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Full paper

Behavioral and pharmacological characteristics of bortezomib-induced peripheral neuropathy in rats

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ABSTRACT

Bortezomib, an effective anticancer drug for multiple myeloma, often causes peripheral neuropathy which is mainly characterized by numbness and painful paresthesia. Nevertheless, there is no effective strategy to escape or treat bortezomib-induced peripheral neuropathy (BIPN), because we have understood few mechanism of this side effect. In this study, we evaluated behavioral and pathological characteristics of BIPN, and investigated pharmacological efficacy of various analgesic drugs and adjuvants on mechanical allodynia induced by bortezomib treatment in rats. The repeated administration of bortezomib induced mechanical and cold allodynia. There was axonal degeneration of sciatic nerve behind these neuropathic symptoms. Furthermore, the exposure to bortezomib shortened neurite length in PC12 cells. Finally, the result of evaluation of anti-allodynic potency, oral administration of tramadol (10 mg/kg), pregabalin (3 mg/kg), duloxetine (30 mg/kg) or mexiletine (100 mg/kg), but not amitriptyline or diclofenac, transiently relieved the mechanical allodynia induced by bortezomib. These results suggest that axonal degeneration of the sciatic nerve is involved in BIPN and that some analgesic drugs and adjuvants are effective in the relief of painful neuropathy.

1. Introduction

Bortezomib is a proteasome inhibitor widely used in chemotherapy to treat multiple myeloma. A side effect of bortezomib is that it often causes severe peripheral neuropathy and is therefore dose limiting. Bortezomib-induced peripheral neuropathy (BIPN) occurs in 35–52% of patients (grade ≥3 in 8–14% of patients) and is mainly characterized by hypoesthesia and painful paresthesia (1–3). At present, the mechanism of BIPN is not well understood and there is no established method to treat it. Thus, the development of peripheral neuropathy is an important issue in bortezomib chemotherapy.

We previously reported that other anticancer drugs such as paclitaxel and oxaliplatin induce pain symptoms that are characteristic of neuropathy including mechanical allodynia associated with axonal degeneration of the sciatic nerve in rats (4,5). These anticancer drugs also inhibited neurite outgrowth in cultured rat pheochromocytoma 12 (PC12) and rat dorsal root ganglion (DRG) cells. Furthermore, we reported that neurotropin, which attenuated the inhibition of neurite outgrowth in these cultured cells, ameliorated neuropathy induced by these anticancer drugs (4,5). Our findings indicate that axonal degeneration in the sciatic nerve is involved in mechanical allodynia induced by paclitaxel and oxaliplatin, and that the effect on neurite outgrowth has the potential to affect axonal degeneration in sciatic nerve.

It has been reported that administration of bortezomib induces mechanical and cold allodynia and pathological changes in sciatic nerve and DRG in rodents (6–9). Recently, it has been revealed a part of mechanisms of BIPN such as changes the expression of tumor necrosis factor-α (TNF-α), transient receptor potential vanilloid 1 (TRPV1), calcitonin gene-related peptide (CGRP), and substance P in DRG neurons, and the contributions of peroxynitrite, and altered discharges of spinal neurons (10–14). Furthermore, bortezomib-induced mechanical allodynia is inhibited by gabapentin in mice (15). However, the effects of various analgesic drugs or adjuvants,

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which approved for use in clinical practice, on pain behaviors in animal models of BIPN have been not well studied.

In this study, we developed an animal model of BIPN, and consequently characterized pain behaviors and pathological changes. In addition, we investigated the effect of bortezomib on neurite length in cultured PC12 cells for adequacy evaluation of bortezomib-induced neurotoxicity. Finally, we evaluated the pharmacological efficacy of various analgesic drugs and adjuvants on painful neuropathy.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing 200–250 g (Kyudo Co., Tosu) were housed in groups of four to five per cage, with lights on from 7:00 to 19:00 h. Animals had free access to food and water in their home cages. All experiments were approved by the Experimental Animal Care and Use Committee of Kyushu University according to the National Institutes of Health guidelines, and we followed the International Association for the Study of Pain Committee for Research and Ethical Issues guidelines for animal research (16).

2.2. Drugs

Bortezomib was purchased from LC Laboratories (Woburn, MA, USA). Tramadol hydrochloride and mexiletine hydrochloride were purchased from Sigma–Aldrich, Inc. (St. Louis, MO, USA). Pregabalin, duloxetine hydrochloride, amitriptyline, and diclofenac sodium were obtained from Pfizer Inc. (New York, NY, USA), Tokyo Chemical Industry Co., Ltd. (Tokyo), LKT Laboratories, Inc. (St. Paul, MN, USA), and Novartis International AG (St. Johann, Basel, Switzerland), respectively. Bortezomib was dissolved in 5% dimethyl sulfoxide (DMSO, Sigma–Aldrich, Inc.) solution. Bortezomib (0.05, 0.1, or 0.2 mg/kg) or vehicle (5% DMSO solution) was administered intraperitoneally (i.p.) twice a week for 2 weeks (on days 1, 4, 8, and 11). The administration schedule and doses of bortezomib were determined based on clinical treatment (1.3 mg/m^2 of bortezomib on days 1, 4, 8, and 11). Tramadol, amitriptyline, and mexiletine were dissolved in sterile water. Pregabalin, duloxetine, and diclofenac were suspended in 0.25% sodium carboxymethylcellulose (CMC-Na) solution. The volume of vehicle or drug solution injected was 1 mL/kg.

2.3. Behavioral studies

2.3.1. Rota-rod test

Rota-rod test was performed to investigate the change in motor coordination. Rats were placed on a rotating rod (Muromachi Kikai Co., Ltd., Tokyo) and the latency to falling was measured for up to 2 min according to the method described previously (17). The test was performed three times, and the rotating speed was 10 rpm.

2.3.2. von Frey test

Mechanical allodynia was assessed by the von Frey test. Each rat was placed in a clear plastic box with a wire mesh floor and allowed to habituate for 30 min prior to testing. von Frey filaments (The Touch Test Sensory Evaluator Set; Linton Instrumentation, Diss, Norfolk, UK) with a range of 1–15 g bending force were applied to the mid-plantar skin of each hind paw six times, with each application held for 6 s. The paw withdrawal threshold was determined by a modified up-down method (4).

2.3.3. Acetone test

Cold allodynia was assessed by the acetone test. Each rat was placed in a clear plastic box and allowed to habituate for 30 min prior to testing. Fifty microliters of acetone (Wako Pure Chemical Industries, Ltd., Osaka) was sprayed onto the plantar skin of each hind paw, and the number of withdrawal responses was counted for 40 s from the start of the acetone spray according to the method described previously (18). The test was performed six times (three times per hind limb).

2.3.4. Hot-plate test

Thermal hyperalgesia was assessed using a hot-plate test. Each rat was placed on a hot/cold-plate apparatus (Ugo Basile Biological Research Apparatus, Gemonio, Varese, Italy), and the latency to licking their hind paw was measured for up to 60 s. The temperature of the hot-plate was kept at 52.5 °C (19). The test was performed three times.

2.4. Drug evaluation

Mechanical allodynia was confirmed on days 12–15 and drug evaluation was carried out the next day. Tramadol, pregabalin, duloxetine, amitriptyline, mexiletine, and diclofenac were administered orally through a stainless steel gavage tube. Control rats were injected with the drug vehicle. For tramadol and diclofenac,

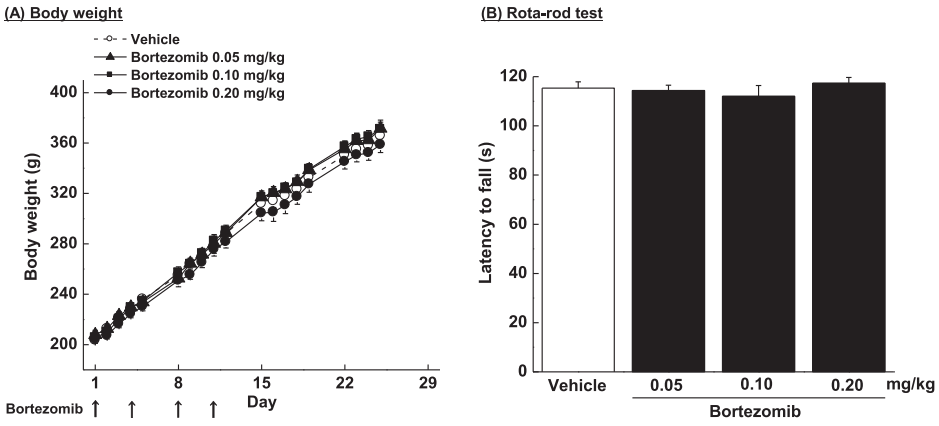

Fig. 1. Effects of bortezomib on body weight (A) and motor coordination (B) in rats. Bortezomib (0.05, 0.1, or 0.2 mg/kg) was administered i.p. twice a week for 2 weeks. Body weight was measured on days 1–5, 8–12, 15–19, and 22–25. The rota-rod test was performed on day 15. Results are expressed as mean ± SEM of 10 rats.