Oxaliplatin and Axonal Na^+ Channel Function *In vivo*

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Abstract

Purpose: The aim of the study was to investigate the pathophysiology of oxaliplatin-induced neurotoxicity using clinical nerve excitability techniques that provide information about axonal ion channel function.

Experimental Design: Excitability studies were combined with standard nerve conduction studies and clinical assessment in 22 patients undergoing treatment with oxaliplatin.

Results: Excitability studies recorded before and immediately after oxaliplatin infusion for 89 treatment cycles revealed significant increases in refractoriness and relative refractory period postinfusion in all patients, consistent with an effect of oxaliplatin on axonal Na^+ channels. However, those patients that developed chronic neuropathy had significantly greater changes. Following cessation of oxaliplatin treatment, 41% of patients had persistent symptoms and nerve conduction abnormalities consistent with the development of chronic neuropathy.

Conclusion: The present study provides evidence that oxaliplatin-induced neurotoxicity is mediated through an effect on axonal Na^+ channels. Clinical nerve excitability techniques may prove beneficial in monitoring for early signs of neurotoxicity and in the assessment of future prophylactic therapies.

Oxaliplatin is a novel chemotherapeutic agent effective against advanced colorectal cancer (1, 2). Unlike other platinum-based agents, it does not induce dose-limiting nephrotoxicity; dose limiting bone marrow toxicity is uncommon (3) but it causes considerable neurotoxicity (4–7). Oxaliplatin-induced neurotoxicity manifests as rapid-onset neuropathic symptoms exacerbated by cold exposure and as chronic neuropathy that develops after several treatment cycles (1, 2).

The incidence of oxaliplatin-induced neurotoxicity has been defined by clinical studies that graded neurotoxicity using the National Cancer Institute Common Toxicity Criteria, with grade 3 neuropathy (severe sensory loss that interfered with function) reported in 12% to 18% of patients (1, 2, 8). Early identification of neurotoxicity may allow for alterations in dose or schedule to prevent the development of chronic symptoms, which, once established, may take many months or years to resolve (7). The development of chronic neurotoxicity becomes especially problematic in the setting of adjuvant therapy where long-term neurologic deficit is an unacceptable outcome. Whereas preliminary *in vitro* studies have documented changes in voltage-dependent Na^+ channel function (9–11) following oxaliplatin, mechanisms responsible for nerve dysfunction in patients have not been established.

Nerve excitability techniques, recently adapted for clinical use (12–14), provide information about axonal membrane ion channel function. Axonal excitability in human subjects is assessed using "threshold tracking," where threshold indicates the stimulus current required to produce a target potential, which can be adjusted online by computer (i.e., tracked) to assess excitability. Excitability studies have shown alterations in axonal Na^+ channel function in toxic and metabolic neuropathies (15, 16) and in patients with genetic mutations in Na^+ channels (17). Such information cannot be gained using standard nerve conduction studies, which provide information about the number of conducting fibers (amplitude) and the speed of the fastest conducting fibers (latency and conduction velocity). More critically, nerve conduction studies may not manifest abnormalities until significant fiber loss has occurred and are therefore unsuitable for predicting the development of neuropathy.

Given that excitability studies undertaken in patients who developed neuropathy following completion of oxaliplatin therapy provided supportive evidence for an effect on axonal Na^+ channels (7), the aim of the present prospective study was to establish whether acute neurotoxicity was mediated by effects on axonal Na^+ channels, and if so, whether axonal excitability measures undertaken in a prospective fashion may help to identify those patients at greatest risk of developing neurotoxicity.

Materials and Methods

Nerve excitability studies were recorded in 22 consecutive patients treated with oxaliplatin for advanced colorectal cancer for a total of 89 treatment cycles. Patients received 2 to 12 cycles of oxaliplatin (Table 1)

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Received 3/21/06; revised 5/11/06; accepted 5/25/06.

Grant support: National Health and Medical Research Council of Australia (Project Grant 400938) and the Australian Association of Neurologists.

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doi:10.1158/1078-0432.CCR-06-0694

Table 1. Clinical and nerve excitability data, oxaliplatin dosages, presence of neuropathic symptoms, and reason for oxaliplatin cessation for each patient

	Single dose range (mg)	Cumulative dose (mg)	Chronic neuropathy	Highest RRP	Initial RRP elevation	NCI-CTC	Reason for cessation of oxaliplatin
#1 (64)	110-140 (15)	1,820	Yes (10th cycle)	4.84	Cycle 3	1	Neurotoxicity
#2 (63)	180 (6)	1,080	No	3.08	—	0	Disease progression
#3 (52)	110-170 (9)	1,250	Yes (9th cycle)	4.09	Cycle 6	3	Neurotoxicity
#4 (63)	125-160 (8)	1,175	Yes (4 wk post)	6.48	Cycle 3	3	Completed therapy
#5 (74)	190 (2)	380	No	3.16	—	0	Declined further therapy
#6 (71)	123-162 (10)	1,269	Yes (9th cycle)	4.02	Cycle 3	2	Neurotoxicity
#7 (74)	135-175 (10)	1,470	Yes (2 wk post)	5.05	Cycle 3	3	Disease quiescent
#8 (57)	180-220 (4)	840	No	3.98	—	0	Disease progression
#9 (54)	177-236 (6)	1,593	No	2.94	—	0	Completed therapy
#10 (57)	100-132 (9)	1,128	No	3.82	—	0	Disease progression
#11 (70)	100-180 (13)	2,020	Yes (9th cycle)	5.75	Cycle 2	3	Disease progression
#12 (54)	250 (7)	1,750	Yes (7th cycle)	3.94	—	3	Neurotoxicity
#13 (76)	100-150 (12)	1,470	No	3.64	—	0	Completed therapy
#14 (42)	105-140 (10)	1,190	Yes	4.06	Cycle 2	2	Completed therapy
#15 (49)	136 (12)	1,632	No	3.14	—	0	Completed therapy
#16 (24)	150-188 (10)	1,690	No	2.82	—	0	Completed therapy
#17 (68)	96-157 (8)	1,158	No	3.97	—	0	Disease quiescent
#18 (72)	155 (9)	1,395	No	3.77	—	0	Disease progression
#19 (22)	165 (10)	1,650	No	3.85	—	0	Completed therapy
#20 (73)	140-175 (9)	1,470	Yes	3.53	—	3	Neurotoxicity
#21 (60)	90-150 (8)	860	No	2.9	—	0	Disease progression
#22 (63)	110-170 (7)	975	No	3.14	—	0	Completed therapy

NOTE: Maximal single infusion dosage is calculated using body surface area to correspond to a dosage of 100 mg/m^2 and the total number of cycles of treatment is indicated in brackets after range of single infusion dose. Chronic symptoms are those which were present at the time of the first review following completion of oxaliplatin treatment. The neurosensory scale of the National Cancer Institute Common Toxicity Criteria (NCI-CTC; version 1) was used with the following grading system (21): 0, no neuropathy; 1, mild paresthesias, loss of deep tendon reflexes; 2, mild or moderate objective sensory loss or moderate paresthesias; and 3, severe objective sensory loss or paresthesias that interfere with function. The number of cycles after which chronic symptoms were first noted is given in brackets. Two patients (#4 and #7) developed chronic symptoms 4 and 2 weeks after the completion of oxaliplatin therapy respectively. Relative refractory period (RRP) duration was >4 ms in 78% patients with chronic neuropathy and the cycle at which this change was first noted is shown. Two patients (#5 and #10) died 4 to 6 weeks after completion of oxaliplatin therapy. Although nerve conduction studies were not undertaken, there were no clinical signs of neuropathy.

at initial dosages of 100 mg/m^2. Clinical assessment incorporating National Cancer Institute Common Toxicity Criteria scale and standard nerve conduction studies of sural, tibial, superficial radial, and median nerves (18) were also undertaken using a Medelec Synergy system (Oxford Instruments, Surrey, United Kingdom) in all patients before oxaliplatin treatment. Patients with clinical symptoms of neuropathy before the commencement of oxaliplatin or baseline nerve conduction abnormalities were excluded. Patients gave written consent to the procedures approved by institutional Research Ethics Committees.

In all excitability studies, the median nerve was stimulated at the wrist and compound muscle action potentials were recorded from abductor pollicis brevis. Multiple excitability variables were recorded using an established protocol (13) that included measures of relative refractory period duration, a marker of inactivation of transient Na^+ channels (19), and refractoriness, which relates to the percentage increase in current required to generate an impulse during the relative refractory period at a conditioning-test interval of 2.5 ms (20).

In total, the following investigations were conducted on all patients: (*a*) baseline clinical assessment, standard clinical nerve conduction studies, and nerve excitability studies before commencement of oxaliplatin treatment; (*b*) nerve excitability studies and recording of relative refractory period before infusion and within 48 hours after oxaliplatin infusion for multiple treatment cycles; and (*c*) clinical assessment and nerve conduction studies 4 weeks after completion of oxaliplatin treatment. Excitability variables were analyzed with Student's paired and unpaired *t* tests and repeated measures ANOVA. Results are expressed as mean ± SE.

Results

Acute symptoms, defined as those occurring immediately following oxaliplatin infusion and typically lasting for less than a week, occurred in 90% patients. Neuropathy, defined as a National Cancer Institute Common Toxicity Criteria neurosensory grade of >0 and accompanied by nerve conduction abnormalities (21), developed in 41% of patients (see Table 1).

Baseline relative refractory period values before the first dose of oxaliplatin were normal in all subjects (reference range, 3.1 ± 0.1 ms; age range, 23-59 years; mean, 39.4 years; $n = 29$) when compared with previously established normative data (13). Following oxaliplatin therapy, alterations were noted in relative refractory period duration and refractoriness, illustrated for a single representative patient in Fig. 1A. When compared with preinfusion recordings for all 89 cycles, postinfusion recordings showed increases in the duration of the relative refractory period (postinfusion, 3.83 ± 0.1 ms; preinfusion, 3.1 ± 0.1 ms; $n = 89$; $P < 0.0005$, paired *t* test) and refractoriness (postinfusion, 52.0 ± 6.5%; preinfusion, 26.1 ± 4.4%; $n = 89$;

$P < 0.05$) consistent with increased Na^+ channel inactivation due to oxaliplatin (Fig. 1B). Similar changes were also noted when recordings were analyzed as a single mean value per patient (Fig. 1C). Repeated measures ANOVA revealed persistence of these changes over the period of treatment ($P < 0.05$).

Following completion of oxaliplatin therapy, the acute alterations in Na^+ channel variables were reviewed in relation to the development of neuropathy. Patients who developed chronic neuropathy manifested greater pre- and post-infusion changes in both refractoriness and relative refractory period duration when analyzed either across all treatment cycles ($P < 0.005$) or as a single mean value per patient ($P < 0.05$; Fig. 1C). Furthermore, preinfusion relative refractory period was a reliable predictor of neuropathy development and was >4 ms on at least one occasion in 78% of patients that subsequently developed neuropathy (Table 1; Fig. 1D) but never exceeded this value in any patient that remained neuropathy-free (i.e., specificity of 100%). The occurrence of this abnormal reading predated the onset of neuropathic symptoms and was noted at an average of seven cycles before the development of symptoms. Furthermore, relative refractory period duration remained elevated in subsequent cycles when analyzed as a single mean value per patient (relative refractory period duration, 4.1 ± 0.3 ms; $n = 7$; $P < 0.05$). In contrast, postinfusion changes in patients who did not develop neuropathy had largely resolved before the next cycle of treatment.

Discussion

The present study has established acute abnormalities of nerve excitability in patients treated with oxaliplatin. Specifically, the changes in Na^+ channel–dependent variables, refractoriness, and relative refractory period duration, following oxaliplatin administration, suggest that the acute form of oxaliplatin-induced neurotoxicity is mediated through an effect on axonal voltage-gated transient Na^+ channels. The fact that these acute excitability changes induced by oxaliplatin were greater in patients who subsequently developed neuropathy supports previous suggestions of an association between acute oxaliplatin-induced neurotoxicity and chronic neuropathy (7, 22). It is proposed that acute alterations in axonal Na^+ channel function are involved in the subsequent process of long-term axonal degeneration, characterized by chronic neuropathic symptoms and reduction in action potential amplitude on standard nerve conduction studies (7).

The increase in refractoriness and relative refractory period duration following oxaliplatin treatment supports the findings of previous *in vitro* studies that have suggested that the acute effects of oxaliplatin are due to effects on voltage-gated transient Na^+ channels (9, 11). Importantly, the present study suggests that patients who accrue abnormalities in Na^+ channel function during the course of therapy are at highest risk for developing chronic neuropathy. Such incremental changes cannot be diagnosed on clinical grounds alone given that almost all patients treated with oxaliplatin manifest acute neurotoxic symptoms, and yet not all develop chronic neuropathy. Furthermore, the finding that patients with a pretreatment relative refractory period of >4 ms invariably developed neuropathy suggests that excitability measures may be of clinical use in determining which patients are at highest risk of chronic neurotoxicity.

Findings from the present study also provide a rationale to the hypothesis that reduction in acute neurotoxicity may potentially reduce the development of chronic neuropathy (22). The only Na^+ channel found at the node of Ranvier in the

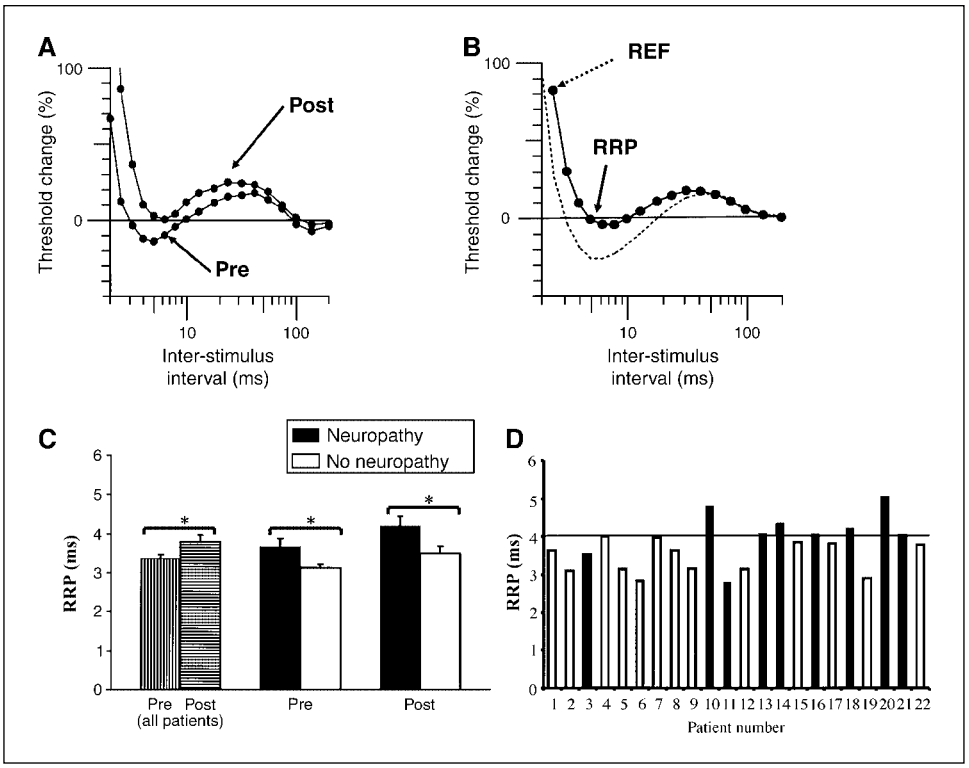

Fig. 1. *A,* recovery cycle in a single representative patient before and after a single infusion of oxaliplatin, showing a postinfusion increase in the degree of refractoriness (*REF*) and the duration of the relative refractory period (*RRP*). Refractoriness was measured as the percentage increase in threshold at a conditioning-test interval of 2.5 ms. Relative refractory period (ms) refers to the first intercept on the *x* axis. *B,* recovery cycles (mean data) from patients for the first paired set of excitability recordings. Postinfusion values (*line with circles*) are compared with preinfusion data (*dotted line*; ref. 13), showing similar changes to those shown for single subject. *C,* comparison of relative refractory period recorded before and after oxaliplatin therapy. Whereas relative refractory period increases following oxaliplatin therapy in all patients (hashed boxes), both preinfusion and postinfusion values are greater in the neuropathy group. Values shown are those obtained using a single mean value per patient to account for differences in the number of treatment cycles (*, $P < 0.05$). *D,* highest relative refractory period value in patients with and without neuropathy. Whereas preinfusion relative refractory period varied during treatment in individual patients, a value >4 ms was invariably associated with the development of neuropathy.

peripheral nervous system (Na_v 1.6) colocalizes with the Na^+/Ca^{2+} exchanger at sites of axonal injury (23). Alterations in axonal Na^+ concentrations may trigger reverse flow of the Na^+/Ca^{2+} exchanger, which activates damaging Ca^{2+}-mediated processes, leading to axonal loss (23, 24). Reductions in membrane-bound Ca^{2+} may contribute to the axonal hyperexcitability, which underlies paraesthesia, cramp, and tetany (25), common symptoms immediately following oxaliplatin infusion, providing a rationale for prophylactic Ca^{2+} infusions. Furthermore, the fact that the excitability abnormalities preceded the onset of clinical symptoms by an average of seven cycles provides a window for prophylactic therapy to be initiated.

The recent report of a randomized trial with xaliproden may have identified an effective prophylactic therapy (26). The use of a predictive test such as ours may enable more focused use of such strategies in addition to maximizing the benefit of the intervention in a cost-effective manner. Recent studies, such as the OPTIMOX1 trial (27), suggest that one strategy for increasing the duration of progression-free interval may be to recycle the oxaliplatin regimen at regular intervals for a fixed duration. Such an approach does, however, run the risk of increased neurotoxicity. Data from the present study suggest that it may be possible to identify which patients can be safely reexposed to an oxaliplatin-based regimen in a recycling strategy to maximize the benefit of each line of therapy.

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Clin Cancer Res 2006;12:4481-4484.