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Regular Article

# Chemotherapy-induced peripheral neurotoxicity in immune-deficient mice: New useful ready-to-use animal models

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ABSTRACT

Cisplatin, paclitaxel and bortezomib are effective chemotherapy drugs in cancer treatment. However, they share severe peripheral neurotoxicity (PN) as one of their major dose-limiting side effects, often impairing cancer patients' quality of life and sometimes being permanent. Even if preclinical oncology is largely based on the use of immune-deficient mice, rodent models used to study the chemotherapy-induced PN are available only in immune-competent animals. In this study we characterized for the first time the PN induced by these chemotherapies through neurophysiological, behavioral, morphological and morphometric studies in athymic nude mice, a commonly employed strain in the preclinical oncology. The animals, divided into four groups, were chronically treated with cisplatin, paclitaxel or bortezomib once or twice a week for 4 or 6 weeks or were left untreated. These schedules were tolerated, neurotoxic and in the range of antineoplastic effectiveness. Despite similarities, differences in the features of PN were evident if compared with immune-competent models under comparable regimens of treatment. The results of this study may provide a basis for future combined analysis of antineoplastic and neurotoxic effects of chemotherapy in the same animals.



## Introduction

Cisplatin, paclitaxel and bortezomib are among the most commonly employed chemotherapy drugs in cancer treatment. They are very effective, but their use is frequently associated with severe peripheral neurotoxicity (PN), now one of their major dose-limiting side effects, often long-lasting or even permanent (Han and Smith, 2013). Since these chemotherapy drugs are not able to cross the blood–brain and blood–nerve barriers while they have access to the dorsal root ganglia (DRG), their PN is primarily characterized by sensory disturbances including paresthesias, dysesthesia and sensory impairment with a "stock-and-glove" distribution (Cata et al., 2007). Only in a small minority of cases also motor fibers are affected, generally only at the subclinical level. Neuropathic pain, if present, can be severe and is among the main reasons for chemotherapy discontinuation and/or withdrawal particularly in bortezomib- or taxane-treated patients (Argyriou et al., 2008).

Cisplatin is still a first line chemotherapy drug in the treatment of several solid tumors (Mollman et al., 1988; Andrè et al., 2004; Bianchi et al., 2007). Its anticancer activity is mainly due to the induction of inter- and intra-strand crosslinks in DNA that cause apoptotic cell death in cancer cells (Huang et al., 1995). The mechanisms underlying cisplatin PN are not known, but direct binding to the neuronal DNA (Cavaletti et al., 1992) and oxidative neuronal damage with mitochondrial dysfunction (Carozzi et al., 2010a) have been proposed. Also paclitaxel is widely employed in the treatment of different solid tumors (Zhang et al., 2013). In cancer cells paclitaxel acts stabilizing microtubules and interfering with their normal breakdown during cell division leading to cell cycle arrest and death (Horwitz, 1992). In neuronal cells microtubule system damage (Cavaletti et al., 1997) causes a disturbed cytoplasmic flow and axonal transport leading to neuronal dysfunction; also, in this case, mitochondrial damage with possible subsequent energy failure has been described (Park et al., 2008). Bortezomib is a cardinal drug used for the treatment of multiple myeloma, but its use has been proposed also for some lymphomas and solid tumors (reviewed in Richardson et al., 2005). It reversibly inhibits chemotryptic-like activity of the 26S proteasome unit, leading to the activation of signaling cascades, cell-cycle arrest and apoptosis (Mitsiades et al., 2002). Besides this effect, an action on tubulin in the DRG and peripheral nerves has been recently described (Meregalli et al., 2014).

*Abbreviations:* PN, peripheral neurotoxicity; DRG, dorsal root ganglia; CIPN, chemotherapy-induced peripheral neurotoxicity; NCV, nerve conduction velocity; AMP, action potential amplitude; BMI, body mass index; CDDP, cisplatin; PACLI, paclitaxel; BTZ, bortezomib; FU, follow-up

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So far no effective preventive strategy is available against chemotherapy-induced peripheral neurotoxicity (CIPN) and its treatment is purely symptomatic and often unsatisfactory. On this background, the co-treatment with neuroprotective agents during chemotherapy might represent a valuable therapeutic approach, provided that their interference with the antitumor activity of chemotherapy is reliably ruled out (Argyriou et al., 2008).

In the past 20 years we developed several chronic rat and mouse models of cisplatin, paclitaxel and bortezomib-induced PN (Cavaletti et al., 1992, 1995; Carozzi et al., 2010b,c, 2013; Meregalli et al., 2010; Renn et al., 2011), where it was possible to investigate the effects of drug administration on the peripheral nervous system at the clinical, neurophysiological and pathological level. However, these models were established in immune-competent animals, while preclinical oncology workout is largely based on the use of immune-deficient mice. In this study we provide a proof-of-concept of the feasibility of the set-up of cisplatin, paclitaxel and bortezomib PN models in immune-deficient animals in order to allow the simultaneous investigation of chemotherapy drug activity on cancer of human origin and PN. Moreover, this study can be the starting point to investigate in the same cancer-bearing animals, the neuroprotective or analgesic effects of new experimental compounds and their non-interference with the antineoplastic activity of chemotherapy.

## Materials and methods

### *Animals*

Female athymic nude-Foxn1$^{nu}$ mice (19–21 g, 4 weeks of age on arrival, Harlan, San Pietro al Natisone, Italy) were used in the study.

Animals were housed in a dedicated room in individually ventilated cages (IVC, TECNIPLAST S.p.a., Varese, Italy) where temperature and relative humidity were set at 22 +/− 2 °C and 50 +/− 10%, respectively. Artificial lighting provided a 24-hour cycle of 12 hour light/12 hour dark (light 7 a.m.–7 p.m.) and food and water were available ad libitum. The animal care and husbandry were in compliance with Italian (D.L.vo 116/1992 and subsequent modifications) and International policies and laws (EEC Council Directive 86/609, OJ L 358, 1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, US National Research Council, 8th ed., 2011). The International Association for the Study of Pain (IASP) guidelines for the investigation of pain in animals were followed. The Ethics Committee for Animal Studies of the University of Milan-Bicocca approved all the experiments. The mice were euthanized at the end of the experimental period under deep isoflurane-induced anesthesia.

### *Anesthesia*

For the neurophysiological recordings, anesthesia was induced in a chamber with 3% isoflurane carried in oxygen followed by 1–1.5% isoflurane through a nose cone for maintenance throughout the procedures. This was adequate to suppress the corneal blink response and any withdrawal response to a noxious stimulus. The body temperature was maintained at ~37 °C using a heating pad (Homoeothermic System, Harvard Apparatus, Holliston, MA) to minimize isoflurane-induced hypothermia.

### *Drugs*

Cisplatin (Pfizer, Nerviano, Italy), paclitaxel (LC Laboratories, Woburn, MA) and bortezomib (LC Laboratories, Woburn, MA) were prepared immediately before each administration. Cisplatin was dissolved in sterile saline solution and intraperitoneally (ip) administered. Paclitaxel and bortezomib were dissolved in a solution composed by ethanol 100%/tween 80/saline (5%/5%/90%) and intravenously (iv) administered via the caudal vein. This solvent was previously tested to ensure the absence of any neurotoxicity (Persohn et al., 2005).

### *Experimental design*

Drug doses and frequencies of administration were chosen on the basis of our previously published data (Carozzi et al., 2010b), oncology studies adapted to our experimental plan and pilot studies (data not shown). As shown in Table 1, forty-eight mice were used and randomized in 4 groups of 12 animals each. One group of animals was treated with paclitaxel 80 mg/kg/weekly for 4 weeks and one group was treated with bortezomib 0.8 mg/kg twice/weekly for 4 weeks. At the end of the fourth week of paclitaxel and bortezomib administration, 4 animals/group were sacrificed for biological sampling while the remaining animals received the drugs for other 2 weeks (for a total of 6 weeks). One group of mice was treated with cisplatin 4 mg/kg twice/weekly for 4 weeks. Three days after the last cisplatin administration, 4 animals were sacrificed for biological sampling while the remaining mice underwent 2 weeks of follow-up. Based on our previous observations, cisplatin treatment was limited to 4 weeks when animals reach the maximal tolerated cumulative dose. A group of 12 healthy control animals was left untreated (naïve). Four of these mice were sacrificed at week 4, while the remaining animals were followed-up to the end of week 6. All the drugs were administered as 10 ml/kg solutions.

In order to investigate the peripheral nervous system functional damage and the onset of neuropathic pain, all mice were tested at baseline, week 4 and week 6 for the caudal and digital nerve conduction studies (NCV) and for dynamic and plantar tests. At the end of weeks 4 and 6 DRG and peripheral nerves obtained from sacrificed animals were employed for light microscopy and morphometrical analysis.

### *General toxicity*

Mice were examined every day for sickness symptoms due to drug treatment. Changes in their appearance (e.g., kyphosis and altered grooming), behavior (altered nesting) and activity (altered exploring) were monitored. Body weight was recorded twice weekly for general toxicity assessment and drug dose adjustment.

### *Peripheral neurotoxicity*

#### *Neurophysiological analysis of the peripheral nerves*

The nerve conduction velocities (NCVs) and maximal action potential amplitudes (AMPs) were measured stimulating the caudal and the digital nerves as previously reported (Carozzi et al., 2010b, 2013; Renn et al., 2011). Briefly, using an electromyography apparatus (Myto2 ABN Neuro, Firenze, Italy) the caudal NCV and AMP were recorded by placing a couple of needle recording electrodes (cathode and anode) at the base of the tail and a couple of stimulating electrodes 3.5 cm far from the recording points. Similarly, the digital NCV and AMP were measured by placing the recording electrodes at the ankle bone and the stimulating electrodes close to the fourth toe near the digital nerve. The NCVs were calculated as a ratio between the latency between the stimulus artifact and the onset of the first peak of the elicited action potential and the distance between the recording and the stimulating points. The corresponding maximal AMP was recorded. Ten responses were averaged for each recording. All the neurophysiological measures were obtained under standard conditions in temperature/humidity controlled rooms. The animals were maintained under deep nose-cone isoflurane anesthesia and their body temperature was continuously monitored during the recordings. The baseline recordings were measured before starting the drug treatments in order to randomize animals into homogeneous groups.