Parathyroid Hormone-related Peptide-producing Multiple Myeloma and Renal Impairment

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Abstract

A 68-year-old man was hospitalized and examined for renal impairment. A laboratory analysis showed hypocalcemia. Although the serum parathyroid hormone and serum 1-25(OH)₂ vitamin D₃ levels were not elevated, the serum parathyroid hormone-related peptide (PTHrP) level was increased. Immunoelectrophoresis of the urine and bone marrow aspiration indicated multiple myeloma (MM). He was diagnosed with the coexistence of cast nephropathy and light chain deposition disease by a renal biopsy. Notably, PTHrP expression was detected in the myeloma cells based on immunohistochemistry and in situ hybridization. It is therefore important to examine the PTHrP concentration in MM patients with hypercalcemia.

Key words: cast nephropathy, light chain deposition disease, PTHrP


Introduction

Multiple myeloma (MM) is characterized by the neoplastic proliferation of a single clone of plasma cells. Kidney injury is a common complication of MM, and the pathogenesis can be separated into the immunoglobulin (Ig)-dependent or Ig-independent mechanisms (1). Cast nephropathy (CN), light chain deposition disease (LCDD) and AL-amyloidosis are common forms of monoclonal Ig-mediated kidney injury. Hypercalcemia is an Ig-independent disorder associated with MM. Myeloma bone disease causes hypercalcemia and it is usually related to the effects of local humoral factors mediating bone resorption and formation (2). On the other hand, several reports have shown parathyroid hormone-related peptide (PTHrP) to be implicated in the onset of hypercalcemia associated with MM (3-6). In this report, we describe a case of MM with CN, LCDD and hypercalcemia associated with the production of PTHrP proteins and mRNA in myeloma cells, as detected on immunohistochemistry (IHC) and in situ hybridization (ISH), respectively.

Case Report

A 68-year-old man who had not been previously diagnosed with proteinuria or hematuria presented to his primary care physician complaining of a poor appetite two months prior to the current admission. A laboratory examination revealed anemia and renal impairment, and he was referred and subsequently admitted to our hospital for a further examination of these symptoms.

At the time of admission, the patient’s body temperature was 35.9°C, his pulse rate was 64 beats per minute and his blood pressure was 120/71 mmHg. He weighed 53.4 kg and had experienced a body weight loss of 5 kg within the preceding three months. No rashes or lymphadenopathy were observed, and no lung rales or heart murmurs were detected on chest auscultation. An examination of the abdomen was unremarkable, and there was no edema in either leg.

A urinalysis revealed proteinuria with a protein level of

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0.61 g/gCr, the absence of occult blood, with 1-4 red blood cells per high-power field, and five granular casts per each entire field. The urinary N-acetyl-beta-D-glucosaminidase (NAG) level was 24.5 IU/gCr and the urinary beta 2-microglobulin level was 78,455 μg/gCr. A blood examination showed normochronic normocytic anemia, with a red cell count of 2.94 million/μL and a hemoglobin level of 10.0 g/dL. The laboratory findings showed renal dysfunction as well as hypercalcemia, with a blood urea nitrogen level of 58 mg/dL, a serum creatinine level of 4.19 mg/dL, an estimated glomerular filtration rate (eGFR) of 12.1 mL/min/1.73 m², a serum calcium level of 11.4 mg/dL, ionized calcium level of 2.88 mEq/L and a serum phosphate level of 4.7 mg/dL. The urinary calcium level was 0.12 g/gCr and the percentage of renal tubular reabsorption of phosphate was 40.4%. In addition, the serum parathyroid hormone (PTH) level was 21 pg/mL (normal range: 10-65), while the serum 1-25(OH)2 vitamin D3 level was decreased (8.2 pg/mL; normal range: 20.0-60.0) and the plasma PTHrP level was increased (6.8 pmol/L; normal range: <1.1).

Immunoelectrophoresis of the urine was positive for kappa-type Bence-Jones proteins. Furthermore, the concentration of the serum kappa-type free light chain (FLC) was increased at 2,180.0 mg/L, and the FLC ratio (kappa/lambda) was 110.1. Bone marrow aspiration showed 34.9% plasma cells, and the patient was diagnosed with MM.

Whole-body computed tomography and 18F-fluorodeoxyglucose positron emission tomography showed no apparent bone lesions. The size and shape of the kidneys did not suggest atrophy on abdominal ultrasonography or computed tomography.

Pathological findings

A renal biopsy was performed to examine the cause of the patient’s renal impairment. A total of 45 glomeruli were observed on light microscopy, 22 of which exhibited global sclerosis, and severe interstitial fibrosis with tubular atrophy (Fig. 1A) and atherosclerosis was noted. Cell infiltration was detected in the renal interstitium, primarily composed of lymphocytes, whereas plasma cells were scarce. Several tubules contained cast material with detached tubular epithelial cells (Fig. 1B). No glomerular nodular lesions were observed. Dyron staining was negative for amyloid deposition (Figure not shown). Immunofluorescence studies using frozen renal sections demonstrated a linear pattern of Ig kappa in the capillaries and tubular basement membrane (TBM) (Fig. 1C). Electron microscopy showed granular electron dense deposits in the glomerular basement membrane (GBM) (Fig. 1D) and TBM (Fig. 1E). Collectively, the patient was diagnosed with the coexistence of CN and LCDD.

Clinical course

The patient’s hypercalcemia was treated with intravenous saline, loop diuretics and elcatonin. After making the diagnosis of MM, we stopped the treatment with loop diuretics and initiated alkalinization of the urine in addition to the administration of three courses of bortezomib (2 mg/body) and dexamethasone (26.4 mg/body). However, due to the appearance of a fever and rash, only dexamethasone (20 mg/week) was continued thereafter. One month after the start of treatment, the serum calcium level decreased to within the normal limits, and the serum PTHrP became negative. The serum creatinine level decreased to 2.74 mg/dL, while the eGFR increased to 19.2 mL/min/1.73 m². After four months of treatment, the serum FLC ratio (kappa/lambda) decreased to 1.45 and the urinary protein/creatinine ratio decreased to 0.09 g/gCr; however, no further improvements were noted in the serum creatinine level.

Expression of PTHrP in the myeloma cells

Because the concentration of PTHrP was decreased by the treatment for MM, we examined whether the patient’s myeloma cells expressed PTHrP using IHC and ISH. On IHC, formalin-fixed, paraffin-embedded tissue sections were dewaxed with xylene and rehydrated via a series of graded alcohols. After blocking the endogenous peroxidase activity with 0.3% H2O2 in methanol, the sections were incubated with an anti-PTHrP (1-34) monoclonal antibody, 4B3, as previously described (7), at a final concentration of 5 μg/mL. The avidin-biotin-peroxidase complex method (8) was employed using the DAKO LSAB kit (DAKO, Carpinteria, USA). Final development of the sections was carried out with 3,3’-diaminobenzidine containing 0.03% H2O2. ISH was performed using formalin-fixed, paraffin-embedded tissue sections with a digoxigenin (DIG)-labeled single-stranded DNA probe for PTHrP, as previously described (3, 9, 10).

The hematopoietic tissue of a bone marrow clot section was found to be infiltrated with atypical plasma cells (Fig. 2A). Meanwhile, the presence of PTHrP was demonstrated in the myeloma cells on IHC (Fig. 2B), and signals for PTHrP mRNA were detected in the myeloma cells on ISH performed with a DIG-labeled single-stranded antisense DNA probe. The inset shows a control sample hybridized with a DIG-labeled single-stranded sense DNA probe (Fig. 2C). Normal hematopoietic cells in the bone marrow did not express PTHrP on IHC or ISH.

Discussion

We herein describe a case of MM with CN, LCDD and PTHrP-induced hypercalcemia. The PTHrP expression was detected in myeloma cells in the bone marrow on IHC and ISH, indicating that PTHrP was produced in the patient’s myeloma cells.

MM is characterized by the clonal proliferation of malignant plasma cells, monoclonal proteins, osteolytic bone lesions and immunodeficiency (11). The mechanism underlying the development of bone lesions and hypercalcemia in patients with MM is considered to involve the production of osteoclast-activating factors, such as receptor activator of nuclear factor-kappa B ligand, macrophage inflammatory protein 1 alpha, tumor necrosis factor alpha and interleukin 6,
and osteoblast inhibitory factors, such as dickkopf-1, soluble frizzled-related protein-3 and hepatocyte growth factor, by myeloma cells, subsequently resulting in accelerated osteoporosis and the onset of bone lysis (2). Hypercalcemia due to malignancy involves another mechanism in which PTHrP is generated by malignant cells and its level is increased in the plasma. PTHrP displays a close homology to PTH in the N-terminal sequence and stimulates the systemic actions of PTH in increasing bone resorption and the renal reabsorption of calcium via a common PTH/PTHrP receptor (12). There are some reports of hypercalcemia associated with a high level of PTHrP with the expression of PTHrP in myeloma cells (3-6). The plasma PTHrP concentration was analyzed using an immunoradiometric assay system (LSI Medience, Tokyo, Japan). The results of PTHrP measurement have been reported to be unaffected by the degree of chronic renal failure for this assay (13). Although we were unable to investigate either osteoclast-activating factors or osteoblast-inhibitory factors in this patient, a high PTHrP level and a low excretion of urinary calcium due to kidney dysfunction probably contributed to the onset of hypercalcemia in this case.

Renal insufficiency is found at presentation in almost 50% of patients with MM. The pathogenesis can be separated into Ig-dependent and Ig-independent mechanisms. CN, LCDD and AL-amyloidosis are common forms of monoclonal Ig-mediated kidney injury (1). In native renal biopsy studies, 40 to 63% of all patients are reported to have CN, 19 to 26% have LCDD and 7 to 30% have AL amyloidosis (14). CN is involved in the development of intratubular obstruction via the precipitation of Ig light chains and other serum proteins, including Tamm-Horsfall proteins, which

Figure 1. The findings of the renal biopsy specimens. Light (A, B), immunofluorescence (C), and electron (D, E) micrographs are shown. (A) Severe interstitial fibrosis with tubular atrophy was noted (original magnification ×100). Several tubules contained cast formation (circles). (B) Detached tubular epithelial cells were observed in the casts (original magnification ×400). (C) Immunofluorescence showing intensive deposition of Ig kappa with a linear pattern in the capillaries and TBM. Electron dense deposits were localized in the GBM (D) and TBM (E) (original magnification B; ×8,000, C; ×6,000).
leads to interstitial inflammation and fibrosis (15). LCDD is diagnosed based on the detection of monoclonal Ig light chains in the GBM or TBM of the kidneys and/or the presence of typical granular electron-dense deposits along the GBM or TBM (16). The most characteristic light microscopic finding in patients with LCDD is nodular glomerulopathy. The present patient was diagnosed with the coexistence of CN and LCDD according to the renal biopsy findings. Overlapping LCDD and CN is a clinically and morphologically distinct entity from pure monoclonal Ig deposition disease, with a higher creatinine level at presentation, low level of proteinuria, reduced edema and fewer nodular lesions in the glomeruli (17, 18). In the current case, we did not detect any nodular sclerosing lesions, a typical finding of pure LCDD on light microscopy. This suggests that the time to develop nodular formation was too short, as the patient exhibited symptoms associated with hypercalcemia and renal impairment.

In this case, the concentration of serum creatinine decreased following treatment for hypercalcemia and MM, although this parameter did not improve to the normal range. Based on the renal biopsy findings, the extent of interstitial fibrosis was more severe than that of cast formation. Compensation for volume depletion after administering appropriate fluid therapy may have washed out the casts. Furthermore, an elevation of the serum PTHrP level may be involved in the development of interstitial fibrosis. The most prevalent form of chronic kidney disease is characterized by the onset of progressive tubulointerstitial fibrosis, irrespective of the etiology, in addition to a large proportion of interstitial myofibroblasts in the injured kidneys derived from tubulothelial cells via epithelial-mesenchymal transition (EMT) (19). Ardura et al. reported that PTHrP may interact with vascular endothelial growth factor, epidermal growth factor and TGF-beta, and thereby induce EMT in renal tubulothelial cells (20).

The therapeutic approach for MM is to target myeloma cells and their microenvironment as well as potential Ig-independent complications, such as volume depletion and hypercalcemia. The administration of bortezomib alone or in combination with dexamethasone is effective in newly diagnosed or relapsed myeloma patients (21, 22). Hypercalcemia is usually treated with intravenous saline, loop diuretics, elcatonin or bisphosphonates. In our patient, bisphosphonates were not used due to the patient’s impaired renal function. After diagnosing MM, we stopped the treatment with loop diuretics and started therapy with alkalization of the urine to prevent cast formation. It is important to adjust the treat-

**Figure 2.** Morphological findings of the bone marrow clot tissue. (A) The hematopoietic tissue was infiltrated with myeloma cells on Hematoxylin and Eosin staining. (B) Presence of PTHrP in the myeloma cells was demonstrated on immunohistochemistry. (C) Signals for PTHrP mRNA were detected in the myeloma cells on in situ hybridization using a DIG-labeled single-stranded antisense DNA probe. The inset shows a control sample hybridized with a DIG-labeled single-stranded sense DNA probe.
ment for hypercalcemia according to the patient’s current disease status.

In conclusion, we herein reported a case of MM accompanied by CN and LCDD that occurred in association with the production of PTHrP in myeloma cells in the bone marrow. This case suggests that a high level of PTHrP contributes to the onset of hypercalcemia and renal fibrosis. It is therefore important to examine the concentration of PTHrP in MM patients with hypercalcemia.

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The authors state that they have no Conflict of Interest (COI).

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