16, 17]. BJP- κ overload into proximal tubular cells may possibly have been a risk that prompted deterioration into FS by another mechanism in the present case.

Conclusion

In summary, we experienced and reported the first case of adefovir-induced FS in a patient with monoclonal gammopathy (mIgG- κ and BJP- κ). ‘Low-dose’ adefovir-induced FS has a tendency to occur several years after starting the drug. The proximal tubular reabsorption function of the present case achieved gradual recovery after discontinuation of adefovir, but the speed and degree were varied for phosphate, uric acid, glucose, and proteins.

The patient requires continuous and careful observation, and it will be necessary to take care of any recurrence of FS by another mechanism, as well as the progression from monoclonal gammopathy to myeloma, cast nephropathy, and any type of monoclonal immunoglobulin deposition disease.

Conflict of interest The authors have declared that no conflict of interest exists.

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