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Research Report

Altered discharges of spinal neurons parallel the behavioral phenotype shown by rats with bortezomib related chemotherapy induced peripheral neuropathy

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ABSTRACT

Bortezomib is a first generation proteasome inhibitor that is the frontline chemotherapy for multiple myeloma with the chief dose-limiting side effect of painful peripheral neuropathy. The goal of this study was to define the behavioral phenotype in a preclinical model of bortezomib chemotherapy-induced peripheral neuropathy (CIPN) and to test whether this is matched by changes in the physiological responses of spinal wide dynamic range neurons. Sprague-Dawley rats were treated with four injections of bortezomib at four doses, 0.05 mg/kg, 0.10 mg/kg, 0.15 mg/kg, 0.20 mg/kg, or equal volume of saline. All doses of bortezomib above 0.05 mg/kg produced showed significant dose-dependent mechanical hyperalgesia that was fully established at 30 days after treatment and that recovered to baseline levels by day 69 after treatment. Thermal, cold, and motor testing were all unaffected by treatment with bortezomib. Spinal wide dynamic range (WDR) neurons in rats with confirmed bortzomib-related CIPN showed an increase in number of evoked discharges to mechanical stimuli and exaggerated after-discharges in rats with bortezomib CIPN.

1. Introduction

Bortezomib is a first generation proteasome inhibitor drug used in the treatment of multiple myeloma and other non-solid malignancies. It may be administered on its own, but is used primarily in conjunction with other drugs such as thalidomide, dexamethasone, melphalan, and prednisone (San Miguel et al., 2008; Kaufman et al., 2010). As with many other chemotherapeutic drugs, bortezomib exhibits a side effect of painful chemotherapy-induced peripheral neuropathy (CIPN) (Aghajanian et al., 2002; Kane et al., 2006). This appears in a stocking and glove distribution with patients reporting the most severe neuropathic symptoms in the glabrous skin of their toes and fingers (Cata et al., 2007). CIPN frequently becomes so severe that patients are given a lower dose than maximally effective or forced off of chemotherapy

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entirely (Cata et al., 2007; Broyl et al., 2012). Understanding the mechanisms of chemotherapy-induced peripheral neuropathy so that it may be alleviated or prevented is therefore critical if patients are to have a higher quality of life and benefit fully from chemotherapy treatment.

Bortezomib was granted a fast-track approval by the FDA for use in treatment of multiple myeloma due to its effectiveness. It is distinct from other chemotherapy drugs in that it targets proteasomes within cells, which consist of a 19S subunit that detects ubiquitination and a 20S subunit that degrades ubiquitinated proteins. Bortezomib competitively binds to the 20S subunit, preventing the normal degradation of misfolded or obsolete proteins by targeting the chymotrypsin-like activity of the proteasome (Adams et al., 1999; Adams, 2004; Adams and Kauffman, 2004). Due to high levels of protein turnover and synthesis, the accumulation of these proteins has a greater consequence in cancer cells than in healthy cells. However, it is unclear how this accumulation of proteins results in apoptosis, as multiple mechanisms have been proposed that could explain this effect. Cell cycle arrest may occur when proteins regulating the cell cycle at critical stages remain at concentrations that do not permit proliferating cells to continue through the cell cycle, such as what may occur with stabilization of IκB, the inhibitory form of the transcription factor NFκB (Hideshima et al., 2001; Voorhees et al., 2003). This would not necessarily account for apoptosis, but could explain a slower progression in treated patients. It is also possible that blocking the cell's ability to digest misfolded or otherwise faulty proteins via bortezomib treatment causes excessive stress on the endoplasmic reticulum, which then leads to caspase-mediated apoptosis (Landowski et al., 2005). Alternatively, bortezomib may trigger apoptosis through the activity of p53, which prompts mitochondrial cytochrome *c*-triggered apoptosis (Hideshima et al., 2003; Voorhees et al., 2003). Malignant cells must continually work against apoptotic signals, and it has been suggested that inhibiting the proteasome shifts this balance in a manner unfavorable to the survival of malignant cells. Many other reasonable mechanisms have been proposed for how proteasome inhibition leads to apoptosis in addition to those listed here. In light of this, it seems likely that there are multiple pathways contributing to apoptosis and inhibited growth of cancer cells treated with bortezomib, as opposed to any single mechanism.

With the current lack of understanding of the activity of bortezomib on cancer cells, it is not surprising that the cause of bortezomib-induced peripheral neuropathy is also not well understood. This poses major difficulties in identifying treatments to prevent or reverse this neuropathy. Understanding the cause of bortezomib CIPN is more complicated than simply treating generalized pain, since CIPN in other drug models does not necessarily affect every modality of somatosensation. Instead, a given drug has a profile of select types of stimuli that produce abnormal numbness, tingling, pain, or other sensations that are similar across drugs, but not identical (Cata et al., 2007; Dougherty et al., 2007; Boyette-Davis et al., 2012). It is therefore necessary in characterizing bortezomib-induced peripheral neuropathy to survey multiple modalities so as to provide insight as to a common mechanism that may underlie affected modalities.

Electrophysiological data are also important to determine whether central sensitization, glutamate transporter dysfunction, or other similar mechanisms are involved, since bortezomib cannot cross the blood brain barrier (Adams, 2004). Studies with other chemotherapy drug models have revealed increased responses and persistent afterdischarges in wide dynamic range (WDR) neurons in the spinal dorsal horn in CIPN (Cata et al., 2006, 2008a). A similar finding in bortezomib would indicate a maladaptive response in these cells that might explain both exaggerated sensitivity and persistent after sensations to cutaneous stimuli such as those seen in patients (Cata et al., 2007; Boyette-Davis et al., 2011). Taken together with behavioral data, the present study was conducted to identify changes in sensory behavioral phenotype and accompanying spinal cellular responses to understand bortezomib CIPN for the sake of its treatment or prevention.

2. Results

2.1. *Mechanical sensitivity*

2.1.1. *0.05 mg/kg Bortezomib*

Rats treated at the 0.05 mg/kg dose of bortezomib had a gradual and slight onset of mechanical hyperalgesia versus baseline behavior that was determined in ANOVA to not differ significantly versus controls (Fig. 1A). Nevertheless, this group was significantly lower versus control at day 19 (naïve: 17.2±2.49 g, bortezomib: 10.4±1.10 g), as well as at days 23, 26, and 34. After this point, rats began to recover from changes in mechanical sensitivity. Although the average withdrawal threshold was higher for this group at later time points than in saline-treated rats, this difference was not statistically significant, and these rats started with a higher baseline threshold than the saline-treated group.

2.1.2. *0.10 mg/kg Bortezomib*

Rats treated at the 0.10 mg/kg dose of bortezomib showed a significantly reduced mechanical withdrawal threshold than the saline-treated and 0.05 mg/kg treated groups ($P<0.01$), but not different from the 0.15 mg/kg or 0.20 mg/kg groups. This group first showed statistically significant mechanical hyperalgesia versus controls at day 6 (naïve: 19.3±2.56 g, bortezomib: 13.9±1.54 g), after the third injection of bortezomib (Fig. 1B). Significant differences were also observed at every following time point until day 54. Peak severity was observed from day 16 to day 34 (approx. 7.5 g). Recovery from changes in mechanical sensitivity was gradual after this point, with statistically equivalent behavior versus saline-treated rats at days 63 and 69.

2.1.3. *0.15 mg/kg Bortezomib*

Rats treated at the 0.15 mg/kg dose of bortezomib showed significantly lowered withdrawal threshold from the saline-treated and 0.05 mg/kg group ($P<0.01$), but not from the 0.10 mg/kg or 0.20 mg/kg groups. This group first showed statistically significant mechanical hyperalgesia versus controls at day 8 (naïve: 17.3±2.48 g, bortezomib: 11.1±2.41 g), after the final injection of bortezomib (Fig. 1C). Significant