Vol.1, No.2, 11-13 (2012)
http://dx.doi.org/10.4236/mc.2012.12003

New antimyeloma drugs in spinal cord infiltration for multiple myeloma. Determination of lenalidomide in cerebrospinal fluid with ultrasensitive high-performance liquid chromatography

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Received 29 August 2012; revised 30 September 2012; accepted 8 October 2012

ABSTRACT

A 64-year-old woman with IgA kappa multiple myeloma was treated with thalidomide-dexamethasone. Due to progression of the disease, bortezomib, doxorubicin and dexamethasone were administered, followed by autologous stem cell transplantation. Although near-complete remission was achieved, 5 months later, neurological symptoms appeared and the patient was diagnosed with multiple myeloma with cells infiltrating the spinal cord. Bis-chloronitrosourea, bortezomib and lenalidomide were then administered and although the patient remained neurologically asymptomatic, she died 3 months later becausee of disease progression. Lenalidomide entered into the cerebrospinal fluid (confirmed by ultrasensitive high-performance liquid chromatography), although did not improve the poor prognosis of multiple myeloma involving the central nervous system.

Keywords: Lenalidomide; Multiple Myeloma; Spinal Cord Infiltration; High-Performance Liquid Chromatography

1. INTRODUCTION

Multiple myeloma (MM) is a malignant plasma cell (PC) neoplasm usually restricted to the bone marrow (BM), although extramedullary spread may occur. Involvement of the central nervous system (CNS) and presence of monoclonal PCs in the cerebrospinal fluid (CSF), occur in approximately 1% of patients [1-3]. Lenalidomide is a second-generation immunomodulatory compound. There are not clinical studies designed to determine whether lenalidomide crosses the Blood-Brain Barrier (BBB). However, preclinical studies in rabbit and mouse models have determined that lenalidomide crosses the BBB [4].

2. CASE REPORT

2.1. Clinical and Laboratory Findings at Diagnosis

A 64-year-old woman was seen in October 2007 because of progressive lumbar and costal pain of 3 months duration. She had undergone hysterectomy, treatment for myeloma in 1996, with a mild anxious-depressive disorder. Laboratory tests showed moderate renal insufficiency, anaemia, 4.2 g/dL serum M-protein (paraprotein IgA-kappa) and immunoparesis; light chain excretion 0.8 g/L in 24-h urine, creatinine 2.1 mg/dL, calcium 13.4 mg/dL, β_2-microglobulin 6.8 mg/dL, haemoglobin 10.2 g/dL and 13% PCs in Peripheral Blood (PB). Multiple cranial osteolytic lesions and diffuse osteoporosis were documented. BM aspiration showed interstitial infiltration (90% atypical PCs). Cytogenetic analysis reported t (4;14) in all investigated cells.

2.2. Clinical Course

Renal insufficiency reversed completely with dexamethasone, zoledronic-acid and fluids administration. MM treatment with thalidomide-dexamethasone was then started. MM progressed after one cycle. The treatment was changed to bortezomib, doxorubicin and dexamethasone for 5 cycles. Near-complete remission was achieved and the patient underwent to autologous stem cell transplantation with melphalan 200 mg/m^2 as conditioning regimen. She achieved near complete remission as maximal response after transplantation. A slowly progressive subacute numb chin syndrome (cutaneous hypo-aesthesia limited to the region served by the mental nerve) with weakness and paraesthesia in the upper ex-

tremities was documented after 5 months. M component level was similar to previous studies, with negative electrophoresis (positive immunofixation). Flow Cytometry (FC) of BM aspiration showed 1.2% pathologic PC. An extrapleural lesion of 3 cm and several new vertebral lesions were also documented. Brain MRI was normal. Spinal MRI showed enlarged nerve roots, diffuse spinal arachnoiditis (L3 to L5 level), and an 8 × 3 cm^2 mass at D7 with enlargement of soft tissues without canal invasion. Lumbar puncture revealed CSF infiltration with 800 atypical PC/μL (CD56+, CD38+ and CD19– by FC).

Consequently, the patient was diagnosed with MM with cells infiltrating the spinal cord, and treatment with bis-chloronitrosourea (BCNU) 100 mg/m^2, bortezomib 1.3 mg/m^2 × 4, lenalidomide 15 mg per 21 days every 4 weeks and dexamethasone was started. After the first cycle of lenalidomide, neurological signs improved: movement, sensitiveness in the upper extremities and numb chin syndrome resolved completely. The patient was asymptomatic. Grade IV haematological toxicity was observed. Only 8 PC/μL were detected by FC on lumbar puncture, with normalization of MRI findings 30 days after the first rescue treatment. An identical course of chemotherapy was administered but systemic disease progression was documented 20 days later. The patient died without neurological symptoms after 3 months.

2.3. Lenalidomide Determination in Plasma and CSF with HPLC

A high-performance liquid chromatography (HPLC) method was developed to measure lenalidomide in PB (serum and lithium heparin-anticoagulated plasma) and CSF. Samples were collected 1 hour after drug administration. Specimens were obtained in the first cycle of treatment. A CFS specimen was also collected in the second therapy cycle. The samples were centrifuged at 1500 g for 10 min and then stored at –80˚C.

We used lenalidomide 3-(4-Amino-1-oxo-1, 3-dihydro -2H-isoindol-2-yl) piperidine-2, 6-dione; MW 259.3; CAS No. 191732-72-6, 99.56% purity) (Selleck Chemicals LLC, TX, USA). Lenalidomide standard was dissolved in ethanol (1 mL) and diluted (200 nM) in the mobile phase. Stock solutions were stored at –80˚C. An internal standard with an appropriate retention time (RT) was selected (Chromsystems, Instruments & Chemicals GmbH, Munich, Germany). All used chemicals were guaranteed reagent or HPLC grade.

A Solid-Phase (SP) extraction was done before inject the analytes in the chromatograph, with octadecy l (C18) cartridges (Speed SPE C18/18%, Applied Separations, PA, USA). The steps followed for the extraction of lenalidomide were: 1) Cartridge conditioning with methanol (1 mL); 2) SP equilibration with 0.02 M KH_2PO_4 (pH 7) buffer (1 mL); 3) Load of serum or plasma (300 μL) or CSF (900 μL) + internal standard (100 μL); 4) Wash-out with 0.02 M KH_2PO_4 (pH 7) buffer (1 mL); 5) Elution with methanol-0.02 M KH_2PO_4 (pH 7) (1 mL) (50:50).

Separation was performed in an isocratic chromatograph with a 119 UV/visible detector (Gilson, Middleton, WI, USA) and an auto sampler (Gilson 231 XL, Gilson). The column was C18 (Mediterranean Sea 18.5 μm, 25 cm × 0.46 cm; Teknokroma, Barcelona, Spain) and a meth-anol/0.02 M KH_2PO_4 (pH 7) (35:65) mobile phase at 1 mL/min flow rate were used. Wavelength was fixed at 220 nm for lenalidomide detection. The RTs were 5.5 min for lenalidomide and 6.8 for the internal standard.

Calibration curves were constructed measuring in duplicate the areas of six increasing concentrations. Within-day variation was determined from five injections at two concentrations of lenalidomide (0.5 and 10 nM). The limit of detection was calculated from the standard deviation of the areas obtained in 10 injections of the drug at the lower concentration of the calibration curve (0.1 nM). SP extraction recovery was performed in triplicate in plasma samples spiked with lenalidomide (7.7 nM).

The quantifiable range was 0.1 - 50 nM (R^2 = 0.993). Within-day variation coefficients were 3.3% for 0.5 nM and 5.4% for 10 nM lenalidomide. The limit of detection was 0.009 nM for lenalidomide concentration. Drug recovery from the previous extraction using SP cartridges was 100%.

3. RESULTS

A peak in lenalidomide RT was observed in the chromatograms for plasma and CSF samples. However, no peak at this RT was detected in serum samples, even with a 1 mL serum volume in SP extraction. The lenalidomide plasma concentration for 1 h after the first cycle of therapy sample was 23.2 ± 1.0 nM. The lenalidomide concentration in CSF was 0.81 ± 0.03 nM for the sample taken after the first cycle and 0.99 ± 0.03 nM for the sample taken after the second cycle.

4. DISCUSSION

MM with diffuse infiltration of the spinal cord is rare, although reports have increased recently [5]. New drugs used for the treatment of MM [6] may contribute to this MM manifestation due to their inability to access certain tissues such as those in the neurological compartment [5-7]. The special characteristics of the CNS and the presence of the BBB could prevent these drugs from reaching the CNS.

This aggressive relapse had a dire prognosis: survival in this situation is 4 - 5 months [2,8]. Our patient was treated with bortezomib, BCNU and lenalidomide. First of them was used as a more systemic active treatment, and the second one as an efficient treatment for tumours

that affect the CNS due to its ability to cross the BBB and is also used in MM. Lenalidomide was included because is an active antimyeloma drug. It is not known whether its kinetics enables it to cross the BBB, but there is indirect evidence that it can [4].

Lenalidomide has antimyeloma activity even at nanomolar concentrations [9]. Lenalidomide concentration in our patient's CSF reached 1 nM, high enough to kill MM cells. In addition, BCNU is active in this setting and bortezomib may also enter the CSF if the BBB is breached.

We developed and standardized a new, simple, ultrasensitive method based on HPLC to measure low concentrations of lenalidomide in plasma and other body fluids (as low as 0.001 μM). An earlier study measured lenalidomide levels with a more complex chromatographic method based on mass spectrometry [10]. Our method is simpler and could be widely applicable in many hospitals because it is based on widely-available HPLC methods.

Although our patient's CNS symptoms and level of CSF infiltration improved with treatment, systemic relapse occurred after the first cycle of therapy. The patient died of disease progression but no new neurologic symptoms appeared. New drugs used together with conventional chemotherapy do not improve the poor prognosis of MM with CNS involvement, even when the drugs enter the CSF at therapeutic doses.

5. ACKNOWLEDGEMENTS

This work received medical writing support from Lidesec S.L. K. Shashok improved the use of English in the manuscript. Lidesec S.L. and Ms. Shashok's fees were supported by Celgene S.L.

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