Hemostatic effects of bortezomib treatment in patients with relapsed or refractory multiple myeloma

Due to increased baseline hypercoagulability, subjects with multiple myeloma (MM) are at increased risk of developing venous thromboembolism (VTE) compared with the general population.[1] Both thalidomide and lenalidomide treatment have been associated with an increased thrombogenecity,[2] however, this was not associated with shorter overall survival.[3] Pre-clinical and clinical reports have suggested a possible anti-thrombotic effect of bortezomib.[4,5] We report the first study which prospectively describes *in vivo* effects of bortezomib on routine tests of blood coagulation and platelet function in MM treated patients. Patients with relapsed /refractory multiple myeloma who failed one line of prior therapy were eligible for the study. After obtaining informed consent approved by the local institutional review board, bortezomib-naïve patients with relapsed/refractory MM were enrolled in the study. Upon enrollment, subjects were required to have a normal prothrombin time (PT) and partial thromboplastin time (PTT) and a platelet count >100×10^9/L.

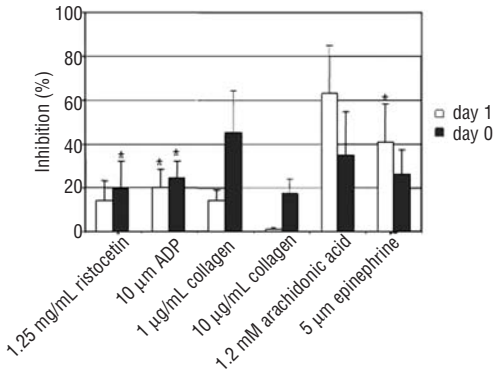
Patients that were known carriers of a hypercoagulable state, had a previous history of cardiovascular disease or had platelet abnormalities were excluded. The use of any agents known to interfere with coagulation and platelet function was prohibited. Factor V Leiden and prothrombin gene mutations were measured only at baseline. All the remaining tests were obtained prior to and 1 hour after the first (day 1) and second (day 4) dose of bortezomib therapy. Platelet counts were obtained using a Hemavet® HV950FS (Drew Scientific, Oxford). The PT, activated partial thromboplastin time (APTT), and thrombin times (TT) were measured using a compact coagulometer (Diagnostica Stago). Proteins C, S and anti-thrombin III activities along with fibrinogen, D-dimer levels were also obtained, (Diagnostica Stago, Inc., Parsippany, NJ, USA).

Acquired resistance to activated protein C was determined using an APTT-based assay with pre-dilution in excess of factor V deficient plasma (Chromogenix Instrumentation Laboratory, Milano, Italy). For platelet aggregation studies, blood was collected in 3.2% citrate centrifuged at 200 g for 10 mins. and platelet-rich plasma (PRP) was obtained. Aggregation associated changes in light transmission was recorded for a total time of 5 mins. using a lumi-aggregometer (Chrono-Log Corp, Havertown, PA, USA) with 1 or 10 µg/mL collagen, 10 µM ADP, 1.2 mM arachidonic acid and 5 µM epinephrine. Ristocetin-induced platelet agglutination was also performed. Expression of P-selectin on the platelet surface at baseline and after ADP and collagen exposure was also measured on the collected samples. Whole blood was incubated with FITC-labeled anti-P-selectin antibody and PE-labeled anti-CD41a antibody (Pharmingen, BD Biosciences, San José, CA, C USA, at#*555523* and Cat #*555467* respectively). Twenty thousand positive events for CD41 were acquired from each sample and analyzed as per standard procedure (FACscan, Becton Dickinson, Franklin Lakes, NJ, USA).[6]

Ten patients with relapsed/refractory multiple myeloma were enrolled in the study (median age 62 years, range 50-72 years), 80% male. Myeloma subtypes were IgG/IgA/IgD/light chain 40%, 20%, 20% and 20% respectively. No mutations for V Leiden or prothrombin G20210 were identified. The median levels of coagulation factors pre and post bortezomib infusion are shown in Table 1; no statistically significant changes associated to bortezomib exposure were reported. As previously reported, we observed a modest decrease in median platelet count after bortezomib infusion (171×10^9/L *vs.* 153×10^9/L).[7] With a median platelet rich plasma (PRP) count of 193×10^9/L, bortezomib infusion platelet aggregation was decreased after bortezomib infusion with most of the agonists used on both days of treatment. The decrease was statistically significant when comparing ADP induced aggregation in samples collected pre- and post-bortezomib infusion on days one, (20% decrease, p=0.033) and day four (29% decrease, p=0.009) of the cycle. Post-bortezomib samples collected on day one of the treatment cycle showed a significant decrease in epinephrine induced platelet aggregation (p=0.034); ristocetin induced agglutination was also decreased on day 4 after bortezomib infusion (p=0.0077). The percentages of inhibition on platelet-aggregation for each of the agonists are shown in Figure 1. Overall the expression level of the surface protein P-selectin was decreased on the platelets' surface after bortezomib treatment; geometrical mean fluorescence values were 16.1 and 12.8 respectively before and after bortezomib doses under resting conditions. Overall, when platelets were activated by collagen or ADP, P-selectin expression was decreased after bortezomib treatment. Using 10 µM ADP as agonist the geometrical mean fluorescence values before and after bortezomib doses were 81.9 and 73.1 respectively.

Table 1. Pre- and post-bortezomib infusion coagulation factor levels.

Test (reference range) n=10 patients	*Day 1 Pre-dose Median (Range)*	*Day 1 Post-dose Median (Range)*	*p value*	*Day 4 Pre-dose Median (Range)*	*Day 4 Post-dose Median (Range)*	*p value*
PT (11-15 sec)	13.4 (12.1-15.5)	13.85 (12.5-15.4)	0.15	13.55 (12.2-15.5)	14.4 (12.4-14.7)	0.66
PTT (28-40 sec)	27.35 (22.8-32)	26.2 (20-31.5)	0.24	26.95 (22.8-32)	25.5 (21.9-29.8)	0.64
Fibrinogen (200-400 mg/dL)	385 (255-448)	360 (271-467)	0.36	386 (247-448)	341.5 (213-437)	0.28
Protein C (70-160%)	114 (84-200)	102 (84-164)	0.07	120 (81-199)	115 (79-197)	0.93
Protein S (65-140)%	89 (71-141)	85 (87-123)	0.3	92 (63-119)	92.5 (83-127)	0.55
Antithrombin III (90-127)%	98 (78-132)	97.5 (87-123)	0.47	100.5 (90-130)	102 (83-127)	0.20
APC Ratio (>2.26)	2.345 (2.23-2.41)	2.33 (2.16-2.41)	0.3	2.3 (2.11-2.49)	2.22 (198-223)	0.17
T.T. (14-21 sec)	18.2 (16.4-43)	17.9 (17.2-21)	0.4	17.7 (17.1-23.2)	19.45 (16.5-20.7)	0.91
D-Dimer (<0.50 µg/mL)	0.56 (0.18-3.8)	0.5 (0.14-3.66)	0.56	0.5 (0.3-3.54)	0.59 (0.28-1.04)	0.87
Homocysteine (5-15 µmol/L)	8.77 (8.28-26.8)	8.99 (6.73-24.3)	0.58	8.69 (4.02-22.1)	8.13 (5.55-12.1)	0.29



*p<0.05, SEM=standard error of the mean

Figure 1. Inhibition of platelet aggregation by bortezomib. For both days 1 and 4, post-treatment are compared with pre-treatment samples. Mean±SEM are shown.

After activation with 10 µg/mL collagen before and after bortezomib treatment we observed a geometric mean fluorescence value of 25.4 and 21.0. However, no statistical difference was reached after bortezomib exposure with any agonist.

This pilot clinical trial has shown for the first time *in vivo* that even a short exposure to bortezomib can affect platelet function. In experimental models, proteasome inhibitors are known to prevent cytokine-induced expression of the endothelial cell adhesion molecules E-selectin, vascular cell adhesion molecule-1, intracellular adhesion molecule-1 and P-selectin expression in activated human endothelial cells.[8,9] *In vitro* bortezomib has also shown an inhibitory effect on platelet aggregation induced by ADP in a dose dependent manner.[10] PSI, a highly specified peptide aldehyde proteasome inhibitor can prevent arterial thrombus formation in renovascular hypertensive rats; it has been shown to inhibit platelet aggregation and to increase the initial blood flow rate after arterial thrombosis induction.[4] We have previously reported a low incidence of deep vein thrombosis during the induction phase of our study combining bortezomib, thalidomide and dexamethasone.[11] In addition, no thromboembolic events were observed in a group of 30 patients with relapsed MM treated with bortezomib, and melphalan, prednisone, thalidomide (MPT) combination despite the absence of any anticoagulant prophylaxis.[12] The incidence of VTE reported by the same authors was significantly higher when MPT was given without bortezomib.[13] This manuscript supports strong *in vivo* evidence that bortezomib exerts anti-thrombotic actions mainly by its effects on platelet function. This effect should be tested after more prolonged bortezomib exposure and related to the clinical events.

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References

1. Srkalovic G, Cameron MG, Rybicki L, Deitcher SR, Kattke-Marchant K, Hussein MA. Monoclonal gammopathy of undetermined significance and multiple myeloma are associated with an increased incidence of venothromboembolic disease. Cancer 2004;101:558-66.
2. Zangari M, Anaissie E, Barlogie B, Badros A, Desikan R, Gopal AV, et al. Increased risk of deep-vein thrombosis in patients with multiple myeloma receiving thalidomide and chemotherapy. Blood 2001;98:1614-5.
3. Zangari M, Barlogie B, Cavallo F, Bolejack V, Fink L, Tricot G. Effect on survival of treatment-associated venous thromboembolism in newly diagnosed multiple myeloma patients. Blood Coagul Fibrinol 2007;18:595-8.
4. Ostrowska JD, Wojtukiewicz MZ, Chabielska E, Buczko W, Ostrowska H. Proteasome inhibitor prevents experimental arterial thrombosis in renovascular hypertensive rats. Thromb Haemost 2004;92:171-7.
5. Wang M, Giralt S, Delasalle K, Handy B, Alexanian R. Bortezomib in combination with thalildomide-dexamethasone for previously untreated multiple myeloma. Hematology 2007;12:235-9.
6. Ruf A, Patsheke H. Flow cytometric detection of activated platelets: comparison of determining shape change, fibrinogen binding, and P-selectin expression. Semin Thromb Haemost 1995;21:146-51.
7. Lonial S, Waller EK, Richardson PG, Jagannath S, Orlowski RZ, Giver CR, et al. Risk factors and kinetics of thrombocytopenia associated with bortezomib for relapsed, refractory multiple myeloma. The SUMMIT/CREST Investigators. Blood 2005;106:3777-84.
8. Read MA, Neish AS, Luscinskas FW, Palobella VJ, Maniatis T. The proteasome pathway is required for cytokine-induced endothelial-leukocyte adhesion molecule expression. Immunity 1995;2:493-506.
9. Xia L, Pan J, Yao L, McEver RP. A proteasome inhibitor, and antioxidant, or a salicylate, but not a glucocorticoid, blocks constitutive and cytokine-inducible expression of P-selectin in human endothelial cells. Blood 1998;91:1625-32.
10. Avcu F, Ural AU, Cetin T, Nevruz O. Effects of bortezomib on platelet aggregation and ATP release in human platelets, in vitro. Thromb Res 2008;121:567-71.
11. Zangari M, Barlogie B, Lee CK. Protective effect of Velcade on thalidomide-associated deep vein thrombosis. Blood 2004;104:310b-1b[Abstract].
12. Palumbo A, Ambrosini MT, Benevolo G, Pregno P, Pescosta N, Callea V, et al. Bortezomib, melphalan, prednisone, and thalidomide for relapsed multiple myeloma. The Italian Multiple Myeloma Network; Gruppo Italiano Malattie Ematologiche dell'Adulto. Blood 2007;109:2767-72.
13. Palumbo A, Bringhen S, Caravita T, Merla E, Capparella V, Callea V, et al. Oral melphalan and prednisone chemotherapy plus thalidomide compared with melphalan and prednisone alone in elderly patients with multiple myeloma: randomized controlled trial. The Italian Multiple Myeloma Network, GIMEMA. Lancet 200a6;367:825-31.

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