A Quantitative Sensory Analysis of Peripheral Neuropathy in Colorectal Cancer and Its Exacerbation by Oxaliplatin Chemotherapy

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Abstract

Peripheral neuropathy caused by cytotoxic chemotherapy, especially platin and taxanes, is a widespread problem among cancer survivors that is likely to continue to expand in the future. However, little work to date has focused on understanding this challenge. The goal in this study was to determine the impact of colorectal cancer and cumulative chemotherapeutic dose on sensory function to gain mechanistic insight into the subtypes of primary afferent fibers damaged by chemotherapy. Patients with colorectal cancer underwent quantitative sensory testing before and then prior to each cycle of oxaliplatin. These data were compared with those from 47 age- and sex-matched healthy volunteers. Patients showed significant subclinical deficits in sensory function before any therapy compared with healthy volunteers, and they became more pronounced in patients who received chemotherapy. Sensory modalities that involved large Aβ myelinated fibers and unmyelinated C fibers were most affected by chemotherapy, whereas sensory modalities conveyed by thinly myelinated Aδ fibers were less sensitive to chemotherapy. Patients with baseline sensory deficits went on to develop more symptom complaints during chemotherapy than those who had no baseline deficit. Patients who were tested again 6 to 12 months after chemotherapy presented with the most numbness and pain and also the most pronounced sensory deficits. Our results illuminate a mechanistic connection between the pattern of effects on sensory function and the nerve fiber types that appear to be most vulnerable to chemotherapy-induced toxicity, with implications for how to focus future work to ameliorate risks of peripheral neuropathy.

Introduction

Neuropathy induced by chemotherapy can seriously impede successful treatment for many cancers as it often leads to reduction or cessation of frontline treatment; and can drastically impact patients' quality of life both during and following therapy (1). The mechanism for chemotherapy-induced peripheral neuropathy (CPN) is poorly understood. Primary afferent neurons seem to be the most vulnerable as most commonly sensory symptoms alone start in the tips of the toes and fingers and then advance over time proximally in a "stocking-glove" distribution (2–5). More specifically, pain is typically reported in the tips of the toes and fingers; numbness and tingling, but not necessarily pain is present in the soles of the feet and palms; while hairy skin is typically outside the areas of patient complaint (3, 6). Yet specific data concerning the vulnerability of primary afferent fiber subtypes to toxic insult by cancer and its treatment are not known. Here, quantitative sensory tests (QST) were conducted in patients before and after various cumulative doses of oxaliplatin to fill this gap in knowledge. QST is a psychophysical method used to study human somatic sensory physiology including pain perception (7). Small-fiber sensory function is assessed by measuring the threshold to detect warmth, hot, and cold pain; thinly myelinated fibers are assessed by measurement of threshold to detect skin cooling and sharpness; and large-fiber sensory function is measured by detection thresholds for cutaneous mechanical stimuli (8). The pattern of effects of chemotherapy on sensory function has clear mechanistic implications for the nerve fiber types that are vulnerable to the toxicity of chemotherapy.

Patients and Methods

Patients

Patients starting initial chemotherapy with oxaliplatin for stage II, III, or IV colorectal cancer at MD Anderson (Houston, TX) were recruited for the study that was approved by the
Institutional Review Board. Seventy-eight patients were enrolled and gave informed consent. Patients with any history of neuropathy or other factors known to contribute to neuropathy including diabetes mellitus, history of alcohol intake more than 100 g per week, vitamin deficiency, nerve compression, or any central nervous system metastasis were excluded. The typical chemotherapy protocol consisted of oxaliplatin administered at a dose of 85 mg/m² on 2-week cycles. Patients underwent QST before each treatment with oxaliplatin. In addition, 47 age- and sex-matched volunteers were recruited to provide comparative data.

QST
QST data were collected with the patients comfortably seated in a quiet, dedicated psychophysics laboratory in the daytime hours with the subjects not on analgesics that might interfere with the tasks. Three sites were tested in each subject, the tip of the index finger, the thenar eminence, and the volar surface of the forearm to encompass the areas of skin that are typically affected in chronic CIPN patients (2–5). QST were conducted by two clinical data coordinators with many years of experience and with previously verified excellent inter-rater reliability (2–5). The specific tests that were performed included the following and were performed in the order described.

Basal skin temperature. Basal skin temperature was measured using an infrared thermistor positioned against the skin at each site.

The Slotted Pegboard test. The Slotted Pegboard test was used to evaluate sensorimotor function (9). Participants filled a 5 × 5 slotted pegboard with spindles in nonrandom fashion by one row or column at a time with the dominant hand and then with the nondominant hand (10). The time for each participant to complete the task was recorded with a 5-minute (300 seconds) cutoff.

Bumps detection. Bumps detection was used to assess low threshold mechanosensation (11, 12). Participants used their index finger to probe a smooth plate that was divided into nine blocks, with each block marked by five colored circles. Over one of the circles in each block, a bump of varying height (500 μm in diameter, 2.5–22.5 μm tall) was concealed such that it was not visible to the patient (3 plates total in the set). The threshold was defined as the lowest height bump correctly detected with the next two higher bumps also correctly detected.

Touch detection threshold. Touch detection threshold was determined using von Frey monofilaments (Semmes–Weinstein) in an up/down manner as previously described (2). The filaments were applied for 1 second at each testing site starting with a force of 0.5 g and the patients were unable to see the stimulus application. If a participant did not feel a given filament, the next higher force filament was applied. If a participant felt a stimulus, the next lower force filament was applied. Threshold was defined as the first filament force detected by the participant three times.

Sharpness detection threshold. Sharpness detection threshold was determined using blunted 30-gauge needles with force determined by weights graded from 8 to 128 g (10, 13). Weighted needles were applied in order from lightest to heaviest at each site for 1 second, and participants were asked to report each stimulus as touch, pressure, sharp, or pain. The lowest force at which the report of "sharp" or "painful" was determined the endpoint for each trial. The final threshold was the mean of three trials separated by 30 to 90 seconds. The starting weight was modified between trials to manage errors in anticipation.

Thermal detection threshold. Thermal detection threshold was determined using a 3.6 × 3.6 cm Peltier probe set at a baseline temperature of 32°C (2). The probe temperature was ramped upward at a rate of 0.30°C/second for detection of warmth and heat pain thresholds, whereas cool detection and cold pain threshold were determined using a decreasing ramp of 0.50°C/second. Participants were not given any cue to the onset of a given trial, nor whether the probe would heat or cool. Participants were instructed to indicate when they could first detect a change in temperature and then when the temperature became painful; at that point, the probe was immediately returned to the baseline temperature. The final threshold was the average of three heating and cooling trials separated by 30 to 90 seconds.

Descriptors of symptoms. Descriptors of symptoms were assessed using questionnaires and a standardized body map presented to the participants at each meeting (2). The participants marked areas where they felt pain with a red pen and areas where they felt tingling or numbness with a green pen. Participants also selected descriptors for their symptoms from a standardized list (2) that was previously validated (14).

Data analysis
Analysis of the data was based on total cumulative oxaliplatin dose that patients received before each test. In this manner, patient data were stratified into baseline (cumulative dose 0), low (115.7–345.1 mg), medium (347.1–737.8 mg), and high dose (739.5–2328.2 mg) categories established by empirical analysis. Patients only contributed one set of data per dose category with that included at the highest dose if sampled more than once within a given category. Finally, patients were also tested at approximately 6 months after chemotherapy. The nonparametric Kruskal–Wallis test was applied to all data. Patient data were compared with that from the health volunteers only for the baseline time point. The patient data collected at the time points during and following chemotherapy were compared with the patient baseline dataset. Significance was defined as any P value < 0.05.

Results
Patient demographics and clinical data are shown in Table 1. The breakdown by treatment category resulted in 51 patients in the baseline group because some had not agreed to take part in the study before starting chemotherapy. Sixty-two patients were included in the low-dose category; 54 in the medium-dose category; and 49 in the high cumulative dose category. Finally, 27 patients underwent QST at a postchemotherapy follow-up examination at a mean of 165 (±12) days after the last chemotherapy. The
numbers vary due to missed visits and/or loss of subjects to the study over time.

**Pegboard test**

Patients at baseline took significantly more time to complete the pegboard test with the dominant hand than volunteers did (Fig. 1A, \( P < 0.001 \)). The same was observed for the nondominant hand (Fig. 1B, \( P < 0.001 \)). Subsequent pegboard tests collected during chemotherapy did not show any differences from the patient baseline value. Indeed, there was a trend in both hands for a slight decline in pegboard time, most likely reflecting a training effect (none of the volunteers were allowed training before their data collection).

**Bumps detection**

The Bumps test is an assessment of large diameter \( \alpha \beta \) fiber mechanoreceptor function best correlated to transduction by Meissner’s corpuscles (12, 15). The patients showed a significantly elevated Bumps threshold at baseline compared with healthy volunteers (Fig. 1C, \( P < 0.01 \)). There was a clear trend for the impairment seen at the patient baseline QST to worsen during therapy, with this difference becoming statistically significant in the fingertips at middle and high chemotherapy doses (Fig. 2A, \( P < 0.05 \)). Similarly, touch threshold in the thenar eminence gradually increased with chemotherapy dose and was statistically significant between the patient

<table>
<thead>
<tr>
<th>Table 1. Demographic and clinical characteristics</th>
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<tr>
<td><strong>Patients</strong> (( N = 78 ))</td>
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<tr>
<td>Age (mean ± SD)</td>
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<td>Male (%)</td>
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<td>Female (%)</td>
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<tr>
<td>Unknown (%)</td>
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<tr>
<td>Smoke &gt; 10 pack years (%)</td>
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<tr>
<td>Married</td>
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<td>TNM stage(^a)</td>
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<td>II</td>
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<tr>
<td>III or IV</td>
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<td>Mean cycles (mean ± SD)</td>
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\(^a\)American Joint Committee on Cancer Staging Manual (7th edition) criteria.

with all other groups (\( P < 0.01\)–\( < 0.0001 \)) consistent with the coasting phenomenon often attributed to platin-based chemotherapeutics.

**Touch detection**

The detection of touch using von Frey filaments engages large diameter \( \alpha \beta \) slowly adapting Merkel complex mechanoreceptors (16, 17). There was no difference between touch detection threshold between healthy volunteers and patients before therapy. Touch threshold did show increasing deficit with dose during chemotherapy that became statistically significant in the fingertips at middle and high chemotherapy doses (Fig. 2A, \( P < 0.05 \)). Similarly, touch threshold in the thenar eminence gradually increased with chemotherapy dose and was statistically significant between the patient
baseline for those patients receiving high-dose chemotherapy (Fig. 2A, \( P < 0.05 \)). Interestingly, these deficits resolved at the 6-month follow-up test. Touch threshold in hairy skin showed no change at any time point.

**Sharpness detection**

Sharpness detection threshold is an assessment of thinly myelinated A\(\delta\) fibers. There were no differences observed in sharpness detection between healthy volunteers and patients before chemotherapy (Fig. 2B). There was also no change shown in sharpness detection in patients at the various cumulative doses of chemotherapy compared with the patient baseline.

**Basal skin temperature**

The patient group showed significantly cooler skin temperature in the fingertips before chemotherapy than that found in the healthy volunteer group (Fig. 3). Interestingly, skin temperature returned toward that of healthy volunteers in the treatment groups, but this change was not significant compared with the patient baseline.

**Temperature detection**

The detection of warming is dependent on inputs from subgroups of unmyelinated C fibers given the relatively slow heat ramps that were used in this study. The patient baseline warm detection threshold was significantly elevated from that of healthy volunteers at all three test sites (Fig. 4A, \( P < 0.05 \)).
Warm detection in the treatment groups showed no change from the patient baseline. However, warm detection threshold was significantly increased from the patient baseline in the 6-month follow-up group (Fig. 4A, \( P < 0.05 \)). Heat pain threshold mediated by unmyelinated C fibers tended to be higher in the patient group at baseline compared with that of the healthy volunteers, but this difference did not achieve statistical significance. No change in heat pain threshold from the patient baseline was observed in any of the treatment groups or at 6-month follow-up (Fig. 4B).

Cool and cold pain detection is mediated by activity in thinly myelinated \( \alpha \delta \) and unmyelinated C fibers, respectively. The patients had a significantly lower threshold to detect skin cooling at the baseline measure before chemotherapy than the healthy volunteers in both the fingertips and the hairy skin of the volar forearm (Fig. 5A, \( P < 0.05 \)). This threshold showed a further deficit at 6-months follow-up compared with the patient baseline. The patient baseline cold pain threshold was significantly elevated compared with the healthy volunteers in both the thenar eminence and volar forearm (Fig. 5B, \( P < 0.01 \)).Chemotherapy treatment increased this deficit such that the moderate and high doses resulted in pain at significantly warmer temperatures than at the patient baseline (Fig. 5B, \( P < 0.05 \)). This deficit showed some resolution at 6-months follow-up back toward the original patient baseline, though cold pain in the volar forearm remained significantly different.
Neuropathy score and symptom complaints

Figure 6A shows the overall neuropathy scores for the healthy volunteers and for each of the patient groups tabulated by determining the number of measures in the QST battery for each subject that were 2 SDs or more outside the healthy volunteer mean values. The mean neuropathy score for the healthy volunteers was predictably very low, and as detailed in the previous sections, the value for the patients at baseline was significantly higher (Fig. 6A, \( P < 0.01 \)). The mean neuropathy scores showed significant further increases from the patient baseline value with chemotherapy dose and had a peak at the 6-month follow-up (Fig. 6A, \( P < 0.05–0.01 \)). Figure 6B shows the percentage of subjects in each group that had QST measures that were 2 SDs or more outside the healthy volunteer mean values (filled circles). Approximately 25% of the healthy volunteers had at least one out-of-range measure compared with roughly three quarters of the patients (\( P < 0.01 \)). Notably, the QST deficits in the patients at baseline were subclinical as none reported any numbness or pain at the baseline measure (Fig. 6B, open circles and filled circles). The percentage of patients with abnormal QST measures showed a continuous increase across the treatment groups with the final peak at the 6-month follow-up (Fig. 6B, filled circles). The decay in sensory function was paralleled by increasing reports of numbness and pain in the patient groups such that by 6-months follow-up, roughly three quarters of the patients reported numbness and roughly one fifth reported pain. Finally, Fig. 6C shows the rates of symptom complaint that developed during chemotherapy or present at follow-up based on whether the patients had a baseline QST deficit. The frequency of both numbness and pain was significantly increased in the patients who presented with subclinical neuropathy versus those who did not.

Discussion

This is the first study to use repeated QST in the study of the development of CIPN bringing a highly sensitive method to detect sensory impairments to this field (8). A key finding from this approach was the detection of preexisting subclinical sensory deficits in a large cohort of patients with colorectal cancer before treatment that seems to be disease driven and that when present seems to increase the risk for the later development of clinical CIPN. This observation, therefore, provides a generalization of a correlation between apparent subclinical pretreatment neuropathy and risk for CIPN as previously suggested in patients with multiple myeloma (10, 11, 15). A caveat, however, is that QST was not performed on the feet for convenience of the patients, yet CIPN often first presents in the feet. Hence, this study may present an overly conservative survey of QST deficits, particularly those that remained subclinical, that occurred in this patient cohort. It should be noted, however, that all of our subjects who became symptomatic complained of symptoms in the hands as well as the feet.

Perhaps the most important findings of this study are the mechanistic implications for impact of chemotherapy on specific groups of primary afferent fibers. Touch detection using the Bumps and von Frey assays is transduced by large diameter myelinated axons that terminate in or near Meissner’s corpuscles (15) and Merkel disk complexes (16–18), respectively. The slotted pegboard task, although also...
dependent to a degree on cutaneous mechanoreceptors, is more dependent on sensorimotor coordination involving neural inputs from muscle and joint mechanoreceptors that engage spinocerebellar and cortical cognitive processing. The pegboard test was significantly worse for patients at baseline compared with healthy controls, but then did not show any decay from that level and even a trend toward improvement. On the other hand, patient mechanoreceptor function tested using the Bumps test not only showed a difference at baseline from healthy volunteers, but also showed significant further deterioration with increasing chemotherapy doses and evidence of coating following the termination of chemotherapy. Mechanoreceptor function assessed using von Frey monofilaments showed much of the same results as in the Bumps test. The difference between the pegboard to the Bumps and von Frey tests could be explained by assuming that the patients learned to cognitively overcome mechanoreceptor deficits in the former, whereas the lack of learning cues in the latter tests prevented this compensation. Alternatively, this paradox in results could also indicate that the myelinated fibers innervating the different tissues involved in these tasks show differential toxic deficit to oxaliplatin.

The Aβ fiber-dependent tasks seem to provide clear psychophysical evidence in support of a differential susceptibility of primary afferent fibers to toxic insult by chemotherapy. Although the percept of sharpness/sharp pain evoked by the weighted needles showed little change at baseline or with chemotherapy, the detection of skin cooling showed a clear impairment. Similarly, various groups of C fibers are recruited in the detection of warm, heat, and cold pain, yet the patients showed preserved heat pain in the context of altered warmth and cold pain detection. Thus, for each group of fibers, psychophysical evidence indicates that the mechanism of toxicity engaged by chemotherapeutics differentially impacts function in different subtypes of primary afferent fibers, resulting in the clinical phenotype that is observed.

One possible explanation that fits this criterion well is the recent demonstration of an interaction of the chemotherapeutic paclitaxel with Toll-like receptor 4 (TLR4; ref. 19). Not all, but only subsets of small (C-fiber) and medium sized (Aβ fiber) DRG neurons express TLR4 following chemotherapy treatment and show signs of an activated innate immune response including an increase in the expression of proinflammatory cytokines such as MCP-1 (20). The effects of MCP-1 and other cytokines on peripheral nerves could account for the clinical presentation of CIPN and the known risk and protective factors. Schwann cells express cytokine receptors that when activated lead to dedifferentiation and downregulation of myelin synthesis (21–24). This would consequently have pronounced functional impact on Aβ fibers that require extensive myelination, but less so on C-fibers, thus generating a clinical picture like that observed in the patient studies as described here. Finally, this mechanism would explain the observed effects of anticytokine treatments, such as minocycline, in preventing CIPN (25, 26) and suggests a clear short-term target for clinical evaluation in preventing a major complication of cancer treatment.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. de Carvalho Barbosa, A.K. Kosturakis, C. Eng, P.M. Dougherty
Writing, review, and/or revision of the manuscript: M. de Carvalho Barbosa, A.K. Kosturakis, C. Eng, C.S. Cleeland, P.M. Dougherty
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. de Carvalho Barbosa, A.K. Kosturakis, P.M. Dougherty
Study supervision: X.S. Wang, P.M. Dougherty
Other (provision of the metrology used, especially "the Bumps," and review and suggestions for changes/corrections of the manuscript): W.R. Kennedy, D.A. Simone

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