Efficacy and Safety of Bortezomib in Multiple Myeloma Patients with Hepatitis B: A Multicenter Retrospective Study

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Introduction

Bortezomib, the first therapeutic proteasome inhibitor, is one of the primary agents in the treatment of multiple myeloma (MM). It selectively and reversibly inhibits the proteolytic activity of the proteasome complex to block the ubiquitin-proteasome pathway.

Bortezomib has been in market in China since 2005 and widely used in the treatment of MM thereafter. A significant improvement of MM treatment in terms of clinical efficacy and long-term survival rate was observed since the use of bortezomib and immunomodulators and the improvement in hematopoietic stem cell transplantation.¹⁻⁵ Bortezomib has significant anti-myeloma activity and manageable toxicity. The most common adverse effects include gastrointestinal symptoms, peripheral neuropathy, neuropathic pain, and thrombocytopenia. It was demonstrated that bortezomib was significantly associated with high incidence of herpes zoster.⁶ However, the effect of bortezomib on the hepatitis B virus (HBV) remains unclear. HBV reactivation was reported in a few cases after the treatment of bortezomib.⁷⁻¹⁰ As the efficacy and safety evidence of bortezomib in MM patients with hepatitis B is vacant, this study aimed to investigate the efficacy and safety of bortezomib in MM patients with hepatitis B in China.

Methods

Population

Patients were included from 2 MM centers from 2006 to 2011, who met the diagnosis criteria of the Guideline for Diagnosis and Treatment for Multiple Myeloma. From 2006 to 2011, 739 newly diagnosed MM patients were screened for serum hepatitis B virus (HBV) biomarkers. HBV-infected patients were followed for HBV reactivation by monitoring of serum alanine transaminase (ALT) and HBV DNA load. The pattern of HBV reactivation in relation to bortezomib was evaluated. Seven hundred thirty-nine MM patients were included in this study.

Results

The prevalence of MM patients infected with HBV was 3.4% (n = 25), of which 17 cases were treated with bortezomib. Bortezomib had no significant influence on liver function (ALT before and after treatment: 36.69 ± 8.90 U/L vs. 11.31 ± 2.74 U/L, P = 0.19) and HBV DNA of MM patients with HBV (detectable HBV DNA percentage: 5.9% vs. 11.8%, P = 0.12).

Conclusions

Bortezomib can be used safely and effectively in MM patients with hepatitis B. HBV prophylaxis and surveillance are recommended during the MM treatment.

Key words: Bortezomib; Hepatitis B; Lamivudine; Myeloma; Thalidomide

Abstract

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Bone marrow was collected for plasma cell count, immunophenotypic analysis, cytogenetic analysis, and interphase fluorescence in situ hybridization (interphase FISH) analysis, and the HBV virology test results at baseline were also collected in MM patients. Immunophenotypic analyses were performed in 269 patients using a four-color direct immunofluorescence staining to identify the immunophenotype of clonal plasma cell from erythrocyte-lysed bone marrow samples. The fluorescence was FITC/PE/PerCP-Cy5.5/APC, whereas the panel was CD38/CD56/CD9/CD45, CD45/CXCR4/CDll7/CD38, CD38/CD138/CD45/CD19, CD38/CD45/cKappa/cLamba, CD38/CD45/CD20/CD28, and CD38/CD45/CD33/CD200. High risk cytogenetic abnormality was defined as at least abnormalities or 17p- by routine cytogenetic analysis. FISH was performed in interphase nuclei, which included 1q21 amplification, D13s319 (13q14.3) deletion, RB1 (13q14) deletion, P53 (17p13) deletion, and IgH (14q32) rearrangement. A minimum of 200 interphase cells were analyzed for each probe. All patients were screened for the presence of HBV surface antigen (HBsAg), surface antibody, e antigen (HBeAg), e antibody (HBeAb), core antibody (HBcAb), and HBV DNA load.

Anti-viral treatment
For the prophylaxis of HBV reactivation, nucleoside analogues were orally administered in 20 HBsAg+ patients as long as the anti-myeloma treatment initiated and continuing 1 year after treatment stop (100 mg lamivudine per day in 12 cases and 0.5 mg entecavir per day in the other 8 cases). Five HBsAg+ patients did not receive any prophylactic therapy for HBV.

Liver function evaluation
The biochemical indexes (liver and renal function), electrolyte, blood glucose, and coagulation function were examined twice weekly during the treatment and once weekly after treatment. The quantification of HBV DNA was detected monthly by polymerase chain reaction during and after treatment.

Response evaluation
The European Group for Blood and Marrow Transplantation criteria was used to define complete response (CR), partial response (PR), minimal response, relapse, and progression in MM patients.

Statistical analysis
Continuous variables were described as mean ± standard deviation (SD). The alanine transaminase (ALT) difference of these patients with undetectable HBV DNA (>300 copies/ml) were evaluated by Chi-square test. All statistical analyses were performed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). P < 0.05 was considered as statistical significance.

RESULTS
Characteristics of multiple myeloma patients with hepatitis B virus
Among 739 MM patients, 25 were infected with HBV and the prevalence of HBV infection in MM patients was 3.4%. Seventeen HBV-infected patients received bortezomib and other agents as therapy (8 patients got bortezomib combined with dexamethasone regime, 6 patients got PAD, that is bortezomib combined with dexamethasone and epirubicin, 1 patient got bortezomib combined with dexamethasone and thalidomide, 1 patient got bortezomib combined with dexamethasone and cyclophosphamide, and 1 patient got B-DCEP. The dose of bortezomib was 1–3 mg/m², days 1 (d1), 4, 8, 11; the dose of dexamethasone was 20 mg/d, d1–4 and d8–11; the dose for epirubicin was 15 mg m⁻²·d⁻¹, d1–4; the dose of cyclophosphamide was 300 mg/d, d1–5; for the B-DCEP regime, the dexamethasone was 40 mg/d, d1–4, the cyclophosphamide was 300 mg m⁻²·d⁻¹, d1–4, etoposide was 50 mg m⁻²·d⁻¹, d1–4, cisplatin was 15 mg m⁻²·d⁻¹, d1–4, and the dose of bortezomib was the same as showed above, among the 17 patients got bortezomib, all of them were HBV carrier, no patient was diagnosed with chronic hepatitis B or hepatitis B with liver cirrhosis. Among them, 16 patients were inactive HBsAg carrier with HBsAg−, HBeAb−, HBcAb−, and normal ALT; 1 patient was chronic HBV carrier with HBsAg+, HBeAg+, HBcAb+, and normal ALT, and an HBV DNA load of 1.98 × 10⁹ copies/ml, this chronic HBV carrier was also infected with hepatitis C identified by abnormal hepatitis C messenger RNA. The characteristics of the 17 MM patients with HBV are shown in Table 1.

Table 1: Characteristics of 17 multiple myeloma patients with hepatitis B

| Indices          | Values       |
|------------------|--------------|
| Age (years), mean (SD) | 58.7 (9.9)   |
| Gender, male (%)  | 70.6         |
| Type (%)          |              |
| IgGK              | 23.5         |
| IgGL              | 23.5         |
| IgAlamda          | 11.8         |
| K                 | 11.8         |
| L                 | 11.8         |
| IgAK              | 5.9          |
| IgDIamda          | 5.9          |
| Nonsecr           | 5.9          |
| DS (%)            |              |
| IIIA              | 70.6         |
| IIIB              | 29.4         |
| ISS (%)           |              |
| I                 | 11.8         |
| II                | 23.5         |
| III               | 64.7         |
| B2 microglobulin (mg/L), mean (SD) | 9.6 (8.5) |

DS: Durie-Salmon stage; ISS: International Staging System; SD: Standard deviation.
Impact of bortezomib on liver function
To identify the influence of bortezomib on liver function, we evaluated the ALT levels of MM patients with HBV before and after bortezomib treatment in the 1st cycle. No significant difference of mean ALT levels was identified after the treatment of bortezomib [36.69 ± 8.90 U/L vs. 11.31 ± 2.74 U/L, \( P = 0.19 \)].

Impact of bortezomib on detectable hepatitis B virus DNA percentage
To find the influence of bortezomib on detectable HBV DNA percentage, we analyzed the percentages of patients with detectable HBV DNA percentage. There is no significant difference for the percentages of patients with detectable HBV DNA before and after bortezomib treatment in the 1st cycle [5.9% vs. 11.8%, \( P = 0.12 \)].

Efficacy of bortezomib in multiple myeloma patients with hepatitis B virus
Of all 17 patients, 3 cases achieved CR and 13 cases achieved PR. The response rate was 94.1%.

Immunophenotype and cytogenetics of multiple myeloma patients with and without hepatitis B virus
The types and stages of MM in the HBV-infected patients were similar with the non-HBV-infected patients. Using interphase FISH, the incidence of the 13q- were 33.3% (2/6) in MM patients with HBV and 39.7% (69/174) in MM patients without HBV. The incidence of 1q21 amplification was 80.0% (4/5) in the HBV patients and 40.2% (70/174) in those without HBV. 10.5% (2/9) of HBV patients had high risk chromosome in the G banding and the rate was 16.7% (42/251) for those without HBV. The positive rates of CD38, CD138, and CD56 among patients with or without HBV were 100% versus 100%, 100% versus 97.7%, 55.6% (5/9) versus 65.5% (160/248), respectively. The positive rates of CD20 and CD117 in patients with or without HBV were 28.6% (2/7) versus 17.4% (37/212) and 50.0% (3/6) versus 42.0% (94/224), respectively.

Survival
Three patients died of disease progression whereas 5 died of lung infection, which were not related to liver failure and hepatic impairment. The median overall survival (OS) was 17 months (4–62 months).

Discussion
This is the largest systematic analysis of the bortezomib treatment in MM patients with HBV in China to date. We found that bortezomib can be used safely and effectively in MM patients with HBV, whereas HBV prophylaxis and surveillance might be recommended in MM treatment.

Epidemiology of multiple myeloma patients with hepatitis B virus
Chronic HBV infection may increase the risk of lymphoid malignancies, but no significant association was found between HBV and MM. In Engels's report, \[53,045 (8.8\%)

were tested HBsAg + among 603,585 people at health screening. HBsAg + subjects had elevated risk of NHL, but not MM. The incidence of MM is 2.04/100,000 per year in HBsAg + population whereas 2.58/100,000 per year in HBsAg − population. In another study from Guangdong Province in China, the HBsAg + rate was significantly higher in MM patients (19.4%) than acute leukemia patients (12.0%), which were higher than the average incidence of hepatitis B (7.2%) announced by Chinese Center for Disease Control (China CDC). The controversy may come from the regional differences. In a nested case–control study in Europe (study EPIC), \[11\] HBV carrier among controls in different countries ranged from 0% to 2.7%. Statistically significant association was found between HBsAg seropositivity and the risk of MM (odds ratio = 4.00; 95% confidence interval: 1.00–16.00) after a 3-year follow-up. Due to the lower prevalence of hepatitis B in Europe, there were only 6 HBsAg + MM patients in the treatment group and 3 in the control group in this study, which may lead to misjudgment due to the small sample size.

Asia is a highly endemic region of HBV infection and the pattern of hepatitis B prevalence is different among regions. The data of patients in this study were from two MM treatment centers in Beijing and 80% of patients were from other parts of the country, representing the actual status and epidemic characteristics of MM patients in China. The prevalence of HBsAg + in MM patients of our study was 3.4%, which was lower than the results of a Singapore study. In this study, HBeAg + patients were also considered as affected by hepatitis B infection in this study. \[12\] We excluded such kind of patients due to potential abortive infection or postinfection.

Bortezomib in the liver function and hepatitis B virus reactivation
After the use of bortezomib, HBV reactivation, even fulminant hepatic failure was reported in MM patients. In one case, a MM patient was considered to be in the replicative phase of chronic hepatitis B with HBsAg +, HBeAg −, a serum HBV DNA level of 350,000 copies/ml and normal transferase levels. \[5\] He was treated with four cycles of vincristine, doxorubicin, and dexamethasone and up to eight 3-week cycles of bortezomib, HBV DNA was negative and aminotransferases were found to be normal during therapy and then the hepatitis B reactivation was demonstrated with a HBV DNA level of 20,000 copies/ml, 18 months after bortezomib therapy. It was unable to judge the causality relationship between the HBV reactivation and bortezomib, and the patient died from fulminant hepatitis. In a study of Singapore, a HBV reactive case was reported 4 months after the bortezomib and dexamethasone treatment. The relationship between the reactive and bortezomib was unclear. \[3\]

Proteasome plays an important role in virus life cycle. \[13–19\] The proteasome inhibitor bortezomib has anti-viral activity for some kinds of viruses despite anti-tumor activity.
Bortezomib could inhibit the duplication of the fibroblast cell in vitro mouse study.\[20\] About 1 mg/kg of bortezomib could decrease copies of HBV in a transgenic mouse in vivo study.\[21\] The inhibition activity was disappeared when the concentration was below 0.2 mg/kg, however, when the concentration increased to 5 mg/kg, it did not further decrease; however, increase the copies of virus. Increased ALT levels in the higher dose group were seen on day 2 after bortezomib treatment and returned to normal on day 6, which indicated that the increase was not caused by virus replication. The opposite results were seen in mouse HBV infection in vitro model that 1 mg/kg of bortezomib treating C57BL/6 mice increased mouse HBV replication due to inhibition of immune cells.\[22\] The controversial results of the two studies might derive from the specific immune status of the transgenic mice. MM patients, whose immune functions are inhibited and are not immune-deficient, might be similar to the immune conditions of transgenic mice.

In the present study, bortezomib had no impact on the HBV reactivation and liver function of MM patients. However, it is unclear that whether bortezomib therapy increase or decrease virus replications in MM patients with HBV infection, and studies in specific animal models are needed to elucidate the question.

Efficacy of bortezomib in multiple myeloma patients with hepatitis B virus

Of all 17 patients, the response rate was 94%, which was similar to the results in HbsAg− patients in the previous trial.\[9,11,12,23-25\]

Immunophenotype and cytogenetics of multiple myeloma patients with and without hepatitis B virus

The 1q21 amplification by the interphase FISH was more common in HbsAg+ patients, and the immunophenotype with increased CD20 expression. The CD20 expression may play an important role in the B cell abnormality induced by HBV and MM eventually, yet the underlying mechanisms remain unclear. Due to the small sample size of patients with the immunophenotype and cytogenetics determinations, further study is needed.

According to previous reports, the risk of HBV reactivation in MM patients was highest at 3–5 months after ASCT during the immune reconstitution phase. Although the reconstitution of cytotoxic HBV-specific T-cells has never been studied, it is likely to be the same as the reconstitution of HBV, whose specific cytotoxic T-cells are substantially reduced during the first 3 months after transplantation and remarkably recovered 3–6 months after the transplantation.

The use of thalidomide is recommended in the maintenance setting after the ASCT, by the fact that in vitro thalidomide could act as a T-cell co-stimulator, enhancing virus-specific CD8(+)-T-cell cytokine production and cytotoxic activity,\[1,25\] which means the immune recovery phase will be even more profound to induce accurate hepatic injury. Bortezomib could impair several immune properties of human monocyte-derived dendritic cells (DCs), suppresses essential immune functions of human CD4+ T-cells activated by allogeneic DCs,\[23\] Despite its potential immune-modulatory effects, it is still unclear if bortezomib could induce HBV reactivation.

Limitations

Several limitations were unavoidable in this study. First, the sample size is very small. Only 17 MM patients with HBV were included in the final analysis. Second, all the analyses were only performed in the 1st cycle. It is not clear if there is any difference in the following cycles. The last but not least, as it is a retrospective study, selection bias may have impact on the final results. Therefore, a perspective study with large sample size together with analysis in multiple cycles should be performed in the future.

In conclusion, our study indicated no higher prevalence of HBV infection in MM patients. The efficacy of bortezomib among the MM patients with HBV infection was comparable with other studies. No HBV reactivation was observed in patients in the treatment of lamivudine or entecavir prophylaxis. Antivirus nucleoside or nucleotide analog is recommended during the treatment of bortezomib in HbsAg+ MM patients.

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Conflicts of interest

There are no conflicts of interest.

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