Oxaliplatin-induced neurotoxicity: changes in axonal excitability precede development of neuropathy

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Administration of oxaliplatin, a platinum-based chemotherapy used extensively in the treatment of colorectal cancer, is complicated by prominent dose-limiting neurotoxicity. Acute neurotoxicity develops following oxaliplatin infusion and resolves within days, while chronic neuropathy develops progressively with higher cumulative doses. To investigate the pathophysiology of oxaliplatin-induced neurotoxicity and neuropathy, clinical grading scales, nerve conduction studies and a total of 905 axonal excitability studies were undertaken in a cohort of 58 consecutive oxaliplatin-treated patients. Acutely following individual oxaliplatin infusions, significant changes were evident in both sensory and motor axons in recovery cycle parameters (P < 0.05), consistent with the development of a functional channelopathy of axonal sodium channels. Longitudinally across treatment (cumulative oxaliplatin dose 776 ± 46 mg/m²), progressive abnormalities developed in sensory axons (refractoriness P < 0.001; superexcitability P < 0.001; hyperpolarizing threshold electrotonus 90–100 ms P < 0.001), while motor axonal excitability remained unchanged (P > 0.05), consistent with the purely sensory symptoms of chronic oxaliplatin-induced neuropathy. Sensory abnormalities occurred prior to significant reduction in compound sensory amplitude and the development of neuropathy (P < 0.01). Sensory excitability abnormalities that developed during early treatment cycles (cumulative dose 294 ± 16 mg/m² oxaliplatin; P < 0.05) were able to predict final clinical outcome on an individual patient basis in 80% of patients. As such, sensory axonal excitability techniques may provide a means to identify pre-clinical oxaliplatin-induced nerve dysfunction prior to the onset of chronic neuropathy. Furthermore, patients with severe neurotoxicity at treatment completion demonstrated greater excitability changes (P < 0.05) than those left with mild or moderate neurotoxicity, suggesting that assessment of sensory excitability parameters may provide a sensitive biomarker of severity for oxaliplatin-induced neurotoxicity.

Keywords: oxaliplatin; neurotoxicity; neuropathy; excitability; channelopathy

Abbreviations: CMAP = compound motor action potential; CSAP = compound sensory action potential; FOLFOX = Treatment regimen including oxaliplatin, leucovorin and 5-fluorouracil; IQR = interquartile range; I/V = current–threshold relationship; NCI = National Cancer Institute; OSNS = Oxaliplatin-Specific Neurotoxicity Scale; RC = recovery cycle; TE = threshold electrotonus; TEh 90–100 ms = threshold electrotonus hyperpolarizing 90–100 ms; XELOX = Treatment regimen including oxaliplatin and capecitabine
Introduction

Colorectal cancer is highly prevalent worldwide, with more than a million cases diagnosed annually (Parkin et al., 2005). In recent years, standard chemotherapy for colorectal cancer has improved through the addition of newly developed cytotoxic therapies. Oxaliplatin, a third generation platinum-based chemotherapy, has demonstrated superior activity as first-line treatment in advanced colorectal cancer (de Gramont et al., 2000) and as adjuvant treatment (Andre et al., 2004), such that it now represents a central component of colorectal cancer treatment.

Platinum-based compounds have long been associated with neurotoxicity, with cisplatin producing a dose-dependent sensory neuropathy in 50%–90% of patients at cumulative doses >500 mg/m² (Mollman, 1990; LoMonaco et al., 1992). From early treatment trials, oxaliplatin-induced neurotoxicity was recognized as a prominent side effect and has now emerged as the major dose-limiting toxicity of treatment (Raymond et al., 1998; Gamelin et al., 2002; Grothey, 2003).

Oxaliplatin-induced neurotoxicity is unique among platinum analogues, producing a distinctive spectrum of symptoms in both acute and chronic presentations. Acute neurotoxicity develops immediately following infusion and is characterized by transient paraesthesia and muscular spasms in the limbs and perioral region (including jaw spasm), typically triggered or exacerbated by cold exposure (Gamelin et al., 2002; Grothey, 2003). Acute, transient symptoms occur in 95% of patients with evidence of peripheral nerve hyperexcitability on electromyography (Wilson et al., 2002). In contrast, chronic neurotoxicity produces predominately sensory dysfunction with distal paraesthesia progressing to sensory ataxia and functional impairment (Gamelin et al., 2002; Grothey, 2003). Severe neurotoxicity is strongly dose-dependent and occurs in 10%–20% of patients at cumulative doses of 750 mg/m² (Andre et al., 1999; Armand et al., 2000; de Gramont et al., 2000) and up to 50% of patients at higher doses (de Gramont et al., 2000). Symptoms may persist long-term (Krishnan et al., 2005; Pietrangeli et al., 2006; Land et al., 2007), representing a significant limitation to treatment, as end-organ neurotoxicity and neuropathy may require discontinuation of effective therapy. More critically, the development of neuropathy poses a problematic issue in the setting of adjuvant therapy, where long term neurotoxicity is an unacceptable outcome.

Axonal excitability techniques have recently been established to provide insight into the pathophysiological mechanisms underlying aberrant axonal function (Bostock et al., 1998; Kieman et al., 2000; Krishnan et al., 2008). While studies of acute oxaliplatin-induced neurotoxicity have provided in vivo evidence of alterations in voltage-gated sodium (Na⁺) channel function (Krishnan et al., 2005, 2006; Park et al., 2009) consistent with in vitro studies in a variety of experimental models (Adelsberger et al., 2000; Grolloea et al., 2001; Webster et al., 2005; Benoit et al., 2006), the relationship of these acute changes to the development of neuropathy has not been fully established. The present study describes the first cohort of oxaliplatin-treated patients to be prospectively assessed with axonal excitability techniques, both acutely following infusion and longitudinally across treatment, to provide insight into the pathophysiology and development of neurotoxicity and chronic neuropathy. As such, the present series of studies aimed to determine whether axonal excitability techniques may provide a sensitive biomarker for the early detection of oxaliplatin-induced nerve damage and thereby a means to identify at-risk patients prior to the development of irreversible neuropathy.

Materials and Methods

Patients

Clinical grading scales, nerve conduction studies and axonal excitability studies were undertaken in oxaliplatin-treated patients routinely referred from the Department of Medical Oncology, Prince of Wales Hospital. Patients were excluded from formal analysis if they had received prior neurotoxic chemotherapy treatment, or had a history or baseline neurophysiological evidence of peripheral neuropathy (see Results section). The study was approved by the South Eastern Sydney Area Health Service (Eastern Section) Human Research Ethics Committee and University of New South Wales Human Research Ethics Committee. Participants provided written informed consent in accordance with the declaration of Helsinki.

Treatment regimen

All patients received standard oxaliplatin-based treatment regimens: FOLFOX 4 (43% patients; de Gramont et al., 2000), FOLFOX 6 (48% patients; Mandard-Goebel et al., 1999) or XELOX (9% patients; Cassidy et al., 2004). In the FOLFOX regimens, oxaliplatin (FOLFOX 4: 85 mg/m² or FOLFOX 6: 100 mg/m²) was given intravenously over 2 h on day 1 every 2 weeks in conjunction with leucovorin (200 mg/m²) and followed by a 5-fluorouracil (5-FU) bolus injection (400 mg/m²). A continuous 24 h infusion of 5-FU (600 mg/m²) was given over days 1 and 2. On day 2, leucovorin (200 mg/m²; over 2 h) and 5-FU bolus (400 mg/m²) were given intravenously. The XELOX regimen included 130 mg/m² oxaliplatin given intravenously over 2 h every 3 weeks followed by oral capecitabine (1000 mg/m²) twice a day for 14 days. Treatment was continued for 6 months (12 bi-monthly cycles) or until maximal clinical benefit was reached or unacceptable toxicity developed. Treatment delays and dose reductions were managed according to standard clinical practice.

Assessment of neurotoxicity

Conventional clinical grading scales were utilized to assess neurotoxicity severity. The Neuropathy Sensory Subscale of the National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events Scale (Version 3) was utilized with the following grading system: Grade 1 (Mild)—loss of deep tendon reflexes or paraesthesia not interfering with function; Grade 2 (Moderate)—sensory alteration or paraesthesia interfering with function but not activities of daily living; Grade 3 (Severe)—sensory alteration or paraesthesia interfering with activities of daily living; and Grade 4—disabling (Trotti et al., 2003).

The Oxaliplatin-Specific Neurotoxicity Scale was utilized to specifically assess oxaliplatin-related symptoms with the following grades: Grade 1—dysesthesia or paraesthesia that completely regresses before the next cycle of therapy; Grade 2—dysesthesia or paraesthesia persisting between courses of therapy; and Grade 3—dysesthesia or paraesthesia causing functional impairment (Cassidy and Misset, 2002).
The Neuropathy Symptom Score was used to grade neuropathic symptom severity, with patients reporting both negative (subset IIa) and positive (subset IIb) sensory symptoms, summed to give a composite score out of five (Dyck et al., 1992), as utilized in previous studies of oxaliplatin-induced neurotoxicity (Krishnan et al., 2005).

Neurophysiological protocols

Standard nerve conduction studies of the tibial, sural and radial nerves were undertaken to obtain peak amplitude, conduction velocity and distal motor latency as appropriate (Kimura, 1983; Krishnan et al., 2005). A Medelec Synergy system (Oxford Instruments, Oxfordshire, UK) was used to record nerve conduction studies in patients at the commencement of oxaliplatin treatment and post-treatment.

Sensory and motor axonal excitability studies were undertaken on the median nerve. Axonal excitability studies were performed using an automated computerized system (Qtrac® Institute of Neurology, Queens Square, UK). Stimulation was computer controlled and converted to current using an isolated linear bipolar constant current simulator (maximal output ± 50 mA; DS5, Digitimer, Welwyn Garden City, UK). The median nerve was stimulated at the wrist via non-polarizable electrodes (4620 M, Unomedical Ltd., Birkerød, Denmark) with an anode electrode placed 10 cm proximal over bone. An electro-surgical neutral earth plate (2406 M, Unomedical Ltd., Birkerød, Denmark) was placed in the palm. Compound motor action potentials (CMAPs) were recorded from the abductor pollicis brevis muscle with the references electrode placed 4 cm distal. Compound sensory action potentials (CSAPs) were recorded from the second digit, utilizing ring electrodes placed at the proximal and distal interphalangeal joints for recording and reference, respectively. Responses were amplified (ICP511 AC amplifier, Grass Technologies, West Warwick, USA) with electronic noise removed (Hum Bug 50/60 Hz Noise Eliminator, Quest Scientific Instruments, North Vancouver, Canada). Temperature was monitored with a thermistor thermometer (5831-A, Omega Engineering, Manchester, UK).

Multiple excitability parameters were recorded via TROND-CMW2 and CSW protocols as described previously (Kiernan et al., 2000, 2001) including: (i) threshold electrotonus (TE), a measure of internodal conductances and membrane potential; (ii) recovery cycle (RC) of excitability, an assessment of the recovery of excitability following an action potential marking the function of nodal Na+ channels and (iii) current–threshold relationship (I/V), a measure of the rectifying properties of the axon (Bostock et al., 1998; Kiernan et al., 2000; Burke et al., 2001). Target amplitude for threshold tracking was automatically set to 30%–40% of supramaximal response amplitude, utilizing the area of steepest slope of the stimulus response curve. Changes in the threshold current required achieving the target amplitude were tracked on-line, with the tracking steps proportional to the error between target amplitude and actual response (Bostock et al., 1998).

TE was assessed using 100 ms subthreshold polarizing currents set to ±40% of control threshold (Kiernan et al., 2000, 2001). The threshold current necessary to maintain target response amplitude was assessed at different time intervals during and after the application of polarizing current. Threshold electrotonus hyperpolarizing 90–100 ms (TEh 90–100 ms) was measured as the threshold change following the onset of hyperpolarizing current and S2 accommodation, the slow second component during depolarization, represents the difference between peak threshold change and plateau threshold change following depolarizing current. Other TE parameters were measured as in previous studies (Kiernan et al., 2000, 2001). The current–threshold relationship was assessed via application of polarizing currents of 200 ms duration, stepped in 10% current intervals from 50% to 100% polarizing currents, with excitability assessed following a 200 ms current step. Hyperpolarizing current–threshold drift (I/V drift) was assessed as the mean threshold reduction of the three most hyperpolarized intervals (corresponding to 80, 90 and 100% of threshold current).

The recovery cycle of excitability utilized a paired pulse paradigm with a supramaximal stimulus followed by a conditioning stimulus at different interstimulus intervals (from 2 to 200 ms) (Kiernan et al., 2000, 2001). Refractoriness was assessed as the threshold change at an interstimulus interval of 2.5 ms. Supercexcitability was measured as the minimum mean threshold change of three adjacent points. Subexcitability was measured as the minimum mean threshold change after interstimulus intervals of 10 ms.

Study design

The present study was designed such that the processes involved in the development of both acute neurotoxicity and chronic neuropathy could be investigated. To strengthen any findings, nerve conduction studies were completed by an investigator who was blinded to the clinical status of patients, to reduce bias in data collection and analysis. Clinical examination and NCI scale grading of patients was completed by clinical oncologists who were blinded to the results of electrophysiological investigations. The data were reviewed by investigators who were unaware of patient clinical details, neurotoxic symptoms and NCI severity grade.

Patients were assessed at each oxaliplatin treatment cycle (Fig. 1A). Assessments were initially undertaken immediately prior to oxaliplatin treatment (typically 1 h prior to infusion). Patients returned for post-infusion assessment within 48 h of completion of each oxaliplatin treatment. To assess the acute changes that developed following oxaliplatin infusion, recordings were paired pre- and post-infusion for each cycle per patient, enabling evaluation of nerve excitability changes that occurred within the 48 h time period immediately following oxaliplatin infusion. Post-infusion recordings were only compared to recordings taken pre-infusion within the same treatment cycle. To assess the development of chronic changes, recordings undertaken immediately prior to each oxaliplatin infusion were compared with each other in a longitudinal fashion across treatment (7–12 treatment cycles over 4–6 months).

Data analysis

All results were expressed as mean ± SEM. Median and interquartile range (IQR) are presented as a measure of variability (Armitage et al., 2001). IQR was calculated as the difference between the upper and lower quartiles, representing the middle 50% of data, as in Pflügshaupt et al. (2009). To assess acute neurotoxicity, recordings were paired pre- and post-oxaliplatin infusion and compared within individuals utilizing Wilcoxon sign-rank tests (two-tailed). Differences in acute changes between early and late treatment cycles were examined with Mann–Whitney U-tests (two-tailed) and sensory and motor pre-post infusion difference scores were correlated within individual patients utilizing Spearman’s rank correlation coefficient. Wilcoxon sign-rank tests (two-tailed) were used to compare paired excitability recordings between early, mid and late treatment and to identify early alterations in excitability. Composite excitability scores were tabulated as a marker of overall change across oxaliplatin treatment, and comprised the summed change in parameters superexcitability, refractoriness at 2.5 ms and TEh 90–100 ms from initial to final treatment.
Composite scores were compared with Mann-Whitney U-tests (two-tailed) between neurotoxicity severity grades. Significance was defined as \( P \leq 0.05 \). All statistics were performed in SPSS (Statistical Package for the Social Sciences: Version 17, SPSS Inc., Chicago, USA).

Results

Clinical findings

A total cohort of 58 consecutive oxaliplatin-treated patients were referred from the Department of Medical Oncology (Fig. 1B) in whom a total of 905 axonal excitability studies were undertaken. Seven patients declined participation, while the remaining 88% of patients agreed to full involvement in the study. Subsequently, eight patients were excluded from analysis due to a combination of early attrition from the study due to disease progression, consequences of therapy or early cessation of oxaliplatin. Of the remaining cohort, seven patients were analysed separately due to the presence of comorbid conditions (diabetes mellitus or pre-existing entrapment neuropathy) or because they had been treated with neurotoxic chemotherapy previously. These patients displayed a qualitatively similar pattern of findings to the patients presented below.

The remaining patients (53% male, 47% female; age range 22–77 years, average age 56.9\( \pm \)2.4 years) were treated with a mean cumulative dose of 777.8\( \pm \)46 mg/m\( ^2 \) oxaliplatin over 9.4\( \pm \)0.5 treatment cycles. Overall, electrophysiological stimulation was well tolerated by the patient group and no patients reported discomfort from electrophysiological stimulation or withdrew from the study. All patients had stage III or IV colorectal cancer (Stage III 39%; Stage IV 61%). There were no differences in total cumulative dose or premature oxaliplatin cessation rates between cancer Stage III or Stage IV patients, and consequently these patient groups were analysed together.

During oxaliplatin treatment, the majority (94%) of patients experienced acute manifestations of neurotoxicity. By completion of treatment, severe chronic neurotoxicity had developed in 20% of patients (NCI grade 3), while 51% of patients completed treatment with moderate neurotoxicity (NCI grade 2) and 29% of patients with mild neurotoxicity (NCI grade 1), consistent with previous reports (de Gramont et al., 2000; Andre et al., 2004). The Oxaliplatin-Specific Neurotoxicity Scale demonstrated the same distribution of neurotoxicity severity grades. Symptoms of
Acute oxaliplatin-induced modulation of sensory and motor excitability

To assess acute changes following oxaliplatin infusion, paired excitability recordings were compared pre- and post-infusion. Skin temperature was similar to pre- and post-infusion (pre-infusion temperature, 32.5 ± 0.2°C; post-infusion temperature, 32.8 ± 0.2°C; P = 0.11). Following oxaliplatin infusion (mean dose per cycle, 81 ± 2.3 mg/m²), sensory axons demonstrated significant reduction in the RC parameters of refractoriness [pre 8.9 ± 1.8%; post 5.0 ± 1.4%; median (IQR) −3.9 (13.6); P < 0.005; Fig. 2A] and superexcitability [pre −22.7 ± 0.8%; post −21.0 ± 1.0%; median (IQR) 1.8 (5.3); P ≤ 0.001], as previously reported (Park et al., 2009). In contrast, motor axons revealed significantly increased refractoriness acutely following infusion [pre 26.6 ± 2.1%; post 38.5 ± 2.8%; median (IQR) 8.7 (32.2); P < 0.001; Fig. 2A], associated with reduced superexcitability [pre −19.0 ± 0.5%; post −10.8 ± 0.8%; median (IQR) 7.9 (9.5); P < 0.001], again consistent with values reported previously by our group (Krishnan et al., 2006).

To clarify the relationship between motor and sensory abnormalities, changes in recovery cycle parameters following oxaliplatin infusion were compared within individual patients. The magnitude of change recorded during maximum threshold reduction in superexcitability was significantly correlated between motor and sensory axons (Spearman’s rank correlation coefficient = 0.542, P < 0.05, Fig. 2B), suggesting that patients who demonstrated the greatest change in sensory axons also experienced the greatest change in motor axons.

Acute sensory changes were significantly greater in earlier (cycles 1–4) treatment [re refractoriness mean reduction −6.5 ± 2.4%; median (IQR) −7.0 (13.3)] compared with later (cycles 5–12) treatment [mean reduction −1.1 ± 1.3; median (IQR) 0.5 (15.5); P < 0.05]. Such findings suggest that with increasing cumulative exposure to oxaliplatin, acute changes may be concealed by progressive abnormalities in sensory axons. However, motor axons did not demonstrate significant differences in acute changes across treatment cycles (refractoriness P = 0.15), indicating the lack of a cumulative effect of oxaliplatin in motor axons.

Longitudinal alterations in axonal excitability with oxaliplatin treatment

To dissect the longitudinal effects of oxaliplatin exposure, initial treatment recordings were compared with final recordings in both motor and sensory axons (10.3 ± 0.4 treatment cycles over 5.2 months; cumulative oxaliplatin dose, 860 ± 38 mg/m²). There was no significant change in temperature across treatment (initial temperature, 32.0 ± 0.3°C; final temperature, 32.4 ± 0.4°C; P = 0.44). By completion of oxaliplatin treatment major abnormalities developed in sensory excitability parameters (Table 1). The RC curve was shifted downwards, with a prominent decline in refractoriness (P ≤ 0.0001; Fig. 3A), associated with increased superexcitability (P < 0.0001; Fig. 3A). Extensive change occurred in hyperpolarizing threshold electrotonus, with a large
enhancement in the 90–100 ms interval (TEh 90–100 ms \( P \leq 0.0001 \); Fig. 3B), associated with increases in several other TE parameters (S2 accommodation \( P \leq 0.0005 \); TEh overshoot \( P = 0.005 \); Threshold electrotonus depolarizing (TEd) peak \( P \leq 0.0005 \); TEd 10–20 ms \( P \leq 0.0005 \); Fig. 3B). Alterations in TE parameters were also associated with significant hyperpolarizing I/V drift (\( P \leq 0.005 \); Fig. 3C). These parameters, particularly superexcitability and TEh 90–100 ms, are sensitive indicators of membrane potential (Kiernan et al., 2000; Kiernan and Bostock, 2000).

In contrast, motor axonal excitability parameters remained unchanged longitudinally across oxaliplatin treatment cycles, including RC parameters (refractoriness \( P = 0.34 \); superexcitability \( P = 0.17 \); Fig. 3D), TE parameters (\( P = 0.13 \); Fig. 3E) and hyperpolarizing I/V drift (\( P = 0.90 \); Fig. 3F). In total, these findings confirmed that motor axonal excitability remained unaffected by chronic oxaliplatin exposure in the clinical dose range, in keeping with the clinical patterns of chronic neurotoxicity and neuropathy. Consistent with these findings, conventional motor nerve conduction parameters revealed no changes in stimulus threshold (initial \( 6.1 \pm 0.7 \) ms; final \( 5.6 \pm 0.7 \) ms; median (IQR) \( -0.9 \) (3.0); \( P = 0.36 \)), response latency (initial \( 6.9 \pm 0.2 \) ms; final \( 6.8 \pm 0.2 \) ms; median (IQR) 0.1 (3.5); \( P = 0.37 \)) or median nerve CMAP peak amplitude (initial \( 7.7 \pm 0.4 \) mV; final \( 7.5 \pm 0.3 \) mV; median (IQR) 0.2 (0.8); \( P = 0.59 \)). Accordingly, tibial nerve CMAP peak amplitude (initial \( 9.8 \pm 0.6 \) mV; final \( 9.6 \pm 0.6 \) mV; median (IQR) 0.2 (9.4); \( P = 0.85 \)), distal motor latency (initial \( 4.0 \pm 0.1 \) ms; final \( 4.0 \pm 0.1 \) ms; median (IQR) \( -0.5 \) (0.8); \( P = 0.89 \)) and motor conduction velocity (initial \( 47.1 \pm 1.1 \) m/s; final \( 45.7 \pm 1.5 \) m/s; median (IQR) 0.2 (8.0); \( P = 0.44 \)) were unchanged at the time of completion of oxaliplatin treatment, confirming that motor axons remained essentially unimpaired throughout the treatment.

Conversely, sensory nerve conduction studies demonstrated significant increases in stimulus threshold (initial \( 5.7 \pm 0.5 \) ms; final \( 7.1 \pm 0.7 \) ms; median (IQR) 1.4 (2.2); \( P < 0.05 \)) and response latency (initial \( 3.9 \pm 0.1 \) ms; final \( 4.6 \pm 0.2 \) ms; median (IQR) 0.7 (0.9); \( P < 0.001 \), and reduction in median nerve CSAP peak amplitude (initial \( 46.1 \pm 5.8 \) μV; final \( 24.7 \pm 3.3 \) μV; median (IQR) \(-13.4 \) (46.9); \( P < 0.001 \), consistent with the development of an axonal sensory neuropathy. Accordingly, these changes were accompanied by markedly reduced amplitudes in other sensory nerves [pre-treatment sural amplitude \( 14.6 \pm 1.8 \) μV; post-treatment sural amplitude \( 5.4 \pm 1.0 \) μV; median (IQR) \(-8.0 \) (8.6); pre-treatment radial amplitude 38.1 ± 4.2 μV; post-treatment radial amplitude 14.3 ± 1.9 μV; median (IQR) \(-16.2 \) (23.7)] when compared to normative values (Burke et al., 1974; Ma and Liveson, 1983), confirming the development of generalized sensory neuropathy.

### Table 1 Changes in sensory excitability parameters across oxaliplatin treatment

<table>
<thead>
<tr>
<th>Excitability parameters</th>
<th>Initial recording</th>
<th>Final recording</th>
<th>Median (IQR)</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
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<tr>
<td>Recovery cycle</td>
<td></td>
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<tr>
<td>Relative refractory period (ms)</td>
<td>3.4 ± 0.1</td>
<td>2.8 ± 0.1</td>
<td>-0.7 (1.5)</td>
<td>( \leq 0.005 )</td>
</tr>
<tr>
<td>Refractoriness at 2.5 ms (%)</td>
<td>19.8 ± 3.7</td>
<td>-2.3 ± 2.4</td>
<td>-21.1 (30.8)</td>
<td>( &lt; 0.001 )</td>
</tr>
<tr>
<td>Superexcitability (%)</td>
<td>-19.7 ± 1.2</td>
<td>-29.4 ± 1.5</td>
<td>-7.8 (12.6)</td>
<td>( &lt; 0.001 )</td>
</tr>
<tr>
<td>Subexcitability (%)</td>
<td>9.1 ± 0.8</td>
<td>9.3 ± 0.8</td>
<td>-1.5 (6.4)</td>
<td>NS</td>
</tr>
<tr>
<td>TE</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>TEh 90–100ms (%)</td>
<td>-132.9 ± 7.0</td>
<td>-166.3 ± 9.0</td>
<td>-31.4 (43.1)</td>
<td>( \leq 0.001 )</td>
</tr>
<tr>
<td>Hyperpolarizing TE overshoot (%)</td>
<td>17.2 ± 0.8</td>
<td>9.9 ± 2.0</td>
<td>-12.3 (14.6)</td>
<td>( &lt; 0.005 )</td>
</tr>
<tr>
<td>S2 accommodation (%)</td>
<td>13.3 ± 0.6</td>
<td>18.8 ± 1.2</td>
<td>4.0 (6.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Depolarizing TE 90–100 ms (%)</td>
<td>51.7 ± 1.0</td>
<td>51.1 ± 1.1</td>
<td>-0.33 (2.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Depolarizing TE 10–20 ms (%)</td>
<td>65.6 ± 1.0</td>
<td>70.2 ± 1.6</td>
<td>5.3 (5.6)</td>
<td>( &lt; 0.001 )</td>
</tr>
<tr>
<td>Depolarizing TE peak (%)</td>
<td>65.1 ± 0.9</td>
<td>69.9 ± 1.6</td>
<td>3.8 (4.7)</td>
<td>( &lt; 0.001 )</td>
</tr>
<tr>
<td>Depolarizing TE undershoot (%)</td>
<td>-20.0 ± 1.0</td>
<td>-22.0 ± 1.3</td>
<td>-2.9 (8.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Current threshold relationship (I/V)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Hyperpolarizing I/V drift (%)</td>
<td>-298.4 ± 8.7</td>
<td>-348.3 ± 26.9</td>
<td>-64.5 (106.3)</td>
<td>( \leq 0.005 )</td>
</tr>
<tr>
<td>Hyperpolarizing I/V slope</td>
<td>0.43 ± 0.03</td>
<td>0.44 ± 0.05</td>
<td>-0.02 (0.2)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = not significant

### Time course of sensory excitability changes

To evaluate more finely the time course and development of sensory axonal changes, excitability parameters were assessed progressively across each oxaliplatin treatment cycle in a subgroup of 12 patients over 10.6 ± 0.5 cycles. Sensory parameters were compared across early (mean oxaliplatin dose 71.2 ± 22 mg/m²), mid (mean oxaliplatin dose 434.6 ± 26 mg/m²) and late treatment cycles (mean oxaliplatin dose 812.3 ± 41 mg/m²).

With increasing cumulative exposure to oxaliplatin, sensory axonal excitability demonstrated early and progressive changes, while conventional neurophysiological parameters did not alter until late treatment. By mid treatment, TE waveforms demonstrated significant enhancement (Fig. 4A, B; TEh 90–100 ms early cycles 147.8 ± 6.3%; mid cycles 162.8 ± 5.2%; median (IQR) 14.2 (25.2); \( P < 0.01 \); S2 accommodation early cycles 12.5 ± 1.0; mid 14.9 ± 1.1; median (IQR) 2.4 (4.2); \( P < 0.05 \)) with further increases by late treatment [TEh 90–100 ms late cycles 182.4 ± 8.6%; median (IQR) 10.3 (17.6); \( P < 0.01 \); S2 accommodation late cycles 19.3 ± 1.4; median (IQR) 2.5 (8.7); \( P < 0.05 \); Fig. 4B]. Similarly, RC parameters were significantly altered by mid treatment [refractoriness early cycles 23.8 ± 5.9%;...
mid 6.3 ± 4.4%; median (IQR) −17.5 (23.0); P ≤ 0.005; superexcitability early cycles −20.4 ± 1.9%; mid −25.6 ± 2.3%; median (IQR) −4.2 (7.8); P < 0.005) with superexcitability further enhanced by late treatment [−31.1 ± 1.9%; −3.6 (8.2); P < 0.05; Fig. 4C].

However, conventional parameters of nerve function including median peak CSAP amplitude [early 44.0 ± 9.0 μV; mid 44.4 ± 7.8 μV; median (IQR) −0.5 (12.1); P = 0.99; late 22.8 ± 3.1 μV; median (IQR) −15.3 (38.5); P < 0.05; Fig. 4D] and response latency [early 4.0 ± 0.2 ms; mid 4.1 ± 0.1 ms; median (IQR) 0.1 (0.4); P = 0.53; late 4.8 ± 0.2 ms; median (IQR) 0.6 (0.5); P < 0.005] did not significantly decline until late in treatment. These findings confirm that conventional neurophysiological parameters do not provide sensitive markers of early oxaliplatin-induced nerve dysfunction.

Figure 3 Longitudinal change in axonal excitability with oxaliplatin treatment, with initial recordings (filled circles) compared to final recordings (open circles) following 860 ± 41 mg/m² oxaliplatin over 10.3 ± 0.4 treatment cycles. (A) Recovery cycle of excitability in sensory axons, demonstrating differences from the initial to final recorded oxaliplatin treatment cycle. Refractoriness (P < 0.001) and superexcitability (P < 0.001) are indicated on the figure. (B) TE measurements in sensory axons demonstrating longitudinal changes across treatment. TEh 90–100 ms (P < 0.001) and S2 accommodation (P < 0.001) are indicated on the figure. (C) Current–threshold relationship in sensory axons across treatment. Hyperpolarizing I/V drift (P < 0.005) is indicated on the figure. (D) Recovery cycle recordings from initial to final treatment in motor axons. (E) TE recordings across treatment in motor axons. (F) Current–threshold relationship in motor axons across treatment.
Early markers of oxaliplatin-induced sensory nerve dysfunction

To identify the earliest markers of excitability change, sensory excitability parameters were evaluated in early treatment cycles. Superexcitability demonstrated early and progressive alterations (Fig. 5), with significant enhancement typically apparent prior to the fourth treatment cycle \[\text{initial } -20.9 \pm 2.0\%; \text{ Cycle 4 } -22.2 \pm 2.1\%; \text{ median (IQR) } -1.8 (2.8); P < 0.05\]. TE measurements also demonstrated significant early change, with S2 accommodation enhanced prior to the fourth treatment cycle \[\text{initial } 12.8 \pm 1.0\%; \text{ Cycle 4 } 16.1 \pm 0.9\%; \text{ median (IQR) } 3.7 (5.7); P < 0.05\].

Of critical importance, these early changes were able to predict final clinical outcome on an individual patient basis. An enhancement of at least 15% from baseline in superexcitability prior to treatment cycle 5 identified 80% of patients who completed treatment with moderate or severe neurotoxicity (sensitivity of 80%) and identified no patients who completed treatment with mild neurotoxicity (specificity of 100%). These findings suggest that sensory excitability techniques provide an early predictor of chronic oxaliplatin-induced neurotoxicity, predicting clinical outcome prior to treatment completion. Indeed, changes in superexcitability occurred on average 3.7 cycles earlier than clinically significant neurotoxic symptoms necessitated an oxaliplatin dose reduction, suggesting that use of excitability techniques would serve to enable earlier identification of neurotoxicity than current standard clinical practice.

Relationship between sensory excitability and neurotoxicity severity

To assess the relationship between clinical outcome at treatment completion and excitability abnormalities, the cumulative changes...
in refractoriness, superexcitability and TEh 90–100 ms in sensory axons were summed for each patient as a composite measure of axonal dysfunction. Patients who completed treatment with mild neurotoxicity displayed the smallest composite excitability change [15.2 ± 8.8; median (IQR) −21.4 (40.9); \(P < 0.005\); Fig. 6], while patients with severe neurotoxicity displayed markedly greater change than patients with moderate neurotoxicity [NCI 3 excitability change 118.8 ± 17.4; median (IQR) −121.6 (66.9); NCI 2 excitability change 69.4 ± 9.5; median (IQR) −60.1 (36.6); \(P < 0.05\); Fig. 6]. These findings demonstrate that the degree of excitability abnormalities developing by completion of treatment was linked to neurotoxicity severity. In addition, patients with the greatest excitability change demonstrated a higher proportion of severe symptom scores on the Neuropathy Symptom Score scale, reinforcing the relationship between symptom severity and excitability abnormalities. On an individual patient basis, a composite excitability change of greater than 45 identified 86% of patients who completed treatment with moderate or severe neurotoxicity (sensitivity of 86%), and identified no patients who completed treatment with mild neurotoxicity (specificity of 100%), indicating that sensory excitability change provides a reliable marker of clinical neurotoxicity outcome.

**Discussion**

The present study has investigated the full spectrum of oxaliplatin-induced neurotoxicity, from acute manifestations through to the development of chronic neuropathy, utilizing novel axonal excitability techniques. The combined findings have established different patterns of acute dysfunction in sensory and motor axons, with the development of progressive changes in sensory axons that accumulated longitudinally across oxaliplatin treatment cycles. Importantly, sensory axonal dysfunction began prior to reduction in peak amplitude or an increase in latency—classical markers of axonal degeneration and loss—sugestng that excitability assessment may provide a biomarker for early identification of oxaliplatin-treated patients at risk of neurotoxicity. Additionally, the present study has illustrated the feasibility of undertaking excitability techniques in a clinical setting involving an oncology patient group, despite the rigors of their treatment.

**Acute Na\(^+\) channel dysfunction following oxaliplatin administration**

Oxaliplatin produces prominent acute neurotoxic symptoms following infusion. Accordingly, following oxaliplatin infusion, sensory and motor axons demonstrated acute changes in recovery cycle parameters, in accordance with previous findings (Krishnan et al., 2005, 2006; Kiernan and Krishnan, 2006; Park et al., 2009). Refractoriness, a measure associated with inactivation of voltage-gated nodal Na\(^+\) channels (Burke et al., 2001; Krishnan et al., 2008), was reduced in sensory axons, suggesting that a toxic effect on axonal Na\(^+\) channels contributed to acute nerve dysfunction. Of relevance, modulation of Na\(^+\) channel function induced through biological toxins (Kiernan et al., 2005a), neuropathic medications (Kuwabara et al., 2005) or genetic mutation (Kiernan et al., 2005b) produces qualitatively similar findings in human axons, with prominent decline in refractoriness.

Why then may motor axons develop increased refractoriness following acute oxaliplatin exposure? Unlike sensory axons, refractoriness in motor axons is dependent on the transmission of impulses through the neuromuscular junction, which may fail to transmit impulses accurately during high frequency impulse trains (Bostock et al., 1998; Krishnan et al., 2009). During high frequency impulse trains or repetitive activity, normal impulse
conduction may be disrupted and refractoriness rapidly increased at short interstimulus intervals (Kuwabara et al., 2001). The well-documented motor nerve hyperexcitability that develops following acute oxaliplatin exposure, including neuromyotonic-type discharges (Wilson et al., 2002; Lekhy et al., 2004), may therefore underlie increased refractoriness in motor axons. However, despite differences in the acute oxaliplatin-induced response between motor and sensory axons, the magnitude of this acute change correlated significantly within individual patients. As such, the extent of any oxaliplatin-induced abnormality in motor axons underlie increased refractoriness in motor axons. However, despite charges (Wilson et al. documented motor nerve hyperexcitability that develops following and inactivation (Webster et al., 2005; Benoit et al., 2006) and reduces overall Na+ current (Grollreau et al., 2001; Benoit et al., 2006). A change in Na+ channel properties may predispose to ectopic activity, producing symptoms of paraesthesia and fasciculations (Webster et al., 2005). Cold exposure will further affect Na+ channel kinetics (Rutkove, 2001) and accordingly, Na+ channel mutations have been described which are functionally aggravated in cold temperatures (Bouhors et al., 2003), a feature that commonly develops in acute oxaliplatin-induced neurotoxicity. However, the present findings provide no support for a separate effect of oxaliplatin on potassium (K+) channels, despite the presence of neuromyotonic-type discharges (Wilson et al., 2002; Lekhy et al., 2004), which are typically associated with K+ channel dysfunction (Kiernan et al., 2001; Hart et al., 2002).

The link between acute and chronic manifestations of oxaliplatin-induced neurotoxicity has not been fully defined. However, acute modulation of Na+ channel properties in both motor and sensory axons influences the final severity of oxaliplatin-induced neurotoxicity (Krishnan et al., 2006; Park et al., 2009). In further support, preliminary trials of potential neuroprotective agents in oxaliplatin-treated patients have suggested that acute and chronic presentations may also be linked (Gamelin et al., 2004; Wang et al., 2007). As a putative mechanism, acute modulation of axonal excitability may induce chronic sensory axonal degeneration via disruption of Na+ channel function, ultimately provoking a cascade leading to excess Ca2+ influx and axonal degeneration (LoPachin and Lehning, 1997; Waxman, 2006). The prominence of sensory symptoms in chronic oxaliplatin-induced neurotoxicity may suggest greater susceptibility of sensory axons to damage in the typical oxaliplatin clinical dose range, although potentially the relatively greater exposure of sensory cell bodies in the dorsal root ganglia may further predispose sensory axons to toxic damage (Allen and Kiernan, 1994).

Chronic oxaliplatin-induced neurotoxicity and neuropathy

The present series has revealed major cumulative changes in sensory axonal excitability longitudinally across oxaliplatin treatment. However, motor axons remained unaffected over the course of treatment. Importantly, this pattern of nerve dysfunction corresponds to the clinical expression of symptoms in chronic oxaliplatin-induced neuropathy, with sensory symptoms typically developing (Cassidy and Misset, 2002). The pattern of changes identified in sensory axons with chronic oxaliplatin exposure (reduced refractoriness, enhanced superexcitability and ‘fanning out’ of TE) has been identified as a hallmark of axonal hyperpolarization in previous human studies (Kiernan et al., 2000). Such abnormalities were also described in experimental models of axonal degeneration (Moldovan and Kranup, 2004; Moldovan et al., 2009), suggesting that these changes reflect the development of significant axonal damage in sensory nerves that develops during oxaliplatin treatment.

Previous studies have demonstrated that conventional neurophysiological markers, such as peak amplitude, lack sensitivity in chemotherapy-induced neurotoxicity (Postma and Heimans, 2000). Furthermore, alterations in such markers do not typically change until late in the course of treatment (Casciu et al., 2002; Lehky et al., 2004). Importantly, changes in excitability parameters from the present series developed significantly earlier than those in conventional measures of nerve function, with abnormalities evident prior to the fourth treatment cycle. In addition, excitability changes occurred before clinically significant neurotoxic symptoms necessitated an oxaliplatin dose reduction. As such, sensory axonal excitability parameters may provide appropriate biomarkers for the development of oxaliplatin-induced neurotoxicity, prior to axonal loss, particularly given the fact that the magnitude of excitability change was related to the severity of neurotoxic symptoms.

In total, findings from the present series confirm that acute oxaliplatin-induced neurotoxicity relates to modulation of axonal membrane Na+ channels, with chronic dysfunction in sensory axonal excitability developing with increasing cumulative dose exposure. While sensory excitability demonstrated marked change with increasing cumulative doses of oxaliplatin, motor excitability remained relatively unchanged, in-line with the clinical presentation of symptoms in chronic oxaliplatin-induced neuropathy. Alteration in oxaliplatin dosing in response to clinical neurological signs and symptoms is a well established practice (Andre et al., 2004). Unfortunately, those alterations are often too late to prevent long term toxicity (Land et al., 2007) which is a major consideration in the adjuvant setting, where the gain in survival from oxaliplatin is more incremental. The present series has established the feasibility of excitability testing in a clinical oncology setting. As such, axonal excitability testing may provide a sensitive measure of oxaliplatin-induced neurotoxicity to enable early detection of neurotoxicity and thereby assist in treatment optimization strategies, providing a clinical in vivo marker of neurotoxicity severity.

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