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CHEMICAL STABILITY OF BORTEZOMIB SOLUTIONS IN ORIGINAL MANUFACTURER VIALS

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ABSTRACT

Bortezomib is recommended for the treatment of patients with multiple myeloma. Clinical investigations have been completed or are under way to evaluate the safety and efficacy of bortezomib alone or in combination with chemotherapy in multiple myeloma, both at relapse and presentation, as well as in other cancer types. Bortezomib binds to the proteasome and does so "reversibly" (this is a chemical term that means that the bortezomib molecule can come free under different micro-cellular chemical conditions.) Normal healthy (non-cancer) cells are not as prone to damage from this binding after the Bortezomib comes off, as cancer cells are. Malignant cells suffer a breakdown even after inhibition of the proteasome for a short time. The objective of this work was to evaluate the stability of bortezomib reconstituted with sterile NaCl 0.9% to a concentration of 2.5 mg mL^{-1} in original manufacturer vial refrigerated at 4 ^{o}C in the dark.

KEYWORDS

Bortezomib, stability study, HPLC, subcutaneous administration.

INTRODUCTION

Bortezomib (INN, originally codenamed PS-341; marketed as Velcade by Millenium Pharmaceuticals) is the first therapeutic proteasome inhibitor to be tested in humans. It is approved in the U.S. for treating relapse multiple myeloma [1] and mantle cell lymphoma. In multiple myeloma, complete clinical responses have been obtained in patients with otherwise refractory or rapidly advancing disease.

Bortezomib was originally synthesized in 1995 (MG-341) at a company called Myogenics, which soon changed its name to ProScript. After promising preclinical results, the drug (PS-341) was tested in a small Phase I clinical trial on patients with multiple myeloma cancer. ProScript ran out of money and was bought by Leukosite in May 1999. Leukosite in turn was bought by Millennium Pharmaceuticals in October 1999. At this point in time, the project had low priority amongst other projects at the company. This changed significantly when one of the first volunteers to receive the drug in the clinical trial achieved a complete response and was still alive four years later. At the time this was a remarkable result. Later clinical experimentation indicates the possibility of a complete response in 15% of patients in a similar condition, when treated with bortezomib. In May 2003, seven years after the initial synthesis, bortezomib (Velcade) was approved in the United States by the Food and Drug Administration (FDA) for use in multiple myeloma, based on the results from the SUMMIT Phase II trial [2], and in April 2004 by the

Committee for Proprietary Medicinal Products of the European Union. Velcade was approved in 2005 for the treatment of patients with multiple myeloma who had received at least one prior therapy. In December 2006 the FDA approved Bortezomib for treatment of mantle cell lymphoma. On June 2008, the FDA approved bortezomib for injection (Velcade, Millennium, Inc. and Johnson and Johnson Pharmaceutical Research and Development) for the treatment of patients with multiple myeloma. This approval provides clinical trial data for the use of Velcade as an initial treatment for patients with multiple myeloma.

Bortezomib in combination with melphalan and prednisone is indicated for the treatment of patients with previously untreated multiple myeloma who are not eligible for high-dose chemotherapy with bone marrow transplant. Bortezomib is also indicated as monotherapy for the treatment of progressive multiple myeloma in patients who have received at least 1 prior therapy and who have already undergone or are unsuitable for bone marrow transplantation [3].

The drug is an N-protected dipeptide and can be written as Pyz-Phe-boroLeu, which stands for pyrazinoic acid, phenylalanine and Leucine with a boronic acid instead of a carboxylic acid. Peptides are written N-terminus to C-terminus, and this convention is used here even though the "C-terminus" is a boronic acid instead of a carboxylic acid.

Bortezomib is a highly selective, reversible inhibitor of the 26S proteasome. This drug is thought to inhibit many proteins (known as proteasomes) that cancer cells need to survive and multiply. It has been shown to have anti-tumour activity in B cell malignancies. The boron atom in bortezomib binds the catalytic site of the 26S proteasome[4] with high affinity and specificity. In normal cells, the proteasome regulates protein expression and function by degradation of ubiquitylated proteins, and also cleanses the cell of abnormal or misfolded proteins. Clinical and preclinical data support a role in maintaining the immortal phenotype of myeloma cells, and cell-culture and xenograft data support a similar function in solid tumour cancers. While multiple mechanisms are likely to be involved, proteasome inhibition may prevent degradation of pro-apoptotic factors, permitting activation of programmed cell death in neoplastic cells dependent upon suppression of pro-apoptotic pathways.

Bortezomib is rapidly cleared following intravenous administration[5]. Peak concentrations are reached at about 30 minutes. Drug levels can no longer be measured after an hour. Pharmacodynamic are measured by measuring proteasome inhibition in peripheral blood mononuclear cells. The much greater sensitivity of myeloma cell lines and mantle cell lines to proteasome inhibition compared with normal peripheral blood mononuclear cells and most other cancer cell lines are poorly understood.

The recommended dose and schedule of bortezomib is 1.3 mg/m² administered as a 3– 5-s bolus intravenous injection on days 1, 4, 8, and 11 of 21-day cycles. This regimen is active and well tolerated in patients with relapsed multiple myeloma. As an alternative to intravenous delivery, subcutaneous administration of bortezomib could be a good option for patients, particularly those with poor venous access. Subcutaneous administration eliminates the need for repeated intravenous access or insertion of long-term central venous access devices, improving convenience for patients and physicians. Subcutaneous administration is used for several antineoplastic agents that are not directly toxic to tissues, such as alemtuzumab.

Intravenous injection is the standard administration route of bortezomib; however, subcutaneous administration is an important alternative. The study by Moreau et al.[6]

represents a further step towards the optimisation of bortezomib use, offering a more convenient route of administration, fewer side-effects, and similar or even improved efficacy.

The aim of this investigation was to assess the stability of bortezomib solution (2.5 mg mL^{-1}) in the manufacturer vial stored at 4°C in the dark for up to 30 days following reconstitution.

EXPERIMENTAL

INSTRUMENTATION

HPLC analysis was performed at room temperature (~25°C) using a Shimadzu LC-6A pump equipped with Rheodine 7125 injection valve 20µL, a Shimadzu SPD-6A spectrophotometric detector working at 270 nm. The signal from the detector was recorder and integrated with a chromatography data system Shimadzu C-R6A chromatopac; a LiChrospher® 100 C18 (5 µm) LiChroCART® 250-4 column was employed. The mobile phase consisted of acetonitrile:water (40:60, v/v).

MATERIALS

Bortezomib is commercialized by Millennium Pharmaceuticals (Mass, USA) in the US and Janssen-Cilag in Europe under the trade name Velcade. The vials are reconstituted with 1.4 mL of sterile NaCl 0.9% to obtain 2.5 mg mL^{-1} solution of bortezomib. The product information states that reconstituted bortezomib at 1.0 mg mL^{-1} is stable for 8 hours when stored at <25°C and protected from light, and for 3 hours in a syringe. No information was available at this moment about the stability of bortezomib solution at 2.5 mg mL^{-1} concentration.

Stability study

On study day 0, two vials of bortezomib were each one reconstituted with 1.4 mL of 0.9% NaCl to prepare solutions of concentration 2.5 mg mL^{-1}. One of the vials was stored in the refrigerator at 4°C for stability study and the other was stored in the freezer for daily preparation of standards; both vials were protected from light.

Physical stability

On the study days 0, 1, 2, 3, 4, 7, 9, 14, 15, 17, 22 and 30, sample was drawn for analysis of concentration and was inspected visually for changes in colour and presence of particulate matter.

Bortezomib analysis

On each study day (0, 1, 2, 3, 4, 7, 9, 14, 15, 17, 22 and 30), a 100 µL aliquot of thawed solution was used to prepare standards with final concentration of 50, 125 and 250 µg mL^{-1} into NaCl 0.9%. These standards combined with a blank solution allowed the construction of a calibration curve. On the other hand, a quality control sample with bortezomib concentration of 125 µg mL^{-1} was prepared from the solution stored at 4°C. Duplicate 20 µL quantities of each prepared sample, quality control sample, standards and blank were injected manually in the column.

The area under the bortezomib peak at 270 nm was subjected to least squares linear regression, and the bortezomib concentration in each sample was determined by interpolation from the calibration curve. The bortezomib was eluted at 3.5 minutes with a flow rate of 1.5 mL min^{-1}.

Accelerated degradation analysis

Five different studies were carried out for this purpose: acid, base, heat, hydrogen peroxide and sodium hypochlorite. The chromatographic separation of the degradation products from bortezomib and the similarity of the ultraviolet spectrum (200-365 nm) between standard and a degraded sample led to conclude that this analytical method was suitable for indicating stability [12-14].

RESULTS AND DISCUSSION

Bortezomib stability study

Physical stability

All solutions, as reconstituted in the original manufacturer's glass vials, were initially clear and colourless and remained so for the duration of the study. Also, no visible particles were observed in any solution throughout the study period.

Bortezomib analysis

During the study period, the concentration in all study sample retained at least 88.18 % of the initial concentration of bortezomib.

Table 1 provides stability data of bortezomib (2.5 mg mL^{-1}) stored at 4°C in the dark over 30 days, tested at a diluted concentration of 125 µg mL^{-1}.

Table 1. Mean concentration and stability of unused reconstituted bortezomib in manufacturer vials stored at 4°C in the dark

Study day	Concentration of bortezomib (mean ± SD, ng mL^{-1})	Percent of bortezomib remaining)
Day 0	122.38 ± 0.99	97.90
Day 1	110.23 ± 1.24	88.18
Day 2	120.13 ± 0.84	96.10
Day 3	116.16 ± 0.80	92.93
Day 4	111.11 ± 2.21	88.89
Day 7	119.49 ± 2.05	95.59
Day 9	118.50 ± 1.89	94.80
Day 14	118. 00 ± 2.10	94.40
Day 22	117.94 ± 2.25	94.35
Day 30	122.68 ± 1. 15	98.14

ACCELERATED DEGRADATION ANALYSIS

pH study

The ultraviolet spectrum of bortezomib (200-365 nm) shows no variation in acid, neutral and basic medium with a maximum wavelength at 270 nm in all cases. On the other hand, an aliquot of 200 µL of 125 ppm of sample were added different amounts of HCl 0.2 M (the concentration of bortezomib in these samples were (50, 62.5, 71.4 and 83.3 ppm), the chromatograms of these samples let to obtain a calibration graph with a slope similar to the chromatograms obtained when were added different amounts of NaOH 0.1 M for to obtain the same concentration of bortezomib in basic medium. Both slopes were of the same order with respect to slope obtained in neutral medium. The higher difference observed in these chromatograms were the presence of diverse peaks corresponding to a degradation products, principally in basic medium (t_R=2.1 min).

Heat study

A sample of 125 ppm of bortezomib was heat at 90ºC during different times. In the chromatogram of bortezomib obtained after heat the sample appears clearly the same degradation product mentioned above as can be seen in **figure 1**. The results obtained were shown in **figure 2**.

Influence of hydrogen peroxide

Degradation of bortezomib with hydrogen peroxide occurs quickly. At ambient temperature, 0.2mL of 125 µg mL^{-1} of bortezomib solution was degraded completely when 50 µL of hydrogen peroxide solution (30%,

3% or 0.3 %) were added and the degradation product appears to 4.7 min. Solutions with lower concentration of hydrogen peroxide degrade bortezomib more slowly, as can be seen in **figure 3**.

Figure 1. Chromatogram obtained after heat the sample

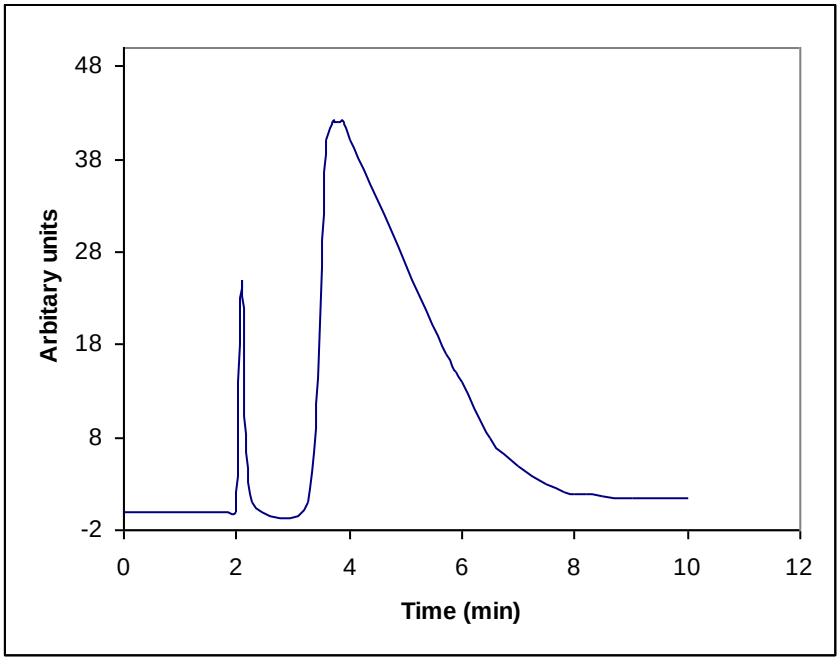


Figure 2. Percentage remains of bortezomib and the degradation product after heat

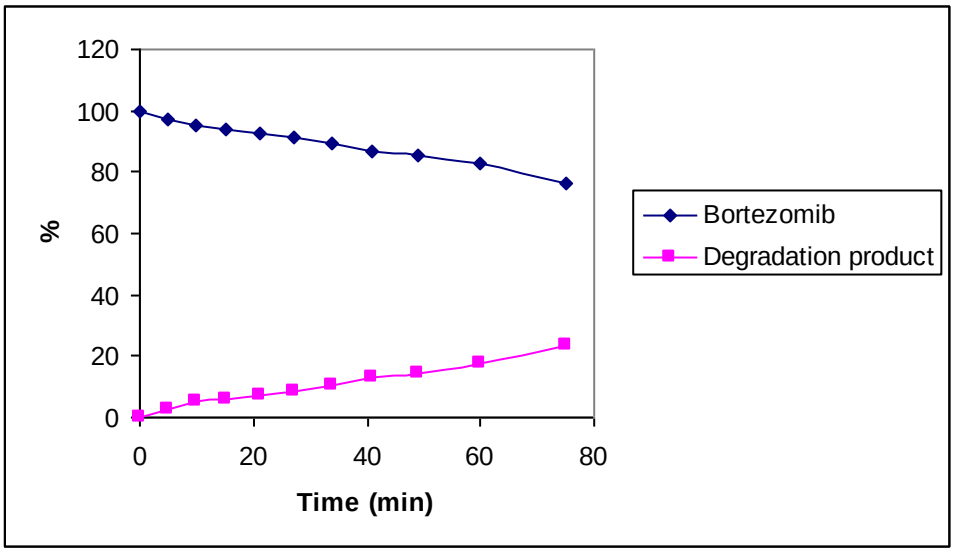
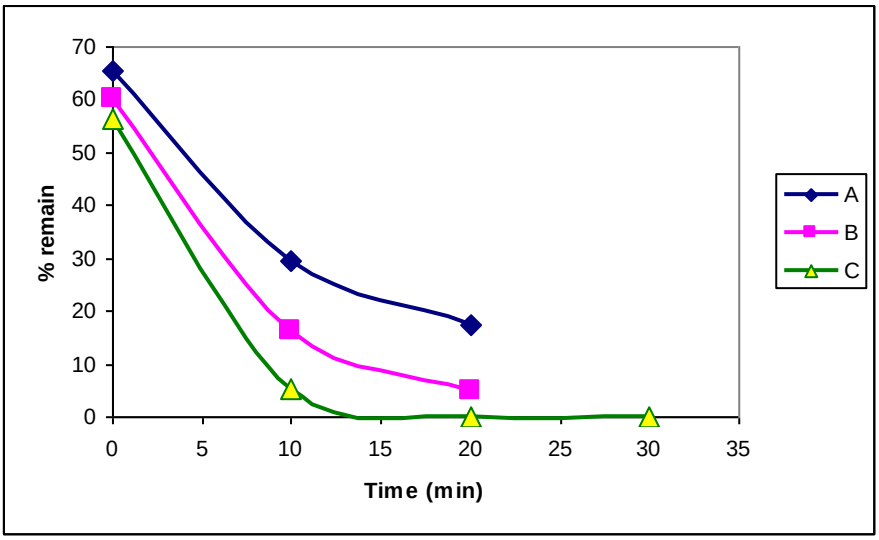


Figure 3. Influence of H_2O_2 (A: 300 µL (50 ppm) + 20 µL 0.03 % H_2O_2)); B: (200 µL (125 ppm) + 50 µL 0.03 % H_2O_2)); C: (300 µL (125 ppm) + 100 µL 0.03 % H_2O_2))



Influence of sodium hypochlorite

Degradation of bortezomib with sodium hypochlorite also occurs quickly. At ambient temperature, 0.7 mL of 125 µg mL^{-1} of bortezomib solution was degraded completely when 50 µL of sodium hypochlorite solution 0.02 M was added and degradation product appears at 5 min when the sample is chromatographied immediately. After 10 min, this degradation product was newly degraded in other products (t_R= 2.1, 3, 3.2, 8.1 min). After 30 min from the addition of sodium hypochlorite solution, new degradation products appear between 3 and 4 min in the chromatogram without resolved.

The addition of 50 µL of sodium hypochlorite solution 0.002 M to 0.8 mL of 125 ppm of sample produced an immediate degradation, until 29% approximately and this percentage stays constant almost up to 45 min.

CONCLUSIONS

Intravenous injection is the standard administration route of bortezomib at 1 mg mL^{-1} concentration, and its stability has been previously studied by different authors. However, subcutaneous administration is an important alternative but the necessary concentration in this case is 2.5 mg mL^{-1}. The stability of these solutions has not been studied at this moment. In this work, we have investigated the stability at this concentration (2.5 mg mL^{-1}).

Reconstituted bortezomib 2.5 mg mL^{-1} was physically and chemically stable at 4°C in the dark at least for 30 days in the original manufacturer vial.

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