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Original Article

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Nephrogenic hypophosphatemic osteomalacia during adefovir monotherapy for chronic hepatitis B monoinfection

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Abstract

Background. In this paper, we explore nephrogenic hypophosphatemic osteomalacia associated with low-dose adefovir dipivoxil (ADV) therapy.

Methods. Five patients who were treated with ADV for >2 years were included in this study. The metabolic index of phosphate and calcium, renal tubular function, renal function and pathological changes of the patients were investigated.

Results. Two male and three female patients were studied. All of the patients presented with a reduced serum phosphate level (0.38–0.60 mmol/L) accompanied with hyperphosphaturia at 10.9–23.8 mmol/24 h. The serum potassium level was also reduced or at lower range (2.56–3.54 mmol/L), but the 24-h urinary potassium was relatively increased. Urinalysis also demonstrated increased excretion of glucose in four patients. Urine protein electrophoresis showed low-to-moderate molecular weight protein. Three patients manifested urine acidification function impairment. Four patients had accompanying renal insufficiency. Three patients had difficulty walking and presented with a reduction in height (2.5–14 cm). Renal biopsy revealed that most of the glomeruli were normal accompanied by mild interstitial fibrosis with inflammatory cell infiltration. ADV treatment was subsequently ceased. Patients were treated with regular phosphate supplementation, citrate acid potassium and calcium bicarbonate. After 6-month treatment, the bone pain was significantly alleviated. Serum creatinine of one patient returned to normal levels and two patients who had difficulty walking were able to walk independently.

Conclusions. The current study showed long-term and low-dose ADV treatment in a Chinese population may lead to proximal tubular impairment, metabolic acidosis, hypophosphatemia, hypokalemia, metabolic bone disease, renal osteopathia and renal functional damage.

Keywords: adefovir; hypophosphatemic osteomalacia; renal function

Introduction

Patients with chronic hepatitis B (CHB) are at a high risk for developing cirrhosis and hepatocellular carcinoma. Long-term viral suppressive therapy provides a critical treatment to prevent disease progression and accompanying complications. Several nucleos(t)ide analogs (NUC) are available for the management of patients with CHB. In most patients, NUC needs to be administered on a long-term basis, thus increasing the risk of adverse effects. Adefovir dipivoxil (ADV) is the first NUC developed to treat CHB. The active intracellular metabolite, adefovir diphosphate, has a longer intracellular half-life and is produced by the addition of two phosphate groups by ubiquitous cellular nucleotide kinases. It causes the inhibition of both reverse transcriptase and HBV-DNA polymerase and is incorporated into DNA inducing chain termination. ADV is effective for patients with both HBeAg(+) and HBeAg(–) CHB and for those who are resistant to lamivudine. Daily treatment with 30 mg ADV may lead to mild-to-moderate renal impairment [1]. However, it is not clear whether longer-term ADV (10 mg/day) treatment leads to further nephrotoxicity. Here, we report five CHB patients who developed proximal renal tubular dysfunction (RTD) and hypophosphatemic osteomalacia following several years of ADV treatment.

Materials and methods

Patients and definitions

All of the patients with CHB were started on therapy with ADV monotherapy for 2–7 years before admission. In each instance, other causes of abnormalities (such as diabetes, drug-induced renal dysfunction, or use of uricosuric medications) were ruled out. The diagnosis of phosphate wasting and RTD was based upon blood and urine testing results and required sustained hypophosphatemia with at least two other manifestations of tubular

dysfunction: hypouricemia, serum creatinine elevation, proteinuria or glucosuria [2].

Methods

Laboratory tests assessed liver and renal function; serum phosphate, calcium, alkaline phosphatase, PTH, 1,25-dihydroxyvitamin D_3 levels and arterial blood gases. Urine analysis included 24-h urine samples for phosphate, calcium, glucose and urine acid–base analysis. Radiological evaluation included determination of bone mineral density (BMD) by dual X-ray absorptiometry (DXA), X-ray, computed tomography (CT) and magnetic resonance imaging (MRI). Glomerular filtration rate (GFR) was calculated based upon serum creatinine, race and age using the updated Modification-of-Diet-in-Renal-Disease (MDRD) equation [$GFR = 175 \times (Scr)^{-1.154} \times (Age)^{-0.203} \times (0.742$ if female)].

Patient treatment

Treatment after diagnosis of nephrogenic hypophosphatemic osteomalacia and RTD included the following: (i) discontinuation of ADV and change to other antiviral medication such as entecavir 500 μg q.d. (ii) regular phosphate supplementation ($Na_2HPO_4 \cdot 12H_2O$ 73.1 g, NaH_2PO_4 6.4 g, H_2O 1000 mL, pH = 7.0, phosphate 778 mg in 100 mL); (iii) 1,25-dihydroxyvitamin D_3 0.75 μ/day; (iv) oral $CaCO_3$ at 1200 mg/day and (v) correction of acidosis.

Results

Clinical profiles (baseline)

Two male and three female patients, aged 33–58 years, were included in this study. They were admitted to our department between January 2008 and December 2011 with the diagnosis of proximal renal tubular disease, hypophosphatemia, osteomalacia and CHB. They had been on ADV treatment (10 mg/day) for 2–7 years before admission. Patient 1 and Patient 2 changed from lamivodine to ADV monotherapy because of viral resistance. The remaining patients were treatment-naive patients starting ADV monotherapy as a first-line treatment. Patient 5 had cirrhosis due to hepatitis B and took 10 mg/day ADV for 6 years after liver transplantation. Patient 4 had surgery for colorectal cancer and postoperative chemotherapy; she had been infected with hepatitis B following a blood transfusion. This patient had taken 10 mg/day ADV for 3 years. Five patients had a viral load of hepatitis B $<1 \times 10^3$ IU/mL at admission. All of the patients developed bone pain involving the ankles, knees and sternum. Some of them had difficulty walking and standing.

Baseline characteristics and renal function evaluation

All of the patients had reduced serum uric acid (95–189 μmol/L) and phosphate levels (0.38–0.60 mmol/L) accompanied by hyperphosphaturia at 10.9–23.8 mmol/24 h (Table 1). The TmPO4/GFR ratio was very low (0.24–0.56 mmol/L), indicating impairment of phosphate reabsorption in the proximal tubule. Serum potassium levels were also low (2.56–3.54 mmol/L), and 24-h urinary potassium was relatively increased (27.5–46.0 7 mmol/24 h). Urine analysis also demonstrated increased excretion of calcium (7.62–9.47 mmol/24 h) in three patients and normoglycemic glycosuria in four patients. Serum chloride was increased or at the high end of the normal range (108.2–112.2 mmol/L) as was urinary bicarbonate (12.3–17.4 mmol/L). Patients 1, 2 and 5 had metabolic acidosis (blood pH: 7.31–7.32). Three patients had an eGFR (MDRD) <50 mL/min/1.73 m^2.

Baseline bone metabolism evaluation

These investigations revealed reduced serum phosphate levels (0.38–0.60 mmol/L) and normal serum calcium (2.03–2.24 mmol/L) (Table 1). Serum 25-hydroxyvitamin D_3 levels were reduced or at the lower end of the normal range (14.6–51.4 nmol/L). Serum alkaline phosphatase levels were significantly elevated (141–430 IU/L). BMD by DEXA scanner indicated severe osteoporosis, osteoporosis and osteopenia. All of the patients complained of bone pain and three patients had difficulty walking. Three patients presented a reduction in height (3–14 cm). In two patients, physical examination revealed sternum, lumbar and rib tenderness, limited hip movements, muscular atrophy in the lower limbs and reduced muscle power and tone. Two patients were shown to have vertebral compression and scoliosis on X-ray, CT and MRI. One patient had osteonecrosis at the distal femur and proximal tibia. Two patients had accompanying rib fracture.

Renal pathology

In Patient 1, light microscopy revealed mostly normal glomeruli except for two to three with sclerosis accompanied by mild interstitial fibrosis with inflammatory cell infiltration. The tubules presented mild necrosis with vacuolar degeneration in parts of the epithelium. Immunofluorescence was negative as were HBsAg (–) and HBcAg (–). In Patient 3, light microscopy revealed mostly normal glomeruli except for one with sclerosis and areas of fibrosis and inflammatory cell infiltration. Tubular necrosis was also observed. Immunofluorescence was negative as were test results for HBsAg (–)and HBcAg (–). Histology showed evidence of interstitial nephritides.

Follow-up

Following the diagnosis of ADV-related hypophosphatemia, ADV treatment was ceased and changed to entecavir 500 μg q.d. (Table 2). Patients were treated with regular phosphate supplementation, 1,25-dihydroxyvitamin D_3, potassium citrate and calcium bicarbonate. After 6 months of treatment, the bone pain was significantly alleviated. Two of the patients who had difficulty walking regained independent mobility. Four patients had follow-up investigations. Laboratory follow-up revealed normal serum phosphate (0.92–1.24 mmol/L), potassium (3.20–3.70 mmol/L) and alkaline phosphatase levels (83–97 IU/L). The TmPO4/GFR ratio improved (0.77–0.86 mmol/L), and HBV-DNA remained controlled ($<1 \times 10^3$ IU/mL). Most of the patients had normal renal function (serum creatinine 76–98 μmol/L), except for mild renal insufficiency in Patient 4 (129 μmol/L). BMD by DXA was normal. MRI of Patient 1 showed normal thoracic and lumbar vertebra, except for mild hyperosteosis along the ridge of the lumbar vertebra. The femoral necrosis of Patient 4 was markedly improved.

Table 1. Baseline characteristics and bone metabolism indices

	Case 1	Case 2	Case 3	Case 4	Case 5
Age (years)	33	35	34	58	45
Gender	Male	Male	Female	Female	Female
Lamivudine treatment	Yes	Yes	No	No	No
ADV monotherapy	10 mg/day	10 mg/day	10 mg/day	10 mg/day	10 mg/day
Cirrhosis	Absent	Absent	Absent	Absent	Present
Serum creatinine (μmol/L)	84	116	127	119	116
eGFR(MDRD mL/min/1.73 m^2)	97.1	66.1	44.5	43	46.6
Uric acid (μmol/L)	95	103	154	189	118
Serum potassium (mmol/L)	3.15	3.54	2.90	2.56	2.58
Serum phosphate (mmol/L)	0.38	0.60	0.64	0.53	0.58
Urinary phosphate (mmol/24 h)	10.9	21.6	23.8	16.74	18.04
TmPO4/GFR (mmol/L)	0.27	0.24	0.56	0.27	0.30
Urinary potassium (mmol/24 h)	27.54	38.88	46.07	32.00	41.00
Urinary glucose (mmol/24 h)	17.82	2.00	20.12	8.09	25.76
Urinary pH	7.28	6.04	7.07	6.3	6.78
Urinary HCO_3 (mmol/L)	16.7	12.3	15.9	17.4	15.2
Serum chloride (mmol/L)	112.2	109.1	108.2	112	110.6
Blood pH	7.32	7.31	7.41	7.35	7.32
Urine protein (mg/24 h)	59	402	601	654	804
Urine electrophoresis	Low to moderate	Low to moderate	Low to moderate	Low to moderate	Low to moderate
Course of ADV (years)	4	7	3	2	6
HBsAg	+	+	+	+	–
HBeAg	+	+	+	–	–
HBV-DNA	$<1\times10^3$	$<1\times10^3$	$<1\times10^3$	$<1\times10^3$	$<1\times10^3$
Serum calcium (μmol/L)	2.1	2.24	2.21	2.03	2.11
Alkaline phosphatase (IU/L)	356	247	147	430	141
PTH (pg/mL)	11.6	27.4	38.4	68.6	95.3
25-Vitamin D (nmol/L)	31.9	14.6	29.1	35.47	51.4
Reduced height (cm)	3.0	14.0	0	2.5	0
Difficulty walking	Present	Present	Absent	Present	Absent
Bone pain	Present	Present	Present	Present	Present
Bone mineral density	Serious osteoporosis	Serious osteoporosis	Osteoporosis	Osteoporosis	Osteopenia

Table 2. Treatment and follow-up (after 6-month treatment)

	Case 1	Case 3	Case 4	Case 5
Serum creatinine (μmol/L)	98	76	129	87
eGFR (MDRD) mL/min	81.3	80.9	39.2	64.9
Serum potassium (mmol/L)	3.20	3.70	3.53	3.59
Serum phosphate (mmol/L)	1.24	1.04	1.05	0.92
Serum calcium (mmol/L)	2.43	2.24	2.37	2.31
TmPO4/GFR (mmol/L)	0.88	0.86	0.77	0.83
Alkaline phosphatase (IU/L)	85	97	91	83
BMD	Normal	Normal	Normal	Normal
Difficulty walking	No	No	No	No
HBV-DNA	$<1\times10^3$	$<1\times10^3$	$<1\times10^3$	$<1\times10^3$

Discussion

Adefovir is given orally as the pro-drug ADV. It is converted intracellularly to the diphosphate adefovir via the phosphorylation of adenylate kinase. The bio-active diphosphate adefovir inhibits the synthesis of hepatitis B virus DNA through competition for the enzyme reverse transcriptase and incorporation into the viral DNA to cease replication of the virus. ADV is effective for patients with both HBeAg(+) and HBeAg(–) CHB and for those who are resistant to lamivudine. In 2002, ADV became the second direct antiviral agent available to treat HBV patients to be approved in the US and later (2003) in the EU at a dose of 10 mg/day. ADV is excreted unchanged in the urine through glomerular filtration and tubular secretion [3]. Although the mechanisms of ADV-induced nephrotoxicity are not fully understood, data suggest a role for alterations in renal tubular transporters, apoptosis or mitochondrial toxicity in renal tubular epithelium. The proximal renal tubule is the target of high drug concentrations because of active uptake of the parent nucleotide ADV from circulating blood directly into proximal renal tubules. Patients with isolated proximal RTD may develop Fanconi's syndrome characterized by metabolic acidosis, hypophosphatemia and glycosuria [4, 5].

While using ADV for treating patients with HIV-1, Fisher *et al.* [6] reported nephrotoxicity rates of 35 and 50% in those receiving 120 mg/day ADV for 48 and 72 weeks, respectively, and 27% for those given 60 mg/day for 42 weeks and 13% for those given 30 mg/day for 48 weeks. Others have reported that 22–50% of patients may develop renal tubular disease while on ADV for 72 weeks at doses over 30 mg/day [6]. The nephrotoxicity is positively related to the dose of ADV. Nephrotoxicity was defined as serum creatinine increases of 44 μmol/L over the baseline and/or serum phosphate <0.48 mmol/L (1.5 mg/dL) in two consecutive measurements. In a retrospective case-controlled real-life study, 145 CHB patients were treated with ADV 10 mg [7]. Seven of these patients (5%) treated for a median of 30 months had >38 μmol/L (0.5 mg/dL) increases in serum creatinine and eGFR reductions <50 mL/min; treatment discontinuation was reported in 5 cases/100 patient-years. In this study, ADV was an independent predictor for significant deterioration of renal function, especially in patients who were older and had baseline renal impairment, hypertension and/ or diabetes. A 5-year study [8] of 10 mg/day ADV in 65 HBeAg(+) patients also showed >38 μmol/L (>0.5 mg/dL) increases in serum creatinine from baseline in five patients (8%), and this required permanent ADV discontinuation in two. Two patients had confirmed hypophosphatemia [from 0.32 to 0.48 mmol/L (1.0–1.5 mg/dL)]. Therefore, real-life studies confirmed an increasing risk of

ADV-related nephrotoxic effects during long-term treatment. Only one study evaluated BMD in patients with CHB who were treated with long-term NUC [9]. The study enrolled 319 patients; 67% had reduced BMD, 19% had osteoporosis and 49% had osteopenia. These findings suggest a need for early bone-oriented surveillance in CHB patients receiving NUC.

In our study, two of the patients changed from lamivudine to ADV because of viral resistance. All five patients were treated with 10 mg/day ADV monotherapy for >2 years (one patient was treated for 7 years). All of the patients manifested severe hypophosphatemia (0.38–0.60 mmol/L) with bone pain, osteoporosis and pathological fracture. Three patients presented with a reduction in height (Patient 1: 3 cm; Patient 2: 14 cm). Serum alkaline phosphatase levels were elevated in all patients (430–147 IU/L) (with concurrent normal ALT, AST and gamma-glutamyl transpeptidase values). Some showed vertebral compression revealed by X-ray and MRI. Patient 4 even developed osteonecrosis, indicating hypophosphatemic osteomalacia.

In normal individuals, urinary phosphate excretion almost completely stops during a period of hypophosphatemia (<0.60 mmol/L), but in our patients, high urinary phosphate excretion rates remained (10.98–23.80 mmol/24 h) and the TmPO4/GFR ratio was very low (0.24–0.56 mmol/L) indicating renal phosphate wasting. Furthermore, serum phosphate levels and TmPO4/GFR ratios improved when therapy was switched to entecavir. Our patients also had high urinary potassium excretion (27.5–46.07 mmol/24 h) when the serum potassium levels were low or at the lower end of the normal range (2.9–3.54 mmol/24 h) and high calcium excretion (7.62–9.47 mmol/24 h) and high glucose excretion (17.82–25.76 mmol/24 h) during normoglycemia and hypouricemia. Urinary protein electrophoresis revealed low-to-moderate molecular weight proteins, indicating impairment of the proximal tubule. These results indicated proximal RTD [2]. Serum chloride levels were increased or at the higher end of the normal range (108.2–112.2 mmol/L). Urinary HCO_3 was also increased or at the higher end of the normal range (12.3–17.4 mmol/L). Patients 1, 2 and 5 showed metabolic acidosis (blood pH 7.31–7.32). Four patients presented with increases in creatinine to above pre-treatment values (serum creatinine 116–127 μmol/L). Gara *et al*. [2] reported that proximal RTD developed in 15% of patients treated with adefovir or tenofovir for 2–9 years which was partially reversible with change to other antivirals.

This review of five patients with CHB demonstrates that long-term therapy with adefovir can be associated with proximal RTD resulting in significant hypophosphatemia, renal insufficiency and osteomalacia. The risk factors for this complication and its frequency are only partially understood. The renal tubular impairments led to metabolic acidosis, hypophosphatemia, hypokalemia and renal bone disease (osteodystrophy and osteomalacia). Renal pathology revealed that most of the glomeruli were normal with mild interstitial fibrosis. The tubules presented mild necrosis with vacuolar degeneration in parts of the epithelium. Immunofluorescence was negative as were testing for HBsAg(–) and HBcAg(–). In a case report, a 58-year-old man who had received ADV 10 mg/day for 3 years developed fatigue, weight loss, bone pain and progressive worsening of renal function along with metabolic acidosis, hypophosphatemia and hypouricemia. He also experienced normoglycemic glycosuria, aminoaciduria, mixed proteinuria and osteoporosis. Kidney biopsy revealed acute tubular necrosis with confluent necrosis of the proximal tubular epithelium and cell vacuolization with a fading of the brush border. After ADV was stopped, plasma levels of bicarbonate, uric acid and urinalysis normalized within 4 months [10]. This combination of nephrotoxicity and proximal RTD was likely caused by ADV treatment given for CHB. It has previously been shown that nephrotoxicity secondary to ADV is dose dependent and that renal function recovers gradually following 16 weeks of discontinuation of ADV; however, 16% of the patients still present renal insufficiency at 41 weeks. It is advisable to discontinue ADV and switch to other antiviral medication when nephrotoxicity develops. Liver function and hepatitis B virus activity should also be closely monitored.

In the current study, the symptoms and laboratory testing for abnormalities were reversed in all patients after 6 months of phosphate supplementation with citrate acid potassium and bicarbonate. Patients 1 and 3 can now walk independently and the body weights have increased. The high serum creatinine level in Patient 4 (129 μmol/L) may be related to advanced age and decreased cardiac function. The current study showed nephrotoxicity and proximal RTD following ADV treatment for CHB in a Chinese population. Similar cases have been reported (two with hypophosphatemic osteomalacia and one with Fanconi's syndrome) [10–12]. We suggest that a close monitoring of electrolyte (phosphate, potassium and calcium) and renal function is essential in patients receiving long-term ADV treatment. To minimize this risk, monitoring of renal function with serum creatinine, creatinine clearance, phosphate and urine analysis should be performed at baseline and every 3 months in the treated patients.

Conflict of interest statement. None declared.

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