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ORIGINAL ARTICLE

Peripheral neuropathy following bortezomib therapy in multiple myeloma patients: association with cumulative dose, heparanase, and TNF-α

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Abstract

Multiple myeloma (MM) is a plasma cell neoplasm which constitutes about 10% of all hematologic malignancies. Despite bortezomib is a promising new generation of drugs for MM, its clinical use is limited by peripheral neurotoxicity in the vast majority of patients, which can be severe and require a reduction of dose or even treatment withdrawal. Tumor necrosis factor-α (TNF-α), as the most important inflammatory factor, could induce the inflammatory response and expression of heparanase (HPSE), which may play a crucial role in peripheral neuropathy after chemotherapy. However, the role of TNF-α in bortezomib-induced peripheral neuropathy (BIPN) has not been reported. In this study, treatment-emergent neuropathy was assessed by total neuropathy score and electrophysiological examination. The expression level of TNF-α and HPSE were evaluated by enzyme-linked immunosorbent assay. The effects of anti-TNF-α on the evolution of neuropathy were tested in rat models of neurotoxicity. The results indicated that with the augment of cumulative dose of bortezomib, the incidence of neuropathy was increased. Moreover, bortezomib administration induced the expression of TNF-α. With the increased expression of TNF-α, neuropathy was exacerbated. TNF-α-induced expression of HSPE was secondary to the development of neuropathy. Co-administration of anti-TNF-α in bortezomib therapy has a potential neuroprotective effect on BIPN in rats. TNF-α participates in the pathogenesis of BIPN, which represents an attractive target for future therapeutic intervention.

Keywords Multiple myeloma · Bortezomib · Peripheral neuropathy · Heparanase · Tumor necrosis factor-α

Introduction

Multiple myeloma (MM) is one of the most common hematologic malignancies, with 103,826 new cases and 72,453 deaths annually, comprising 0.8% and 1% of all cancers, respectively [1]. Bortezomib, a boronic acid dipeptide, is a new generation antineoplastic drug that inhibits cellular protein degradation by blocking the proteasome system. It is largely used in the treatment of refractory or newly diagnosed MM [2–4], with a potential application in the treatment of a variety of tumor types [5]. Although its efficacy is significant, the incidence of peripheral neuropathy (PN) induced by bortezomib vary from 29 to 64% [6–8]. Grades 1–2 PN can occur in up to 75% patients who have relapsed after or were refractory to bortezomib therapy [2, 9], while grade ≥ 3 neurotoxicity was between 2 and 23% [10]. In addition, dose reduction algorithm, longer intervals between cycles, and a weekly instead of a twice-weekly administration schedule are effective strategies to prevent aggravation of symptoms and significantly reduce the incidence of grade 3–4 PN [11, 12]. However, modification of dose and schedule plans severely affects efficacy and daily life [8, 13–15]. Therefore, it is necessary to further deepen the knowledge of the toxicity and develop new therapies designed to avoid the side effect without sacrificing quality of life.

Recent researches suggest that the extended treatment leads to better outcomes for MM patients, while side effects are also increasing. This is particularly relevant to the cumulative dose in the administration of bortezomib [15–18]. Accumulating evidence indicated that inflammatory response plays a key role in the control of responses during nerve degeneration

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and regeneration. Proteasome inhibition can induce pro-inflammatory response in neuronal cells and alterations in the inflammatory processes, which have been related to bortezomib-induced peripheral neuropathy (BIPN) in patients [19, 20]. Tumor necrosis factor-α (TNF-α) is a soluble 17-kDa protein that predominately mediates immune and inflammatory responses and plays a critical role in facilitating the development of neuropathic pain [21, 22]. Previous study indicated that TNF-α induced upregulation of heparanase (HPSE) and activated the production of heparan sulfate (HS) fragments, which triggered the release of TNF-α and other inflammatory cytokines [23–25]. Studies have also demonstrated that trauma or nerve toxic agents such as chemotherapeutic drugs, paclitaxel, or vincristine upregulated the expression of TNF-α after peripheral nerve injury [26]. These findings suggested that TNF-α might be involved in chemotherapeutic drug-induced PN. However, it remains unclear whether TNF-α also participates in BIPN.

In this research, we studied the correlation between cumulative dose of bortezomib, TNF-α, and HPSE with PN. Moreover, we first combined the expression of TNF-α and HPSE in peripheral blood of patients with PN following bortezomib treatment. We also established animal models to observe the effect of anti-TNF-α therapy on neurotoxicity in rats.

Materials and methods

Patients and therapy

Patients were eligible for enrollment in the trial if they were newly diagnosed multiple myeloma according to International Myeloma Working Group criteria [27] and had no received therapy. Patients were excluded if they had PN of grade 2, active infection, or other primary malignancy diagnosed within 5 years [28]. All patients provided written informed consent before study enrollment. Patients received up to eight cycles of bortezomib 1.3 mg/m^2 by subcutaneous injection over 3 to 5 s on days 1, 4, 8, and 11, and oral dexamethasone 20 mg on days 1, 2, 4, 5, 8, 9, 11, and 12 of a 21-day cycle [29–31]. Dose modification of bortezomib was according to the guidelines for the management of BIPN. The dose was decreased from 1.3 to 1 mg/m^2 for grade 1 PN with pain or grade 2 PN patients who are interfered with function but not with daily activities. Withdraw bortezomib until toxicity returned to at least grade 2 PN, then restarted at 0.7 mg/m^2 for grade 2 PN with pain or grade 3 PN patients who are interfered with daily activities. Discontinue bortezomib or combination chemotherapy for grade 4 PN patients who are permanent sensory loss interfering with function [11, 32, 33]. The planned duration of bortezomib therapy was 24 weeks; the maximum planned cumulative dose of bortezomib was 57.6 mg/m^2. Response and progression were assessed according to the standard criteria [28, 34].

Neurologic assessments

Treatment-emergent neuropathy was graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events version 4.03 [35]. Patients were assessable for neural toxicity at the beginning and at the end of each treatment cycle. This evaluation including sensory, motor symptoms, and functional impairment were used to calculate a total neuropathy score (TNS) [8]. In addition, the group of patients in our study underwent motor and sensory nerve conduction studies and quantitative sensory tests at baseline and at study completion, including sensory action potential (SAP), compound muscle action potential (CMAP), sensory nerve conduction velocity (SNCV), and motor nerve conduction velocity (MNCV) [36].

Determination of TNF-α, HPSE, syndecan-1, and HS

Blood samples were collected before and after bortezomib treatment in each cycle. Blood was placed in preservative-free heparin and kept on ice. Serum and plasma were separated by centrifugation at 1000 g/L at 4 °C within 1 h and frozen at − 80 °C until analysis. Serum level of TNF-α at baseline, before, and after each cycle therapy was determined by enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's instructions, as well as the plasma level of HPSE, syndecan-1, and HS on admission and every 2 weeks subsequently [37, 38].

Animal studies

Drugs administration

A total of 36 adult female Wistar rats (Harbin, China) were used for our study. Each group was composed of 12 rats. The care and husbandry of animals were in conformity with the institutional guidelines in compliance with national and international laws and policies (Guide for the Care and Use of Laboratory Animals, US National Research Council, 1996). Animals were housed in a limited access animal facility where animal room temperature and relative humidity were set at 22 ± 2 °C and 55 ± 10%, respectively. Artificial lighting provided a 24-h cycle of 12-h light/dark (light 7 a.m.–7 p.m.). Bortezomib (Settle biological company, Harbin, China) was dissolved in 5% Tween 80, 5% ethanol, and 90% sterile saline, and administered subcutaneously at dose of 0.2 mg/kg three times a week for 8 weeks. Murine monoclonal antibody specific for rat TNF-α (CNTO1081) (Settle biological company, Harbin, China) was dissolved in phosphate-buffered saline and administered intraperitoneally [39–41]. At the end of

treatment period, some of the rats were severely ill and were sacrificed for ethical reasons, while the remaining animals were left untreated and followed up for an additional 4 weeks. The general condition of the animals was assessed daily, while the body weight was measured once weekly during the treatment and follow-up period.

Neurophysiological assessment

The nerve conduction velocity (NCV) was determined in the tail nerve of each animal as previously described in several experiments [40, 42, 43] after 4 and 8 weeks of treatment (n = 12 animals/group) and at the end of the 4-week follow-up period in the surviving rats (n = 8 animals/group). In brief, the NCV in the tail nerve was assessed by placing recording ring electrodes distally in the tail, while the stimulating ring electrodes were placed 5 cm and 10 cm proximally with respect to the recording point. The latencies of the potentials recorded at the 2 sites after nerve stimulation was determined (peak-to-peak) and nerve conduction velocity was calculated accordingly. All the neurophysiological determinations were performed under standard conditions in a temperature-controlled room.

Behavioral measures

Mechanical tests were performed at baseline, after 4 and 8 weeks, and at the end of the follow-up period using a Dynamic Plantar Aesthesiometer test. Rats were placed on an elevated wire mesh floor in a plexiglas chamber and were adapted to the environment for about 15 min. The withdrawal threshold in rats was determined by calculating the mean value of six repeated applications of a 0.5-mm diameter metal filament to the plantar surface, which exerted a progressively increasing puncture pressure, reaching up to 50 g within 20 s. The pressure evoking a clear voluntary hind paw withdrawal response (withdrawal latency) was recorded automatically. Mechanical allodynia was defined as a significant decrease in the dynamic test threshold evoked by mechanical stimuli.

Statistical analysis

Data from experiments were expressed as mean ± SD. Statistical analyses were performed by SPSS 20.0 statistical software using one-way analysis of variance (ANOVA). The significance of paired data was determined by t test, χ^2, or Fisher exact test for comparing categorical variables, and values that showed $P \leq 0.05$ were considered statistically significant. All experiments were repeated at least three times, with similar results obtained.

Results

Patient characteristic

A total of 35 newly diagnosed MM patients in our department were enrolled from January 2015 to December 2017. Across the therapy group, the median age of the patients was 60 years (range, 35 to 78); 22 patients (62.9%) were male, 13 patients (37.1%) were female. Clinical characteristics are listed in Table 1. All patients received one or more cycles of bortezomib therapy. Five patients required therapy discontinuation due to clinical progression of disease (3 patients) and severe PN (2 patients). The patients received a median of five-cycle treatment ranging from one to eight cycles. Over all the cycles, the median cumulative dose of bortezomib administered was 39 mg/m^2 (range, 5.2 to 57.6 mg/m^2).

Comparison of efficacy between two different cumulative dose groups of bortezomib

Thirty-five patients were assessable for response. There were three patients undergoing discontinued therapy due to the progression of disease. Two of them received less than five cycles of treatment, with one progressed in the second cycle and the other one in the third cycle. In addition, there was another

Table 1 Clinical characteristics of the patients

Characteristic	Patients (n = 35)
Age (years)	
Median	60
Range	35–78
Sex (n)	
Male	22
Female	13
β2-microglobulin (mg/L, n)	
Median	6.5
Range	3.2–13.1
Albumin (g/L, n)	
Median	28
Range	22–38
Type of measurable disease (n)	
IgG	20
IgA	13
Kappa light chain	2
International Staging System stage (n)	
I	6
II	11
III	18

There was no significant difference in the characteristics evaluated at baseline of patients in different stages of I, II, and III based on International Staging System

patient progressed in the sixth cycle. Patients with early progressing had no chance to receive more doses. Thus, the two patients with early progressions were removed from the analysis. As shown in Table 2, 27 (81.8%) of all patients achieved a response, with 10 complete response (30.3%), 9 very good partial response (27.3%), and 8 partial response (24.2%). Most patients were managed expectantly after five cycles of therapy, and the median cumulative bortezomib dose received was 39 mg/m^2. Therefore, the cumulative bortezomib dose of 39 mg/m^2 was selected as the cutoff for defining the two cumulative bortezomib dose groups for the response analyses. A total of 16 patients (48.5%) in the lower cumulative dose group and 17 patients (51.5%) in the higher cumulative dose group received subsequent therapy. The overall response rate was somewhat higher in patients in the higher (≥ 39 mg/m^2) versus lower (< 39 mg/m^2) cumulative bortezomib dose group (88.2% vs. 75.0%, respectively). Moreover, with more intensive cycles, complete response rate was higher (35.3% vs. 25.0%, respectively). There was a statistically significant difference in response between the two groups ($P < 0.05$).

Comparison of peripheral neuropathy between two different cumulative dose groups of bortezomib

Incidence and severity of peripheral neuropathy

BIPN observed for patients in the higher (≥ 39 mg/m^2) and lower (< 39 mg/m^2) cumulative bortezomib dose groups are summarized in Table 3. The overall incidence of BIPN in the high cumulative dose group was obvious higher than the low cumulative dose group (82.4% vs. 43.8%, respectively, $P < 0.05$). PN of grade 2 or 3 was more frequently observed in the high cumulative bortezomib dose group than in the low cumulative bortezomib dose group (41.2% vs. 12.5%; 11.8% vs. 0%, respectively, $P < 0.05$). During bortezomib administration, 21 patients (63.6%) developed neurologic adverse effects, which were mostly grade 1 or 2 PN (57.6%), and grade 3 PN was reported in only 2 patients (6.1%). The median onset of PN occurred following the fifth cycle of bortezomib therapy, corresponding to a cumulative dose of approximately 39 mg/m^2, which required neuropathic medications or reduction of the dose of bortezomib. Nine patients (27.3%) experienced grade 2 peripheral sensory disturbance or neuropathic pain, primarily involving the lower limbs; three of them required dose reduction to 1.0 mg/m^2. Two patients (6.1%) developed grade 3 neuropathy, complaining of painful paresthesias at the lower limbs and at the hands, requiring discontinuation.

Clinical and electrophysiologic characteristics in patients

The electrophysiological findings of BIPN patients were detailed in Table 4. The median TNSr for all of the patients at baseline was 0 (range 0–5). Seven patients (21.2%) with TNSr > 2 were symptomatic with mild numbness or weakness. Two patients with a baseline TNSr = 5: one patient developed painful grade 3 sensory neuropathy and required discontinuation, and another one required dose reduction to 1.0 mg/m^2. Comparison of baseline with end of treatment data for sural and peroneal nerves (SAP), CMAP amplitudes, and SNCV reduced in various degrees ($P < 0.05$). Meanwhile, they were significantly lower in the patients with grade 3 PN than in those with grade 1 or grade 2 PN ($P < 0.05$). MNCV was generally minimally reduced in the remaining peroneal nerves ($P > 0.05$). Although severe neuropathy is usually more frequent in the presence of pre-existent nerve damage, bortezomib-related neuropathy seems to be independent of baseline neuropathy.

Table 2 Summary of responses among patients after bortezomib treatment

Responses	Cumulative bortezomib dose (cycles)		*P* value
	< 39 mg/m^2 (< 5)	≥ 39 mg/m^2 (≥ 5)	
Response-assessable patients (*n* (%))	16 (48.5)	17 (51.5)	
Overall response (*n* (%))	12 (75.0)*	15 (88.2)*	$P < 0.05$
Complete response (*n* (%))	4 (25.0)*	6 (35.3)*	$P < 0.05$
Very good partial response (*n* (%))	5 (31.3)	4 (23.5)	
Partial response (*n* (%))	3 (18.8)	5 (29.4)	
Stable disease (*n* (%))	4 (25.0)	1 (5.9)	
Progressive disease (*n* (%))	-	1 (5.9)	

Response was assessed on the basis of uniform criteria of International Myeloma Working Group. The patients were evaluated for response with a confirmed diagnosis of multiple myeloma and must have received at least one dose of bortezomib treatment

*$P < 0.05$

Table 3 Incidence and severity of peripheral neuropathy in patients receiving bortezomib therapy

PN	Cumulative bortezomib dose (cycles)		*P* value
	< 39 mg/m^2 (< 5)	≥ 39 mg/m^2 (≥ 5)	
PN-evaluable patients (*n* (%))	16 (48.5)	17 (51.5)	
Overall incidence of PN (*n* (%))	7 (43.8)*	14 (82.4)*	$P < 0.05$
Severity of PN (*n* (%))			
Grade 1	5 (31.3)	5 (29.4)	
Grade 2	2 (12.5)*	7 (41.2)*	$P < 0.05$
Grade 3	0 (0)*	2 (11.8)*	$P < 0.05$

Peripheral neuropathy was assessed on the basis of uniform criteria of National Comprehensive Cancer Network.
* P < 0.05

Effect of bortezomib on the serum concentration of TNF-α

Comparison serum concentration of TNF-α with cumulative dose of bortezomib

In order to investigate the mechanism of BIPN, TNF-α was measured in patients with MM, all of whom were in the clinical trial and twenty-one of whom experienced PN. TNF-α was detectable in all patients before therapy, with a mean value of 32.34 pg/mL (range 28.55 to 36.13 pg/mL). There was no significant difference between levels of TNF-α in PN and non-PN patients at baseline. Prolonged treatment with bortezomib resulted in a slight augment in TNF-α in patients who had non-PN, with a mean augment of 65.3% after eight cycles of therapy (Fig. 1a). In contrast, there was a more than twofold increase in TNF-α of patients who developed PN after five cycles of bortezomib therapy. These data indicated that patients suffered from PN at the end of the fifth cycle of therapy were accompanied with a corresponding increase in serum TNF-α.

Comparison serum concentration of TNF-α in different PN groups

There was no significant difference in the serum concentration of TNF-α between non-PN and PN groups at baseline ($P > 0.05$). In the process of treatment, further analysis showed that there was still no significant difference in the serum concentration of TNF-α between non-PN and grade 1 PN groups ($P > 0.05$), while the serum concentration of TNF-α in grade 2 PN and grade 3 PN groups were significantly higher than non-PN group ($P < 0.05$). In grade 2 and grade 3 PN groups, the serum concentration of TNF-α increased obviously after PN occurred ($P < 0.05$). The serum concentration of TNF-α slightly increased after treatment in non-PN group. No difference of TNF-α at baseline, before, and after PN occurred was observed in grade 1 PN group ($P > 0.05$). These results suggested that TNF-α was associated with the development of grade 2 PN and grade 3 PN ($P < 0.05$) (Table 5). If the serum concentration of TNF-α was less than 65 pg/mL (red line in Fig. 1b), almost all of patients had no complaints of neurological symptoms. In contrast, complaints of neuropathy began to

Table 4 Electrophysiological findings in patients with neuropathy

Electrophysiology	Grade 1		Grade 2		Grade 3	
	B	End	B	End	B	End
TNSr (median (range))	0 (0–3)	5 (2–8)	0 (0–5)	12 (9–16)	3 (1–5)	18 (17–19)
Sural nerve (median(range))						
SAP (μV)	9.5 (6.5–13.0)	5.7 (3.1–10.6)	8.2 (5.7–15.2)	3.8 (2.0–9.4)	7.5 (5.8–9.2)	3.0* (1.5–4.5)
SNCV (m/s)	47.2 (43.3–53.6)	44.3 (39.4–45.5)	47.1 (41.2–55.4)	43.5 (34.2–45.4)	47.0 (45.1–48.9)	42.6* (42.2–43.0)
Peroneal nerve (median(range))						
CMAP (mV)	8.5 (4.9–13.4)	6.0 (3.6–9.3)	8.0 (4.4–10.7)	4.2 (2.6–7.1)	7.8 (4.8–10.8)	3.2* (1.7–4.7)
MNCV (m/s)	49.0 (42.6–58.3)	48.2 (41.7–50.6)	48.5 (42.8–55.1)	46.1 (40.6–48.3)	47.4 (42.3–52.5)	45.7 (39.5–51.9)

B, baseline; *End*, end of treatment; *TNSr*, total neuropathy score reduced version; *SAP*, sensory action potential (mV); *SNCV*, sensory nerve conduction velocity (m/s); *CMAP*, compound muscle action potential (mV); *MNCV*, motor nerve conduction velocity (m/s)
*$P < 0.05$

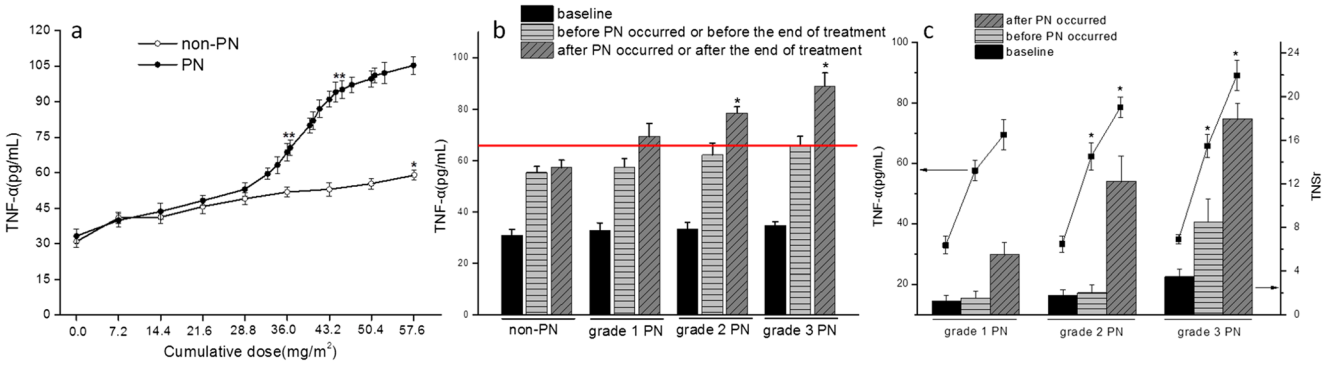

Fig. 1 **a** Relationship between serum TNF-α level and the cumulative dose of bortezomib. Serum TNF-α level in PN and non-PN patients was measured by ELISA. **b** Differences of serum TNF-α concentration in each group. Data represent mean ± SD of three separate experiments. *$P < 0.05$ vs. controls, **$P < 0.01$ vs. controls. **c** Comparison of TNF-α level and TNSr (total neuropathy score reduced version) in different groups of patients at baseline, before, and after PN occurred. Data represent mean ± SD of three separate experiments. *$P < 0.05$ vs. controls, **$P < 0.01$ vs. controls

emerge in majority of patients when TNF-α was over 65 pg/mL (Fig. 1b). A temporal analysis of TNF-α level and development of PN was presented in Fig. 1c. The results indicated that there was a distinct increase of TNF-α level before development of PN and even further increased after the PN occurred. As shown in Fig. 1c, TNF-α level was below 40 pg/mL at baseline, while it increased significantly before the obvious development of PN, especially in grades 2 and 3 PN patients, even more than 60 pg/mL. In addition, level of TNF-α further increased after PN was detected, which exceeded 80 pg/mL in grade 3 PN patients.

Comparison HPSE, syndecan-1, and HS level in different PN groups

Herein, we also evaluated the level of HPSE in the plasma of patients with PN and non-PN subjects by ELISA every 2 weeks. After PN occurred, HPSE level was increased by nearly 2-fold in patients with grade 2 PN and 3-fold in patients with grade 3 PN as compared with non-PN subjects (71, 145, and 218 pg/mL for non-PN, grade 2 PN, and grade 3 PN, respectively) ($P < 0.05$). The level of HPSE in the plasma of patients exhibiting grade 1 PN (84 pg/mL) versus non-PN (71 pg/mL) was not different ($P > 0.05$). Meanwhile, the serum TNF-α in collaboration with HPSE increased when PN occurred. High level of HPSE is associated with TNF-α and grades 2 and 3 PN (Fig. 2a).

HPSE elevates shedding of syndecan-1 and release of soluble HS fragments by trimming the HS chains on syndecan-1. Hence, the level of syndecan-1 and HS among groups was evaluated at baseline, and both of them were of no significant differences. Nevertheless, the ELISA data indicated that comparing with non-PN patients, a conspicuous shedding of syndecan-1 in grade 2 and grade 3 PN patients occurred with bortezomib therapy (21.59, 69.43, and 80.33 pg/mL for non-PN, grade 2, and grade 3 PN, respectively, $P < 0.05$) (Fig. 2b). In addition, after PN occurred, the level of HS essentially showed a complete increase in patients with grade 2 or grade 3 PN, compared with the background level of non-PN patients (34, 137, and 281 pg/mL for non-PN, grade 2 PN, and grade 3 PN, respectively, $P < 0.05$) (Fig. 2c). However, it should be noted that after PN occurred with bortezomib treatment, the level of HS was greatly elevated, indicating that HPSE was cleaving HS.

Effects of anti-TNF-α treatment on BIPN in a rat model

General toxicity

To further confirm the role of TNF-α in the neurotoxicity of bortezomib, we established rat model to study the effects of

Table 5 The serum concentration of TNF-α in different PN groups

	non-PN	Grade 1 PN	Grade 2 PN	Grade 3 PN
TNF-α (B, pg/mL)	30.85 ± 2.32	32.82 ± 2.91	33.23 ± 2.73	34.76 ± 1.56
TNF-α (before, pg/mL)	55.36 ± 2.37	57.53 ± 3.38	62.29 ± 4.59	65.72 ± 3.82
TNF-α (after, pg/mL)	57.32 ± 2.58	69.46 ± 4.97	78.54 ± 2.47*	89.02 ± 5.12*

B, baseline; *before*, before PN occurred for PN patients and before the end of treatment for non-PN patients; *after*, after PN occurred for PN patients and after the end of treatment for non-PN patients. Data represent mean ± SD of three separate experiments

*$P < 0.05$ vs. controls

**$P < 0.01$ vs. controls

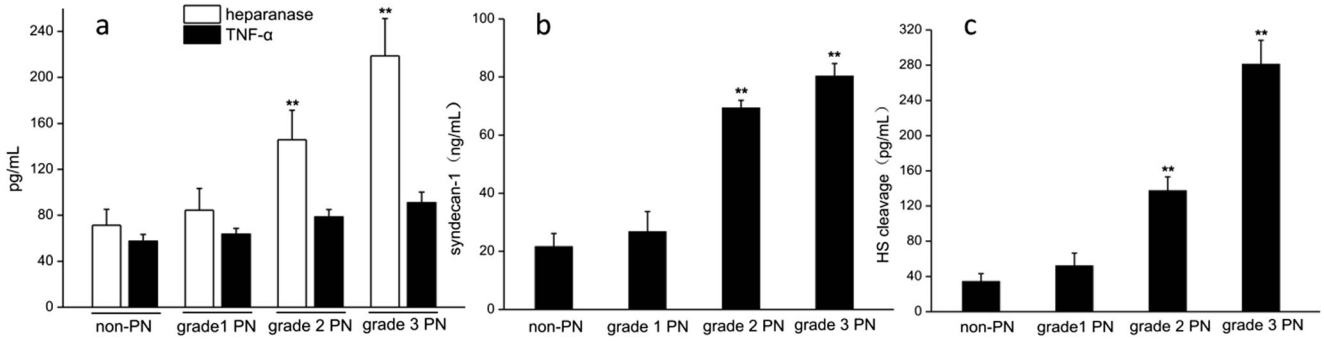

Fig. 2 **a** Evaluation of HPSE level in the plasma of patients in each PN groups. Plasma samples of patients were collected on admission and every 2 weeks subsequently. HPSE level was measured by ELISA. **b** Evaluation of syndecan-1 level in the plasma of patients in each PN groups. Plasma samples of patients were collected on admission and every 2 weeks subsequently. Syndecan-1 level was measured by ELISA. **c** Evaluation of HS level in the plasma of patients in each PN groups. Plasma samples of patients were collected on admission and every 2 weeks subsequently. HS level was measured by ELISA. Data represent mean ± SD of three separate experiments. $*P < 0.05$ vs. controls, $**P < 0.01$ vs. controls

anti-TNF-α on neuropathy. The administration of 0.20 mg/kg bortezomib was well tolerated in rats using the 3q7d schedules during the experiment. Although some of the rats showed prostration and reduced motility, they recovered rapidly in the subsequent treatments. The rats in bortezomib group and anti-TNF-α group tended to have a reduced weight gain versus controls (particularly after 4 weeks of treatment), while at the end of the follow-up period, bortezomib-treated rats had an even higher increase in body weight as compared with controls. No significant difference in weight gain was observed between groups (Fig. 3).

Neurophysiological evaluation

There was no statistically significant difference of neurophysiological determinations evaluated at baseline. NCV of healthy control rats had a slight increase due to the final maturation of the peripheral nerves occurring in these young adult rats.

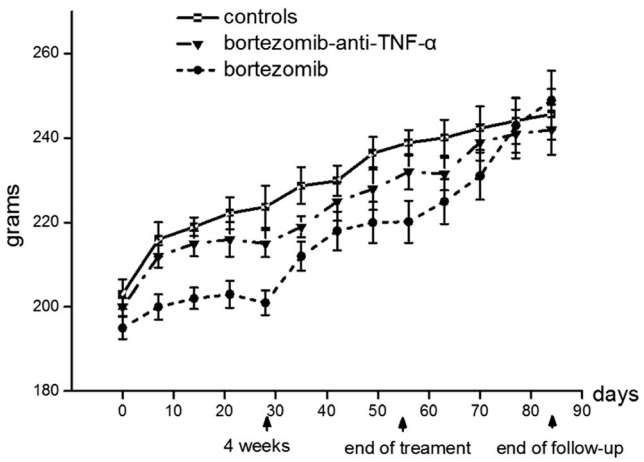

Fig. 3 Body weight changes throughout the experiment. Mean values ± SD, $n = 12$ in each group during the treatment period, $n = 8$ at the end of follow-up

Although NCV of the three groups recovered partially at the end of follow-up period, the tail NCV in bortezomib group significantly reduced after 4 weeks and at the end of administration ($P < 0.05$ vs. controls). It is noteworthy that a significant improvement was observed in tail NCV after co-administration with anti-TNF-α ($P < 0.05$ vs. controls) (Fig. 4a).

Behavioral studies

The determinations of dynamic test performed at baseline showed that there was no statistically significant difference in the mean withdrawal threshold among groups. After 4 weeks and at the end of treatment, there was a marked reduction in the mechanical paw withdrawal threshold, indicating the occurrence of mechanical allodynia in bortezomib-treated group versus controls ($P < 0.05$). However, co-administration with anti-TNF-α from the beginning of bortezomib therapy reduced allodynia than rats treated with bortezomib alone ($P < 0.05$). At the end of the follow-up period, the difference was no longer significant. Our findings indicated that monoclonal antibodies against TNF-α has a significant protective effect against BIPN (Fig. 4b).

Discussion

In this research, a majority of MM patients achieved significant remission after bortezomib treatment (Table 2). Despite bortezomib is a highly effective anticancer drug, it causes serious dose-limiting PN after several subcutaneous administration (Table 3). As shown in Table 4, nervous system was also obviously damaged in PN patients, decrease of nerve conduction velocity, and action potential enhanced with the increase of PN degree. Nowadays, bortezomib, as the frontline anti-myeloma drug, is widely used worldwide; meanwhile,

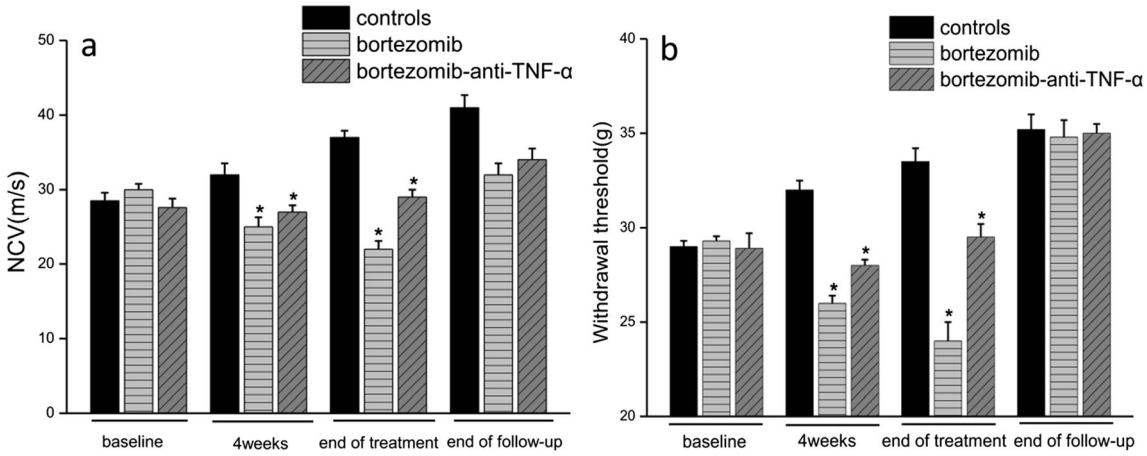

Fig. 4 **a** Nerve conduction velocity (NCV) changes during the experiment. NCV of rats was measured in each group at baseline, after 4 and 8 weeks of treatment and at the end of the follow-up period. Data are expressed as mean values ± SD, $n = 12$ in each group during the treatment period, $n = 8$ at the end of follow-up. **b** Withdrawal threshold changes throughout the experiment. The mechanical threshold was measured at baseline, after 4 and 8 weeks of treatment, and at the end of the follow-up period in bortezomib group and anti-TNF-α group. Data are expressed as mean values ± SD, $n = 12$ in each group during the treatment period, $n = 8$ at the end of follow-up. *$P < 0.05$ vs. controls

side effects of bortezomib have also received considerable attention, especially PN, which affects clinical efficacy and daily life. Thus, in-depth study with respect to pathogenesis of BIPN is foremost for clinic.

Although a great many experiments about BIPN have been performed, the underlying mechanism regarding this side effect is not entirely clear. In this study, we proposed a new mechanism of BIPN. A large number of clinical observations suggest that inflammation and immune response might be crucial factors in BIPN [44]. TNF-α, as the major inflammatory cytokine, mediated PN directly or indirectly by induction of other cytokines or activation of Ca^{2+} and Na^{+} channels, increasing the excitability of nerve cells and resulting in persistent hyperalgesia [45]. Our recent clinical studies have demonstrated that bortezomib-induced pain is sensory and related to cumulative dose, HPSE, and TNF-α. We have recently reported that with a cumulative dose of bortezomib more than 39 mg/m^2, the incidence of BIPN increased markedly and examination of electrophysiology decreased apparently, especially in grade 2 or 3 PN (Tables 3 and 4), requiring neuropathic medications or reduction of the dose of bortezomib, but overall response rate appeared significantly higher in the meanwhile (Table 2).

In former research, proteasome inhibition could induce a series of pro-inflammatory response in neuronal cells and alterations in the inflammatory processes, which may be related to PN in patients [19, 20]. TNF-α, as the most important inflammatory cytokines, predominately mediates immune and inflammatory responses and plays a critical role in facilitating the development of neuropathic pain [21, 22]. Our results indicated that administration of bortezomib resulted in a progressive increase of TNF-α in patients who developed PN after several cycles of therapy (Fig. 1a). Beyond a certain range of TNF-α level, patients began to complain about neurologic symptoms and demand reduction of drug (Fig. 1b). Moreover, we made a temporal analysis of TNF-α level and development of PN, TNF-α increased significantly before the obvious development of PN, especially in grades 2 and 3 PN patients (Fig. 1c). Besides, the serum concentration of TNF-α further increased evidently after PN occurred, which was also significantly higher in grade 2 PN and grade 3 PN patients than non-PN (Table 5). Hence, the development of grade 2 PN and grade 3 PN were associated with a corresponding increase of TNF-α. The increase in the level of TNF-α could be used as an effective biomarker to early anticipate the development of PN. Regular evaluation of TNF-α and early diagnosis of PN are important to manage symptoms and prevent the development of more severe neuropathy. This is of great significance for guiding clinical work and ensuring that patients can continue to benefit from therapy.

In addition, previous studies indicated that TNF-α-induced upregulation of HPSE expression and release from tumor cells in many cancers compared with baseline level was measured prior to therapy [23–25]. Of note, activated HPSE produced HS fragments that trigger the production of TNF-α and other inflammatory cytokines. A soluble form of HPSE was taken up by macrophages in a HS-dependent manner. Then, activated macrophages could induce TNF-α expression increasingly [46]. Focusing on hematological malignancies, we found that the level of HPSE expression was increased nearly 2- or 3-fold in patients with grade 2 or 3 PN as compared with non-PN subjects following bortezomib therapy (Fig. 2a). These data implied that high level of HPSE may be associated with grades 2 and 3 PN, and HPSE was secondary to the development of neuropathy.

HPSE is the only known mammalian endoglycosidase, which cleaves the HS side chains of syndecan-1 expressed in cells such as tumor cells [47]. HPSE-mediated shortening

of HS chains on syndecan-1 also enhances shedding of HS and syndecan-1 [48]. This was consistent with our findings that an augment in HPSE expression increased HS and syndecan-1 shedding from cells in the majority of MM subjects. Our results indicated that comparing with non-PN patient, an almost complete shedding of syndecan-1 and a marked increase of HS level appeared in grade 2 PN and grade 3 PN patients following treatment with bortezomib (Fig. 2b and c). Beyond that, endocytosis of a soluble form of HPSE by both macrophages and tumor cells in a HS-dependent manner affects several inflammatory reactions, including release of cytokines within cell surfaces [46]. Thus, HPSE, HS, and syndecan-1 could be the auxiliary cues for early diagnosis of PN.

Although bortezomib modulates the expression of TNF-α, few animal models of BIPN have been reported. To further identify the role of TNF-α in the neurotoxicity of bortezomib, we studied the effects of anti-TNF-α on the development of the neuropathy. Recently, several studies have described the pathological features associated with antineoplastic drug-induced neurotoxicity. Both damages in DRG and peripheral nerves suggested axonal degeneration suffer from the bortezomib administration [40, 41]. Herein, we tested the neurophysiological and behavioral results of rats in the administration of bortezomib alone and co-administration with anti-TNF-α treatment. Accordingly, the onset of peripheral neurotoxicity started from the 4th week of treatment and continued until the end of treatment. Bortezomib administration induced significant decrease in tail nerve NCV versus controls, which was improved by anti-TNF-α (Fig. 4a). Moreover, anti-TNF-α treatment partially restored the decline of withdrawal threshold of rats (Fig. 4b). However, at the end of the follow-up period, neurophysiological alterations in rats treated with bortezomib showed a complete recovery. Our results were in keeping with several studies demonstrated that anti-TNF-α administration had a protective effect on bortezomib neurotoxicity.

Conclusion

In conclusion, our findings indicated that bortezomib administration induced a chronic increase of TNF-α expression. TNF-α participated in the occurrence and development of neuropathy. In addition, it could induce the increase of HPSE which was secondary associate with PN. In animal experiments, anti-TNF-α treatment reduced neurotoxicity induced by bortezomib. Co-administration of anti-TNF-α in bortezomib therapy had a potential neuroprotective effect on BIPN. Cumulative dose of bortezomib and TNF-α play crucial roles in neuropathy induced by bortezomib. Development of new neuroprotective agents against TNF-α may be a promising therapeutic strategy to prevent the development of neuropathy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict interests.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All institutional and national guidelines for the care and use of laboratory animals were followed.

Informed consent Informed consent was obtained from all individual participants included in the study.

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