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Myelin structure is unaltered in chemotherapy-induced peripheral neuropathy

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ABSTRACT

Purpose: Alterations in mRNA for myelin proteins are reported in animal models of chemotherapy-induced peripheral neuropathies (CIPN); however, ultrastructural changes in aldehyde-fixed and plastic-embedded myelin are not evident by electron microscopy. Therefore, we used X-ray diffraction (XRD) to investigate more subtle changes in myelin sheath structure from unfixed nerves.
Experimental design: We used *in vivo* chronic animal models of CIPN in female Wistar rats, administering cisplatin (CDDP 2 mg/kg, i.p. twice/week), paclitaxel (PT 10 mg/kg, i.v. once/week) or bortezomib (0.20 mg/kg, i.v. three times/week) over a total period of 4 weeks. Animal weights were monitored, and tail nerve conduction velocity (NCV) was determined at the end of the treatments to assess the occurrence of peripheral neuropathy. Sciatic nerves were collected and the myelin structure was analyzed using electron microscopy (EM) and XRD.
Results: All the rats treated with the chemotherapy agents developed peripheral neuropathy, as indicated by a decrease in NCV values; however, light and electron microscopy indicated no severe pathological alterations of the myelin morphology. XRD also did not demonstrate significant differences between sciatic nerves in treated vs. control rats with respect to myelin period, relative amount of myelin, membrane structure, and regularity of membrane packing.
Conclusions: These results indicate that experimental peripheral neuropathy caused by CDDP, PT, and bortezomib—which are among the most widely used chemotherapy agents—does not significantly affect the structure of internodal myelin in peripheral nerve.

1. Introduction

Myelin is a lipid- and protein-rich membrane that insulates axons, thereby increasing conduction velocity of nerve impulses. During maturation, myelin becomes compacted by the close appositioning of membranes giving rise to a periodic ultrastructure (Agrawal et al., 2009). Changes in myelin sheath structure due to genetic defects (Auer-Grumbach, 2004; Carter et al., 2008), metabolic disorders (Tracy and Dyck, 2008), infections (leprosy, HIV), inflammation (GBS-CIDP), or toxic agents (Windebank and Grisold, 2008) can result in peripheral nerve damage.

Chemotherapy-induced peripheral neuropathy (CIPN) is a dose-limiting side effect of anticancer therapy that significantly compromises the quality of life of cancer patients. The usual slow recovery of peripheral nerve from damage does not always occur in CIPN, where damage can persist (Peltier and Russell, 2002). Despite knowledge about the anticancer mechanisms of action of cisplatin (CDDP), paclitaxel (PT), and bortezomib (BOR), the detailed mechanisms in peripheral neurotoxicities are not yet completely elucidated. In clinical practice all these drugs induce primarily an axonopathy, as deduced almost exclusively from neurophysiological data; however, morphological/ultrastructural observations on human biopsies are lacking.

In an experimental animal model of CIPN, there are little or no microscopically detectable effects on myelin structure, however subtle alterations of myelin protein synthesis and composition (Magnaghi et al., 2008; Roglio et al., 2008, 2009; Kawashima et al., 2007; Wrabetz et al., 2000) and genetic mutations leading to amino acid substitutions in myelin proteins (Avila et al., 2010)

Abbreviations: Å, Angstrom; BOR, bortezomid (velcade); CDDP, Cisplatin; CIDP, chronic inflammatory demyelinating polyneuropathy; CIPN, chemotherapy-induced peripheral neuropathy; Ctrl, control; DRG, dorsal root ganglia; EM, electron microscopy; FDA, United States Food and Drug Administration; GBS, Guillain-Barré Syndrome; HIV, human immunodeficiency virus; NCV, nerve conduction velocity; PMP22, peripheral myelin protein-22; PNS, peripheral nervous system; PT, Paclitaxel; XRD, X-ray diffraction.

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could lead to myelin instability and demyelination. To assess whether changes resulting from treatment with antineoplastic agents could affect internodal myelin structure, we used X-ray diffraction (XRD) to examine the amount of myelin, its periodicity and membrane packing, and bilayer structure in an experimental animal model. We found that neither CDDP, PT, nor bortezomib produced significant alteration in internodal myelin structure.

2. Materials and methods

2.1. In vivo models

A total of 40 adult (200–220 g at the beginning of the study) female Wistar rats (Charles River Laboratories, Calco, Italy) were used for the study. The care and husbandry of animals were in conformity with the institutional guidelines and in compliance with national (D.L. n. 116, *Gazzetta Ufficiale della Repubblica Italiana*, suppl. 40, February 18, 1992) and international laws and policies (EEC Council Directive 86/609, OJ L 358, 1, December 12, 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996). The experimental plan was approved by the Institutional Animal Care and Use Committee (IACUC) of Boston College. Animals were housed in a limited access animal facility where room temperature and relative humidity were set at 22 ± 2 °C and $55 \pm 10\%$, respectively. Artificial lighting provided a 24-h cycle of 12 h light/12 h dark.

CDDP (Sigma–Aldrich, St. Louis, MO), dissolved in saline solution at a concentration of 2 mg/kg (i.e., 2 mg/ml) was injected i.p. twice/week. PT powder (Sigma–Aldrich, St. Louis, MO) at 10 mg/kg (i.e., 10 mg/ml) was administered i.v. once/week. As PT powder is difficult to dissolve, we used a saline solution of 5% ethanol/5% Tween 80. Bortezomib (LC Laboratories, Woburn, MA 01801) was prepared in saline solution at a final concentration of 0.20 mg/kg (i.e., 0.20 mg/ml) and injected i.v three times/week. All the drugs were administered for four weeks. The general condition of the animals was assessed daily and body weight was recorded before each drug administration. There were ten animals in each regimen.

2.2. Neurophysiology

At the end of the treatment, NCV of tail nerve was determined for each animal as previously described (Cavaletti et al., 1997, 1998, 2000a,b; Pisano et al., 2003; Tredici et al., 1999). Briefly, NCV was assessed by placing recording ring electrodes around the tail distally, 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 two sites after nerve stimulation were determined (peak-to-peak) and nerve conduction velocity was calculated accordingly. All the neurophysiologic determinations were performed under standard conditions in a temperature-controlled room.

2.3. Morphology and structure

At the end of the 4-week treatment period, animals were sacrificed under ether anesthesia by means of cervical dislocation; both sciatic nerves from each animal were collected and used for light and electron microscopy and for XRD. Optic nerves were used as internal controls to assess any nerve damage caused by dissecting and handling of samples.

2.3.1. X-ray diffraction (XRD)

During dissection both sciatic and optic nerves were continually rinsed with saline solution (154 mM NaCl, 5 mM Tris-buffer, pH 7.4), and the nerves were tied off at both ends with fine silk suture. For diffraction, the sciatic nerve from the right side was slightly stretched and inserted into a 1.0-mm capillary tube (Charles Supper Co., Natick, MA) with medium, and the capillary was sealed at both ends with wax; for optic nerves, 0.7-mm capillary tubes were used. The left sciatic nerve from each animal was fixed by immersion in 3% glutaraldehyde solution in 0.12 M phosphate buffer at pH 7.4; one-half of this nerve was used for XRD (as described above), while the other half was used for microscopy.

XRD patterns were obtained using nickel-filtered, single-mirror focused Cu K_α radiation from a fine-line source on a 3.0 kW Rigaku X-ray generator (Rigaku/MSC Inc., The Woodlands, TX) operated at 40 kV by 14–22 mA. Each diffracting pattern was recorded for 30 min for the fresh samples and 1 h for the fixed ones, using a linear position-sensitive detector (Molecular Metrology, Inc., Northampton, MA) as previously described (Avila et al., 2005). The patterns were analyzed with the program PeakFit (Jandel Scientific, Inc., San Rafael, CA). The myelin periodicity (d) was determined from the position of the reflections, the relative amount of multilamellar myelin for each sample was obtained by calculating the total integrated intensities (M) above background (B) and expressed as $M/M + B$, and the relative disorder in the stacking of membranes in multilamellar myelin was determined by plotting w^2 vs. h^4, where w is the integral width of Bragg order h (see Avila et al., 2005 for details). The slope of this linear plot is proportional to the fluctuation in period d (Inouye et al., 1989).

2.3.2. Light and electron microscopy

Fixed optic and sciatic nerves were processed for resin embedding (Fluka, Sigma–Aldrich, Saint-Louis, MO) as described in previous studies (Cavaletti et al., 1992, 1997, 1998, 2007; Cece et al., 1995; Persohn et al., 2005). Semi-thin sections were prepared from at least two tissue blocks for each animal. The sections were stained with toluidine blue and examined with a Nikon Coolscope light microscope (Nikon Instruments, Inc.). Based on the light microscopic findings, ultrathin sections were obtained from selected tissue blocks using a Reichert-Jung Ultracut E (Leica, Vienna, Austria), counterstained with uranyl acetate and lead citrate, and examined with a Philips CM-10 transmission electron microscope. For each treatment as well as for controls, 3 animals were evaluated by light and electron microscopy.

2.4. Statistical analysis

Of the ten animals in each regimen, some were excluded from further analysis: one control exhibited an extreme weight gain; and a number of the treated animals (two CDDP, six PT, two BOR) did not show any toxic effects. All the data collected were analyzed by ANOVA and a Tukey–Kramer post-test (significant value set at $p < 0.05$).

3. Results

3.1. Animals developed CIPN

No mortality was present in any of the animal groups. PT- and BOR-treated rats did not show changes in weight gains, while rats receiving CDDP showed a significant reduction in weight gain vs. untreated rats, suggesting the onset of a systemic toxic effect of CDDP (data not shown). Despite the variable response among individuals after drug administration, mean NCV values decreased significantly in all the treated groups (Fig. 1, $p < 0.001$ vs. Ctrl), thus confirming the onset of CIPN in the treated rats.