Cancer Chemotherapy and Pharmacology (2019) 84:1195–1200
https://doi.org/10.1007/s00280-019-03956-6

ORIGINAL ARTICLE

# Skin platinum deposition in colorectal cancer patients following oxaliplatin-based therapy

Yanshuo Cao[1,2] · Qing Chang[3] · Wenjiang Zhang[1] · Olga Ornatsky[3] · David Hedley[1,2] · Eric X. Chen[1,2]

Received: 14 July 2019 / Accepted: 4 September 2019 / Published online: 13 September 2019
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## Abstract

**Background** Oxaliplatin is widely used in the treatment of gastrointestinal malignancies. One of the most common and dose-limiting side effects of oxaliplatin is the chronic peripheral sensory neuropathy. The mechanism of this neurotoxicity is poorly understood and there are no effective preventive or treatment strategies, other than oxaliplatin dose interruption or reduction.

**Methods** Colorectal cancer patients who completed FOLFOX at least 6 months prior to enrollment were eligible. EORTC QLQ-CIPN20 questionnaire was used for assessing self-reported neuropathic symptom. Blood samples and skin biopsies were obtained and analyzed for platinum.

**Results** Twelve patients were enrolled. The mean cumulative dose of oxaliplatin was $818 \pm 54$ mg/m$^2$, and the median time from last dose of oxaliplatin was 38.7 months (range: 7.2–65.6 months). The QLQ-CIPN20 sensory score was 18 or less in 10 patients and 19 and 25, respectively, in 2 patients. Platinum was detectable in plasma from 4/12 patients up to 63.3 months after the completion of FOLFOX. In all six patients with skin biopsies, platinum was present in the skin with imaging mass cytometry.

**Conclusions** QLQ-CIPN20 scores and plasma platinum concentrations were not related to cumulative doses of oxaliplatin or interval from the last dose of oxaliplatin. Platinum was readily detectable in skin biopsies more than 60 months post-completion of FOLFOX. This is the first demonstration of platinum deposition in skin post-oxaliplatin treatment and it provides a possible mechanism for oxaliplatin-induced peripheral sensory neuropathy and its persistence.

**Keywords** Colorectal cancer · Platinum · Peripheral sensory neuropathy · Oxaliplatin · Image mass cytometry

## Introduction

Oxaliplatin, a third-generation platinum compound, is widely used in the treatment of gastrointestinal malignancies, such as colorectal cancer, [1–3] pancreatic cancer [4, 5], and gastroesophageal cancer [6, 7]. Treatment with oxaliplatin is associated with the development of a chronic distal sensory neuropathy, which is cumulative and dose limiting. In patients receiving oxaliplatin and 5-FU as adjuvant chemotherapy for locally advanced colorectal cancer, approximately 13% develop grade 3 peripheral neuropathy during treatment, 1.3% at 12 months and 0.7% at 4 years post-treatment, resulting in significant impairment in the quality of life for these patients [8].

The mechanism of oxaliplatin-mediated peripheral sensory neuropathy remains elusive. The transient dysfunction of voltage-gated Na(+) channel has been postulated to play an important role [9]. However, current theories do not explain the persistence and location of oxaliplatin-induced peripheral sensory neuropathy. As a result, pharmacology interventions to prevent and reduce oxaliplatin-induced neuropathy, such as calcium and magnesium infusion and pregabalin, have been unsuccessful [10, 11]. Strategies employing intermittent oxaliplatin administrations have been evaluated in randomized studies with mixed findings [12–14]. The impetus for the International Duration Evaluation of Adjuvant Therapy (IDEA) collaboration was to reduce oxaliplatin-induced neuropathy by shortening the duration of adjuvant chemotherapy from 12 cycles to 6 cycles [15].

✉ Eric X. Chen
eric.chen@uhn.ca

1 Department of Medical Oncology and Hematology, Princess Margaret Cancer Centre, University Health Network, Room 7-824, 700 University Avenue, Toronto, ON M5G 1X6, Canada

2 Faculty of Medicine, University of Toronto, Toronto, Canada

3 Fluidigm Canada Inc., Markham, Canada

This study was conducted to determine whether platinum persisted in skin in patients with colorectal cancer after oxaliplatin-containing chemotherapy by utilizing a recent development technology, imaging mass cytometry (IMC).

## Patients and methods

Patients with colorectal cancer were eligible if they completed adjuvant oxaliplatin and 5-FU (mFOLFOX-6) at least 6 months prior to enrollment [16]. Baseline demographic and treatment information was collected, including gender, age, total oxaliplatin doses received, and interval from the last cycle of chemotherapy to enrollment. The study was approved by the institutional research ethics review board and conducted according to the Declaration of Helsinki. A written informed consent was obtained from each patient prior to enrollment.

Self-reported peripheral sensory neuropathy symptoms were assessed using EORTC QLQ-CIPN20 questionnaire [17]. Patients with CIPN20 sensory score of 18 or below were assigned as mild to moderate PSN (group A), and those with score of 19 or above as severe PSN (group B).

Peripheral venous blood samples were collected and plasma was separated, and analyzed for total platinum levels with high-performance liquid chromatography with tandem mass spectrometry (HPLC/MS–MS). The limit of detection was 0.15 ng/ml.

A skin sample was obtained from the outer aspect of a lower leg of each consented patient using the standard skin punch biopsy technique [18]. One half of each sample was weighed, homogenized and analyzed for total platinum, while the other half was processed immediately into formalin-fixed paraffin-embedded (FFEP) tissue blocks for sectioning. One slide with 5-µm thickness from each patient was stained with the standard H&E technique. A second slide was stained with a metal-conjugated antibody cocktail and analyzed by IMC to visualize platinum deposition ($^{195}$Pt) [19]. Briefly, dewaxed and rehydrated FFPE sections were stained with metal-containing antibodies to various structural epithelial and stroma components. Selected areas from each tissue section were then ablated with a deep UV laser focused to a spot size of 1 µm in diameter, creating individual plumes of particles from each area. These particles were then transferred with high time fidelity to the Helios™ mass cytometer (Fluidigm Canada Inc., Markham, Canada). Individual isotopes of each metal were simultaneously detected and indexed against the source location, yielding an intensity and spatial map of the distribution of biomarkers, including platinum in tissue [20, 21]. The most abundant isotope of platinum, $^{195}$Pt, was used for illustration. Antibody to collagen I, labeled with thulium isotope ($^{169}$Tm-collagen I), and antibody against alpha-smooth muscle actin, labeled with praseodymium isotope ($^{141}$Pr-αSMA), were used for staining stromal components.

## Results

QLQ-CIPN20 scores and blood samples were available from 12 patients. Among these patients, skin samples were obtained from six patients. Patients' demographic characteristics are listed in Table 1. Median age of patients was 50.6 years (range 34.5–69.4 years).

The mean cumulative dose of oxaliplatin was $818.4 \pm 185.8$ mg/m$^2$, and the median time from the last dose of oxaliplatin to enrollment was 38.7 months (range 7.2–65.6 months). Platinum was detectable in plasma in 4/12 patients. For eight patients with non-detectable plasma platinum, the time interval from the last dose of oxaliplatin ranged from 18.6 to 65.5 months. Plasma platinum concentrations did not correlate with the total dose or the time from the last dose of treatment (Fig. 1). There were ten patients with QLQ-CIPN20 sensory score of 18 or less (Group A) and two patients with scores of 19 and 25, respectively (Group B) (Table 1). There was no correlation between QLQ-CIPN20 sensory score and total doses of oxaliplatin or the time from the last dose of treatment (Fig. 2).

Six skin samples were collected and analyzed using IMC. In these six patients, mean dose of oxaliplatin received was $788.3 \pm 64.8$ mg/m$^2$. All six patients were in group A, with mean CIPN 20 sensory score of 14.5 (range 10–18). There was extensive deposit of platinum in skin samples from all six patients, although platinum was not detectable in any of these patients by HPLC/MS–MS and plasma platinum was detectable in only two patients. IMC images from two patients are shown in Fig. 3. One patient had a plasma platinum concentration of 0.27 ng/ml, while the plasma platinum

**Table 1** Demographic and clinical characteristics of patients

| Characteristics | No. of patients ($N = 12$) |
|---|---|
| Sex | |
| Male | 7 |
| Female | 5 |
| Tumor site | |
| Colon | 7 |
| Rectum | 5 |
| Oxaliplatin dose (mg/m$^2$) | |
| Mean | $818.4 \pm 185.8$ |
| Range | 509.4–1014.1 |
| QLQ-CIPN20 score | |
| 9–18 | 10 |
| 19–36 | 2 |

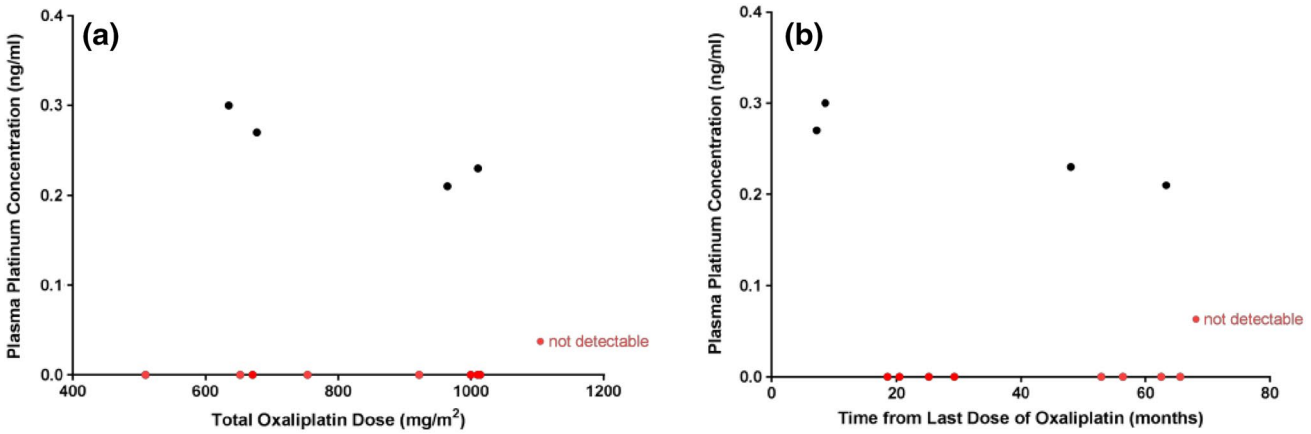


**Fig. 1** Plasma platinum concentration. **a** Total dose of oxaliplatin each patient received, **b** time from the last dose of oxaliplatin

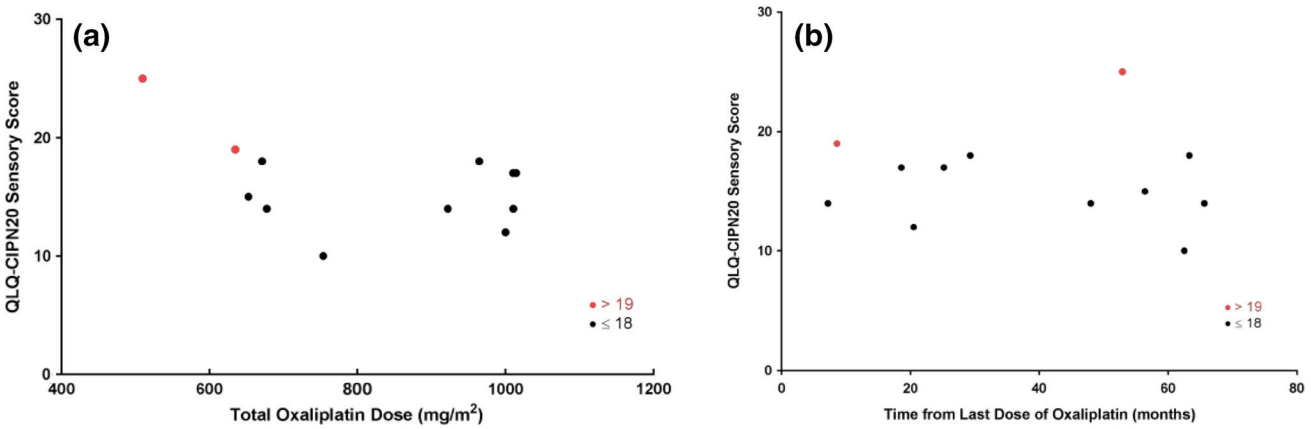


**Fig. 2** QLQ-CIPN20 sensory scores. **a** The total dose of oxaliplatin each patient received, **b** the time from the last dose of oxaliplatin

concentration was not detectable in the 2nd patient. Platinum co-localized with collagen fibers in dermis layers (Fig. 3b, d).

## Discussion

A common toxicity of oxaliplatin-based chemotherapy is the development of a distal sensory neuropathy, which is cumulative and dose limiting. Mechanisms of oxaliplatin-induced peripheral sensory neuropathy remain poorly understood. Nerve conduction, nerve excitability, and needle electromyography studies suggest that the voltage-gated Na(+)-channel may be involved in the development of peripheral neuropathy [22, 23]. Due to this lack of understanding, various strategies in mitigating oxaliplatin-induced peripheral neuropathy have largely been unsuccessful. Loprinzi et al. randomized 353 patients undergoing adjuvant therapy with FOLFOX to intravenous calcium/magnesium before and after oxaliplatin, a placebo or calcium/magnesium before and placebo after oxaliplatin [10]. There were no statistically significant differences in neuropathy among the study arms. Similarly, preemptive use of pregabalin during oxaliplatin infusion did not decrease the incidence of oxaliplatin-induced peripheral neuropathy [11]. The IDEA collaboration enrolled 12,843 patients and evaluated 3 months vs 6 months of oxaliplatin-based adjuvant treatment in patients with Stage III colorectal cancer. Shortening duration of oxaliplatin therapy from 6 months to 3 months significantly reduced the incidences and severity of adverse events, especially neurotoxicity [15]. However, a shorter duration of treatment may only be feasible for patients with lower risks of recurrence, such as those with T1-3 and $N_1$ disease. In addition, oxaliplatin is widely used in treating patients with advanced colorectal cancer, and in other gastrointestinal malignancies such as pancreatic cancer and gastroesophageal cancer.

Oxaliplatin undergoes rapid biotransformation after intravenous administration, resulting in various

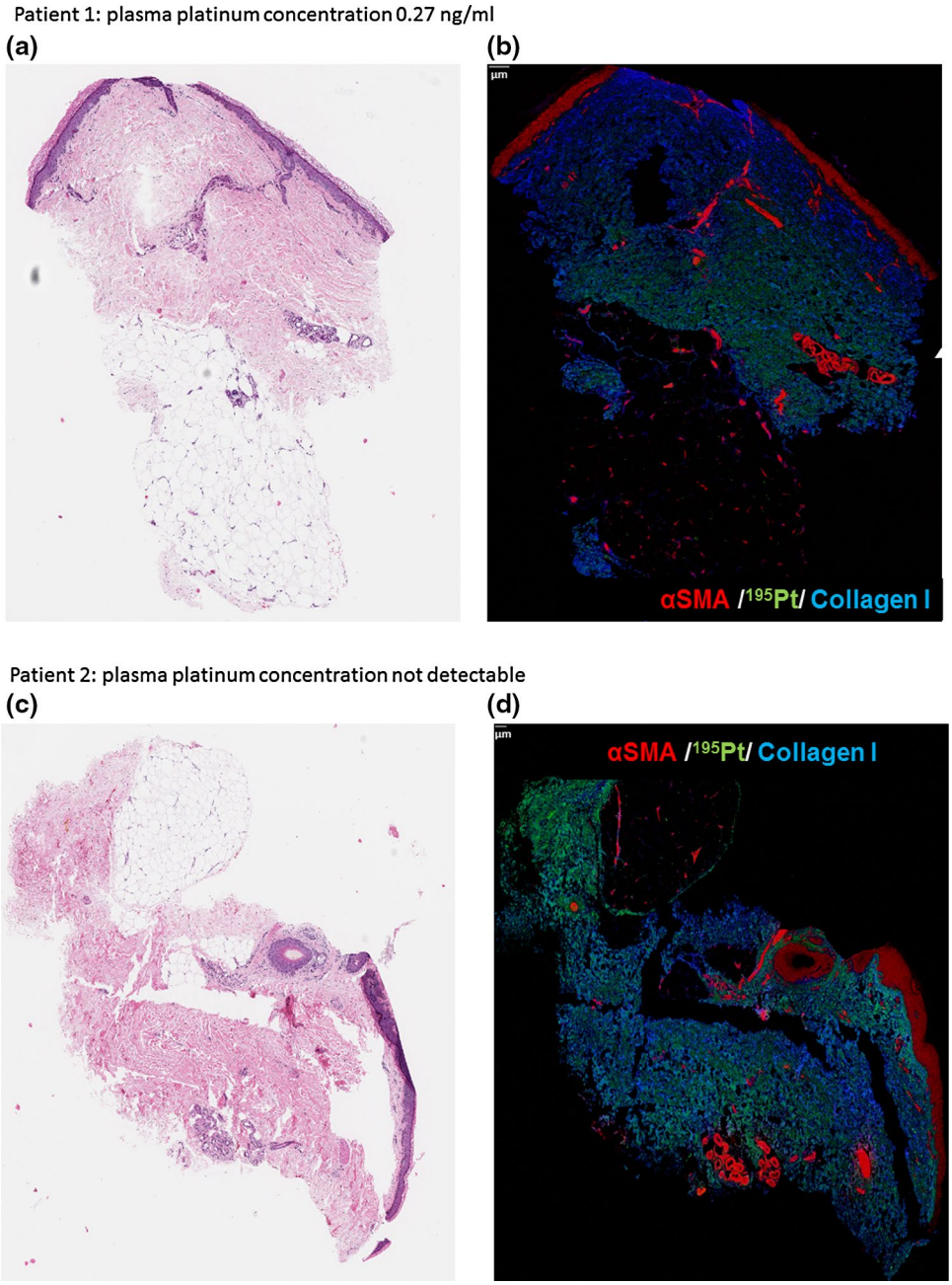


**Fig. 3** Images of skin biopsy samples from two patients. **a**, **c** H & E staining. Scale bar = 100 μm. **b**, **d** IMC images: collagen in blue/platinum in green/αSMA in red. Scale bar = 100 μm

platinum-containing chemical species [24]. These platinum-containing reactive species can form complexes with sulfur-containing compounds and plasma proteins. The terminal elimination half-life of oxaliplatin has been previously reported to be 9 days [25]. However, more recent findings suggest that platinum bound to tissue may persist for much longer periods of time. In patients with testicular cancer, platinum can be detected in serum more than 20 years after completion of cisplatin-containing chemotherapy [26]. Our group previously demonstrated that platinum could be detected in tumor tissues up to 72 days after completion of cisplatin-containing chemotherapy for gastroesophageal cancer [20]. In the current study, platinum was detectable in blood as long as 60 months after the last dose of oxaliplatin.

Breglio et al. recently reported that platinum was retained in human cochlea for many months after cisplatin-containing chemotherapy and postulated that this long retention was related to cisplatin-induced ototoxicity [27]. Gorodetsky et al. showed that platinum could be detected in skin up to 4 months after cisplatin administration with diagnostic X-ray spectrometry [28]. Here, we show the presence of platinum in skin for as long as 60 months after oxaliplatin using a novel imaging technology. The recent development of IMC makes it possible to directly visualize metals, such as platinum in tissues

[21]. IMC appears to be more sensitive since platinum levels in skin are below the level of detection of HPLC/MS–MS. The presence of platinum in skin suggests that a direct toxicity to peripheral nerves may contribute to oxaliplatin-induced neurotoxicity. Our finding is consistent with the cumulative nature of oxaliplatin-induced neurotoxicity. Furthermore, it partly explains the failure of previous attempts at ameliorating oxaliplatin-induced neurotoxicity since none of these attempts addressed the direct presence of platinum in skin and its persistence. Future studies should take into account the persistence of platinum and evaluate strategies of platinum removal to reduce oxaliplatin-induced peripheral sensory neuropathy.

QLQ-CIPN20 scores demonstrated that mild to moderate oxaliplatin-induced peripheral neuropathy was common. However, the score did not correlate with the total dose of oxaliplatin each patient received or the time from the last dose of oxaliplatin administration.

Our findings are limited by the small number of patients enrolled in this study. IMC is a technology in development. At the present time, its analysis is qualitative, rather than quantitative. As a result, we were not able to quantitate the absolute amount of platinum present in the skin. Oxaliplatin causes apoptosis and death of dorsal root ganglion neurons [29, 30]. We did not study the deposition of platinum in dorsal root ganglia since patients enrolled in the study received adjuvant oxaliplatin. Finally, we have not established that skin platinum deposition plays a direct role in oxaliplatin-induced peripheral neuropathy.

In conclusion, we demonstrate the presence of platinum in the skin up to 60 months after oxaliplatin administration. The presence of platinum in the skin may play a role in oxaliplatin-induced peripheral neuropathy. Future studies on mitigating oxaliplatin-induced peripheral sensory neurotoxicity should take into consideration this observation.

**Acknowledgements** We thank Jing Xu for valuable technical assistance.

**Funding** Funding for this study was provided by Princess Margaret Cancer Center Foundation.

## Compliance with ethical standards

**Conflict of interest** Qing Chang and Olga Ornatsky are employees of Fluidigm Canada Inc. Olga Ornatsky is one of the co-founders of DVS Sciences Inc. (now part of Fluidigm) that invented, developed and manufactures mass cytometry technologies, including the Helios CyTOF system, the Imaging Mass Cytometer and metal-conjugated reagents. No other authors declared any potential conflicts of interest.

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