Paclitaxel-Resistant Neurons in Dorsal Root Ganglia

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Abstract

Paclitaxel is an antineoplastic drug, and its usage is often limited by severe peripheral polyneuropathy characterized by symptoms in remote extremities. It is suggested that subpopulations of dorsal root ganglion (DRG) neurons are impacted functionally and/or structurally. In this study, the neurotoxic effect of this drug in cultured rat DRG neurons was analyzed by measuring their neurite length and electrophysiological properties. It was observed that exposure of the cultured DRG neurons to 3, 10 and 30 µM paclitaxel for 24 or 48h caused a dose-dependent shortening of length or loss of neurite, typically in the “dying back” pattern. Eight of 28 large DRG neuron populations with extensive neurites showed less sensitivity or were entirely resistant to paclitaxel treatment. Whole-cell patch clamp experiments revealed that these paclitaxel-resistant neurons may fall in the category of A δ or A β neurons. Deeper understanding as to why this particular subclass of neurons is resistant could reveal novel approaches to protect the primary sensory neurons from chemotherapy agent-induced toxicity and screening methods to identify chemical agents that will not induce peripheral neuropathy in cancer patients.

Keywords: Taxol; Wistar Han Rat; Chemotherapy-Induced Peripheral Neuropathy (CIPN); Dorsal Root Ganglion

Introduction

Paclitaxel (Taxol) is a widely prescribed chemotherapeutic agent that binds to β-tubulin and stabilizes its polymerization [1,2]. This is a critical process for the dynamics of microtubules, which are a key component of the structure of the neuronal soma and axon [3,4]. Paclitaxel is one of the most effective FDA-approved chemotherapeutics for the treatment of several solid tumors including metastatic breast, ovarian, prostate and non-small cell lung cancer [5]. However, its use is often associated with the generation of length-dependent axonal polyneuropathy expressed as sensory abnormalities and debilitating neuropathic pain in humans and animal models with less prominent motor involvement [6-10]. Patients develop unique localized pain in their distal extremities, referred to as the “stocking-glove” pattern, and often complain of numbness and tingling in the painful area [11]. The incidence of paclitaxel-induced neuropathy is reported to be 30 - 50% following a single dose, increasing to > 50% after a second dose [12]. This side effect in human is often dose-dependent [13] and reversible in both human [14] and rodent models [15].

Paclitaxel-induced malfunction in tubulin dynamics hampers axonal transport, leading to axonal degradation and axonopathy in sensory neurons [1,6,17]. Although paclitaxel can affect all sensory modalities, large fibers are probably the most affected [11,13,18]. Large DRG neurons with A-fiber axons are proposed to be particularly impacted by paclitaxel. For example, in in vivo rodent studies, mechanical allodynia and A-fiber hypersensitization were observed following a single treatment of paclitaxel [19,20]. Furthermore, after paclitaxel administration, an increase in caspase-3 immunoreactivity in NF200 colocalized myelinated neurons was observed [21]. In other rat studies following paclitaxel treatment, however, TRPV1 mRNA was up-regulated [22] and high voltage-dependent calcium channel conductance was increased in small or medium sized DRG neurons [23]. Using paclitaxel-treated rats, Xiao and Bennett recorded significantly increased spontaneous discharges in both A-fibers and C-fibers of the sural nerve [24]. They also observed an increase in the incidence of swollen and vacuolated mitochondria in the myelinated and unmyelinated sensory axons of the dorsal root [25]. Additionally, in post-
mitotic non-dividing cells, such as DRG neurons, paclitaxel-induced tubulin stabilization is thought to interfere with axonal transport, causing peripheral neuropathy [2]. However, a previous study showed that the highest concentration of paclitaxel was found in the DRG and not in the peripheral nerves [26]. Results from a study involving human nerve biopsy after treatment with paclitaxel revealed findings also supporting ganglionopathy rather than axonopathy as the most likely pathogenetic mechanism associated with taxane-induced peripheral neuropathy [27].

The clinical observations of the particular sensory symptoms in both human patients and experimental animals led us to hypothesize that paclitaxel damages subpopulations of peripheral sensory neurons differently. Studies of the different subpopulations of DRG neurons are essential for understanding the mechanism of neurotoxicity induced by paclitaxel. In the current study, we took a primary cell culture approach, which allowed us to observe the neurite damage and the electrophysiological properties of somata. We compared the responses of differently-sized primary sensory neurons to paclitaxel treatment in culture and also characterized the electrophysiological properties of these neurons. We showed that paclitaxel treatment caused significant retraction of neurites in small-sized DRG neurons but less reduction in the neurites of the larger neurons. Some larger neuron subpopulations with broader action potentials were resistant to paclitaxel treatment altogether for up to 48 hours in culture.

Materials and Methods

All experimental procedures regarding animal use in this study conformed to the NIH Guide for the Care and Use of Laboratory Animals and the guidelines of the Pfizer Institutional Animal Care and Use Committee (IACUC). Efforts were made to minimize animal suffering and to reduce the number of animals used.

Cell culture and treatment

Lumbar 4 and 5 DRGs, in which the primary sensory neurons innervate the leg via long axons, were harvested from adult Wistar Han rats (200 - 250g) and dissociated enzymatically and mechanically as described previously [28]. Briefly, after light anesthesia with 2.5% isoflurane, rats were rapidly decapitated and the vertebral column was exposed by dorsal laminectomy. The bilateral lumbar ganglia (L4 and L5) were excised, treated with collagenase A (1 mg/ml, Roche Diagnostics GmbH, Germany) for 19 minutes and collagenase D (1 mg/ml, Roche Diagnostics GmbH) with trypsin (0.4 mg/ml, Sigma-Aldrich) for 20 minutes, dissociated in L-15 culture media (ThermoFisher Scientific, Waltham, MA) with 10% heat activated horse serum (ThermoFisher Scientific) and 5% fetal bovine serum (ThermoFisher Scientific) and plated on glass cover slips (Warner Instrument Corp., Hamden, CT) coated overnight with poly-D-lysine (100 µg/mL, Cultrex®) and laminin (20 µg/mL, 3 - 4h, ThermoFisher Scientific) [29]. The dissociated neurons were then maintained in a 5% CO₂-95% O₂ incubator at 37°C for 30 minutes - 1h, allowing cells to adhere [30]. Then, the coverslips were flooded with DRG growth media (15% DMEM (ThermoFisher Scientific) and 85% Sam’s F-14 media (EuroBio Inc, Les Ulis cedex B, France) supplemented with penicillin, streptomycin, nerve growth factor (murine 2.5S NGF, 10 ng/mL, ThermoFisher Scientific) and 10% horse serum to promote neurite growth [31]. NGF concentration in the media was carefully chosen, because high concentration of NGF has been found to be neuroprotective for DRG neurons [32]. After 24h, the cell culture was incubated with vehicle or paclitaxel at various concentrations. Paclitaxel (Sigma-Aldrich, St. Louis, MO) was dissolved in vehicle (cremophor EL/ethanol, 50/50, v/v, Sigma-Aldrich) to make a stock concentration of 100 mM and was then diluted with culture media to obtain the different working concentrations (3, 10 and 30 µM). The maximal concentration of vehicle was below 0.05%.

Neuron and dendrite measurement

After 24h in culture, half of the coverslips carrying DRG neurons were treated with paclitaxel (3, 10, or 30 µM [33]; the other half of the neurons from the same cell suspension were treated with the vehicle and were used as a control [33]. Low power (10 × magnification) phase contrast light micrographs were obtained after 24h and 48h and cell diameter and neurite length were measured using a Zeiss inverted microscope (AXIO Observer. A1) and AxioVison LE64 software (Zeiss, Germany). For each neuron, at least 3 primary neurites were identified as neurite processes that arose from the ganglion cell soma and extended a distance greater than the diameter of the cell soma.
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The average length of the neurites of the neurons were measured and analyzed using the measuring features of AxioVision LE. Three diameter ranges of neuron soma (small [20 - 27 µm], medium [33 - 38 µm], and large [45 - 51 µm]) were selected and analyzed based on the literature [34,35].

**Whole-cell patch clamp recordings**

Whole cell patch-clamp recordings were obtained 24 hours after culture as reported in our previous paper [36]. Briefly, the cultured neurons on glass cover slips were placed in a recording chamber on the stage of an inverted microscope and continuously superfused with a modified Krebs’ solution [composition (in mM): 124 NaCl, 26 NaHCO₃, 3 KCl, 1.3 NaH₂PO₄, 2 MgCl₂, 2 CaCl₂, and 10 dextrose, pH 7.4; osmolarity, 305 - 315 mOsm] at room temperature (~23°C) and bubbled continuously with 95% O₂-5% CO₂ using a flow rate of 0.5 - 1 ml/min [37]. Micropipettes were pulled from borosilicate glass (World Precision Instruments, Sarasota, FL) with a P97 micropipette puller (Sutter Instrument, San Rafael, CA) and polished with a microforge (Narishige, Tokyo). Electrode resistances ranged from 2 to 6 MΩ. The pipette solution contained (in mM): 140 KCl, 2 MgCl₂, 1 CaCl₂, 11 EGTA, 2 Mg-ATP, and 10 HEPES; pH 7.3; osmolarity, 300 - 310 mOsm. Tight seals of 1-5 GΩ were established in the cell-attached configuration. The whole-cell configuration was established by giving a further brief pulse of suction, having previously compensated capacitive transients with a Multiclamp 700B patch-clamp amplifier (Molecular Devices, Sunnyvale, CA). Once a whole-cell configuration was established, the voltage-clamp mode was changed to current-clamp mode. The voltage signals were filtered at 10 kHz and acquired at 50 kHz using Clampex 10 software (Molecular Devices). The Digidata 1322A interface (Molecular Devices) was used for A-D conversion.

Action potentials were elicited from resting potential ($V_r$) levels by delivering depolarizing step pulses of 20-40 ms duration generated by Clampex 10. Neurons were examined in order, as patched, but we accepted only those exhibiting $V_r$ more negative than -45 mV and with an overshooting action potential. The following parameters were measured: spike amplitude, measured from the baseline $V_r$ to the positive peak of the spike; spike duration, measured at 50% of the amplitude (APD50); afterhyperpolarization (AHP) amplitude, measured from the baseline; and AHP duration measured at 75% decay [38].

**Statistical analysis**

Statistical evaluation of the differences in neurite length and cellular electrophysiological properties was performed using one-way analysis of variance (ANOVA) and Tukey-Kramer post-test with GraphPad Prism 6 (GraphPad Software, La Jolla, CA). P < 0.05 was considered as statistically significant.

**Results**

**Description of the cultured DRG neurons and neurites 24 hours after dissociation**

Immediately after dissociation, the cultures consisted of single cells and degenerating fragments of myelinated nerve fibers. The majority of the neurons had a smooth, round outline and occasionally had a short axon stump under phase contrast microscopy, making them readily distinguishable from the much smaller glial cells and other non-neuronal cells. To promote the survival and neurite outgrowth of the neurons isolated from adult animals, we plated the dissociated neurons on glass coverslip coated with higher concentration of poly-D-lysine and laminin. As expected, at 24h, the myelin fragments disintegrated and the resulting fine debris were gradually removed after feeding. At this time, many neurons exhibited 3 - 5 neurites with variable lengths (Figure 1A, Table 1). Some had straight neurites as described by Scott [39]; while others had more abundant neurites in an interconnected network formation (Figure 1A, right). The cell culture at this time point was used as the baseline and the paclitaxel and vehicle treatments were started.
Table 1: Baseline neurite length of selected different sized DRG neurons at 24 hours in culture.

<table>
<thead>
<tr>
<th></th>
<th>Small</th>
<th>Medium</th>
<th>Large</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soma diameter range (µm)</td>
<td>20.0 - 27.0</td>
<td>33.0 - 38.0</td>
<td>45.0 - 51.0</td>
</tr>
<tr>
<td>Mean soma diameter (µm)a</td>
<td>24.3 ± 1.8</td>
<td>35.3 ± 1.7</td>
<td>50.6 ± 4.9</td>
</tr>
<tr>
<td>Mean neurite length (µm)a</td>
<td>171.4 ± 67.6</td>
<td>216.5 ± 81.5</td>
<td>192.9 ± 64.0</td>
</tr>
<tr>
<td>N</td>
<td>45</td>
<td>40</td>
<td>44</td>
</tr>
</tbody>
</table>

a: All values are means ± SD

Figure 1: (A) Typical images of large (L), medium (M) and small (S) DRG neurons with neurites cultured in a growth media for 24 hours. Note the differences in the neurite length and soma shape among three classes of DRG neurons. (B) A representative neuron cultured with 10 µM paclitaxel for 48 hours. Note the characteristic endings of a neurite (arrow) seen often after incubation with paclitaxel.
Paclitaxel inhibits neurite outgrowth in three differently-sized DRG neurons

Exposure of the cultured rat DRG neurons to different concentrations of paclitaxel for 24 or 48h caused a dose-dependent shortening of length or loss of neurite, typically in the “dying back” pattern as was proved by a time-lapse imaging experiment (data not shown). The neurites of the medium and small-sized DRG neurons tested with vehicle were stabilized in their lengths at 48h compared to 24h (P > 0.05, Figure 2, left clusters in B vs. E and C vs. F), while the neurite length was significantly decreased only in the large sized group (P < 0.05, Figure 2 left clusters in A vs. D). After paclitaxel treatment, many neurites exhibited small swelling or baseball bat-like endings at their tips (Figure 1B). The maximal reduction was obtained with 30 µM paclitaxel (Figure 2, right columns in each panel). Surprisingly, at 48 h, small neurons were more affected than the larger-sized DRG neurons (P < 0.001. Figure 3D-F, right columns).

Figure 2: Paclitaxel-induced neural toxicity in DRG neurons. Paclitaxel causes shrinkage of neurite of large (A and D), medium (B and E), and small sized DRG neurons (C and F) at 24 hour (A-C) and 48 hour (D-F) at different concentrations. The reduction of neurites in all three size groups was more evident after 48 hour of paclitaxel treatment. Note some large neurons were resistant to paclitaxel treatment with little changes in their neurite length (outliers in A and D). * P < 0.05; ** P < 0.01; *** P < 0.001, One-way ANOVA, compared with the corresponding vehicle groups.

Citation: Chang-Ning Liu, et al. “Paclitaxel-Resistant Neurons in Dorsal Root Ganglia”. EC Pharmacology and Toxicology 6.3 (2018): 128-140.
A subpopulation of large-sized DRG neurons is resistant to paclitaxel

Following 48 hours of incubation with vehicle, the average neurite length of the large DRG neurons was 161.6 ± 49.5 µm (n = 9) and ranged from 119.1 to 280.4 µm (Figure 2D and Figure 4C, both left columns). Of the 28 large neurons incubated with 10 or 30 µM paclitaxel (Figure 2D, right 2 columns), 10 neurons had average neurite length longer than 119 µm, while other 18 neurons had average neurite length shorter than 119 µm. Among the 10 neurons with average neurite length > 119 µm, 7 (70%) neurons were characterized by polygonal soma and extensive neurite network (Figure 4B). Such neurons had more primary neurites, which were thicker, especially at the junctions with the soma. Of the 18 neurons with average neurite length < 119 µm, 17 (94%) were those with round somas (Figure 4A, Table 2). This distribution is statistically significant (P < 0.001, Chi-square test). In other words, the average length of neurites of the polygonal neurons (159.4 ± 44.4, n = 8) was significantly longer than that of the round neurons (65.0 ± 52.4, n = 20, P < 0.001, Figure 4C) following 48 hours of paclitaxel exposure. Only the round-shaped neurons show significant shortening of neurite compared with those treated with vehicle (P < 0.01, ANOVA, Figure 4C). These results indicate that paclitaxel mainly impact the round-shaped neurons while the majority of the polygonal neurons were less impacted or resistant to it.

Citation: Chang-Ning Liu, et al. “Paclitaxel-Resistant Neurons in Dorsal Root Ganglia”. EC Pharmacology and Toxicology 6.3 (2018): 128-140.
Figure 4: Typical images of polygonal-shaped large DRG neurons (B, arrows) and a regular round-shaped large DRG neuron (A, arrow) captured after 48 hours incubation with 10 µM paclitaxel. (C) At 48 hours cultured with paclitaxel, the polygonal-shaped neurons (n = 8) had statistically significant (P < 0.01) longer neurite than the regular round-shaped neurons (n = 22). Only the round-shaped neurons show statistically significantly shorter neurites compared to the vehicle-treated neurons (P < 0.01). (D) shows a typical action potential waveform elicited by a intracellular current injection-induced depolarization in a polygonal DRG neuron. Usually there was a hump on the failing phase of the action potential.

Following 48 hours of incubation with vehicle, the average neurite length of the mid-sized DRG neurons was 157.7 ± 36.8 µm (n = 11) and ranged from 107.7 to 247.5 µm (Figure 2E, left columns). Of the 22 mid-sized neurons incubated with 10 or 30 µM paclitaxel (Figure 2E, right 2 columns), 5 neurons had average neurite length longer than 108 µm, while other 17 neurons had average neurite length shorter than 108 µm. All the 22 neurons were characterized by round soma and regular or sparse neurite network and no polygonal neu-
rons were observed in this category (Table 2). The average neurite length of the small-sized DRG neurons was $150.0 \pm 37.3 \, \mu m$ ($n = 13$) and ranged from 105.9 to 193.3 µm (Figure 2F, left columns). Of the 26 small-sized neurons incubated with 10 or 30 µM paclitaxel (Figure 2F, right 2 columns), only 4 neurons had average neurite length longer than 106 µm and no polygonal neurons were noticed in this size category, either (Table 2).

<table>
<thead>
<tr>
<th>Neurite length (µm)</th>
<th>&lt; 119</th>
<th>&gt; 119</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of neurons with round soma</td>
<td>17</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Number of neurons with polygonal soma</td>
<td>1</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>10</td>
<td>28</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Neurite length (µm)</th>
<th>&lt; 108</th>
<th>&gt; 108</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of neurons with round soma</td>
<td>17</td>
<td>5</td>
<td>22</td>
</tr>
<tr>
<td>Number of neurons with polygonal soma</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>5</td>
<td>22</td>
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</table>

<table>
<thead>
<tr>
<th>Neurite length (µm)</th>
<th>&lt; 106</th>
<th>&gt; 106</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of neurons with round soma</td>
<td>22</td>
<td>4</td>
<td>26</td>
</tr>
<tr>
<td>Number of neurons with polygonal soma</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>4</td>
<td>26</td>
</tr>
</tbody>
</table>

**Table 2:** Count of the DRG neurons following 48 hours of incubation with 10 or 30 µM paclitaxel.

**Electrophysiological properties of the paclitaxel-resistant neurons**

To identify which subpopulation the paclitaxel-resistant neurons belong to, we measured action potentials by whole-cell patch clamp technique in current mode on the neurons larger than 45 µm in diameters (lower end of large-sized neurons) at 24 hours in culture. Compared with the round shaped large neurons, the polygonal neurons usually had more negative resting potentials (-66.5 ± 4.4 mV vs. -55.1 ± 7.6 mV, $P < 0.001$) as well as AHP (-13.7 ± 7.0 mV vs. -7.4 ± 4.6 mV, $P < 0.05$) and the vast majority (91%) had humps on the falling phase of action potentials. Humps, along with larger AHP, have been previously reported to be present more in $\alpha_3$ and $\alpha_4$ types of neurons in rat DRG [40]. Table 4 lists the electrophysiological properties of the neurons and figure 4D shows a representative action potential waveform obtained from this type of neuron. No spontaneous action potential was observed in any of the neurons.

<table>
<thead>
<tr>
<th>Neuron type</th>
<th>Neuron Number (%)</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of neurons with round soma</td>
<td>35(80%)</td>
<td>20 (71%)</td>
<td></td>
</tr>
<tr>
<td>Number of neurons with polygonal soma</td>
<td>9(20%)</td>
<td>8 (29%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>28</td>
<td></td>
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</table>

**Table 3:** Distribution of round and polygonal neurons before and after 10 or 30 µM paclitaxel treatment.

$a$: $P > 0.05$, Chi-square test compared with those before treatment

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<table>
<thead>
<tr>
<th></th>
<th>Resting membrane potential, (-mV)</th>
<th>Action Potential</th>
<th>AHP</th>
<th>Single spike threshold (nA)</th>
<th>Multiple spikes, (%)</th>
<th>Hump (0/0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polygonal- shaped</td>
<td>66.5 ± 4.4 (11)</td>
<td>80.0 ± 21.7 (11)</td>
<td>28 ± 1.6 (11)</td>
<td>13.7 ± 7.0 (11)</td>
<td>9.6 ± 3.3 (11)</td>
<td>5.9 ± 3.2 (11)</td>
</tr>
<tr>
<td>(paclitaxol- resistant) neurons</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Round- shaped</td>
<td>55.1 ± 7.6 (8)</td>
<td>60.3 ± 21.7 (8)</td>
<td>5.0 ± 3.7 (8)</td>
<td>7.4 ± 4.6 (8)</td>
<td>7.7 ± 1.6 (8)</td>
<td>3.7 ± 3.3 (8)</td>
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<tr>
<td>(paclitaxel sensitive) neurons</td>
<td></td>
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Table 4: Properties of polygonal neurons and comparison with regular round-shaped neurons.

All values are means ± SD with n in parentheses; AHP: afterhyperpolarization; NS: P > 0.05, 2-tailed t-test

Discussion

Using an in vitro model of DRG neurons isolated from adult rats, we demonstrated that these primary sensory neurons respond differently to 3 - 30 µM paclitaxel with the small-sized neurons being readily affected, while a subpopulation of large neurons was relatively resistant to the paclitaxel treatment for up to 48 h at 30 um compared to the controls. Similar to the literature, distal axonal swelling [16] and die back [41,42] were observed in the majority of the neurons exposed to paclitaxel. Indeed, we use a higher concentration of paclitaxel than some published literature [32], because we tested on the neurons isolated from adult animals and plated on glass with a high concentration of neurite-promoting substrates, e.g. poly-D-lysine and laminin. The same concentration range up to 30 µM has also used by other investigators [43]. The soma size distributions were not compared between the vehicle-treated and paclitaxel-treated neurons, since no recognizable differences in number and diameter of A- or B-type DRG neurons were detected in an earlier rat paclitaxel study [44].

The different sensitivities of cultured DRG neurons to paclitaxel may be associated with the microtubule content in differently-sized or shaped neurons (round vs polygonal). Cytoplasmic microtubules are involved in the formation and maintenance of neuronal morphology and axonal transport [45,46]. It is likely that there are few microtubule contents in the small or round-shaped neurons. Thus, this group of neurons may be more susceptible to the microtubule dynamic instability caused by paclitaxel. However, the larger, particularly the polygonal-shaped neurons, may have rich microtubule content, and thus may be resistant to microtubule dynamic instability caused by paclitaxel. The ratio of the round- vs. polygonal-shaped neurons was not statistically significantly affected by paclitaxel treatment up to 48 h (3.9 vs. 2.5, Table 3) indicating that paclitaxel did not alter the shape of the neuronal somata in this experiment.

As in all cell culture work, one may argue about the paclitaxel concentration used in this study and its relevance to clinical exposure. Indeed, wide ranges of concentrations have been utilized by different investigators; Huizing, et al. [47] reported that the peak concentrations of paclitaxel in the blood after common infusion duration in ovarian cancer patients are 195 to 3650 ng/ml (228 nM - 4.3 µM). With 3-30 µM paclitaxel, we did observe clear neurites effects, which is consistent with an in vivo study in which the intraepidermal nerve fiber density was reported to be significantly reduced in the animals following paclitaxel treatment [48,49].

Another focus of this study was to determine what type of neurons are resistant to paclitaxel. Conventionally, the cell type [Aβ, Aδ and C type [40,50,51] or A and B type [52,53]], cell size, myelination, axon size, conduction velocity and type of sensory modality carried by the axon are usually used for DRG cell classification. Given the nature of our cell culture model, determination of the conduction velocity along the neurites is challenging. This model does provide an advantage distinguishing based on the morphology of neurite and shape of soma action potential and other electrophysiological properties obtained by single cell whole-cell patch clamp technique. For example, Villiere and McLachlan [54] intracellularly recorded the DRG neurons with a nerve attached at 35ºC. Based on their results, Aβ low threshold mechanoreceptive neurons had action potential duration that ranged from 0.5 - 2 ms and C neuron action potential that ranged from 1.25 - 4 ms. Rose and coworkers [55] examined action potentials induced by peripheral nerve stimulation from stomata in cat DRG and suggested that neurons that have broader action potentials, especially with a hump on the falling phase, appear to be soma of the Aδ or Ać high-threshold mechanoreceptors. Also, in in vivo pigeon DRG preparations, the humps found in the falling phase of action potentials appeared in slow conducting A-cells (Group III cells) [56]. In our current study, we recorded the action potential at room temperature.
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temperature and found that the majority of the paclitaxel-resistant neurons had action potentials with average APD50 of 2.8 ±1.6 ms. Given that all the paclitaxel-resistant neurons we selected were larger than 45 µm in their diameters, we believe they roughly fit in the Aβ or Aδ high-threshold mechanoreceptors or nociceptors. The paclitaxel-resistant neurons also showed more negative resting potential and AHP than the regular neurons, indicating there are more M-current or other Kv channel activities, such as sustained (K' current) or transient K+ (A type) current [57,58].

Physiologically, cold sensation is mediated by Aδ fiber and this may explain why paclitaxel-dosed patients experience cold hypersensitivity, but not warm hypersensation [11]. Pathophysiologically, compelling evidence from animal models suggests a role of Aβ fiber in mediating neuropathic pain. For example, in an axotomy model [59], and our spinal nerve ligation model [36,37], the affected large Aβ fiber connected DRG neurons showed enhanced excitability or spontaneous activities. In the streptozotocin model of diabetic neuropathy, single Aβ and Aδ fiber sensory axons in the tibial nerve developed ectopic discharges, higher spontaneous activity, and exhibited lower activation threshold to mechanical stimuli [60]. Our hypothesis is that in rats with paclitaxel-induced neuropathy, the paclitaxel-resistant Aδ or Aβ neurons may contribute to hyperalgesia, allodynia and dysesthesia.

A weakness of our investigation is the smaller cultured neuron sample size due to the technical challenge in dissociation and culture of DRG neurons from adult rats over 5 - 6 weeks of age, which usually produces low yield especially in larger DRG subpopulation. Neonatal or younger animals could provide much more neurons for testing and possibly a more powerful statistical analysis method [61], other than the Chi-Square test that was applied in this study. However, we believe that the use of older animals represent a better in vivo model to interrogate a process occurring in an older patient population.

Conclusion

We conclude that paclitaxel impacts the neurite length of the cultured DRG neurons differently. Small sized neurons are more readily affected by paclitaxel. Moreover, select large-sized DRG neurons, presumably Aβ or Aδ high-threshold neurons, are resistant to paclitaxel treatment up to 48 hours under our culture conditions. Further investigation of the resistant mechanism observed in this particular sub-class of DRG neurons could reveal a novel approach to protect sensory neurons from chemotherapy-induced neurotoxicity, form the basis for setting up a screening method for new chemotherapy agents, and ultimately prevent chemotherapy-induced peripheral neuropathy in cancer patients.

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