Platinum accumulation and changes in mitochondrial function of the longitudinal muscle & myenteric plexus following oxaliplatin administration

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Introduction: Oxaliplatin is a platinum-based chemotherapeutic agent widely used for the treatment of cancer. Cytotoxicity of this drug is mediated by the formation of platinum adducts on nuclear and mitochondrial DNA. Although effective, oxaliplatin induces chronic gastrointestinal (GI) side-effects including nausea, vomiting, constipation and diarrhoea which can limit dosage and/or result in total treatment cessation. Platinum metabolites from oxaliplatin can be retained in blood plasma and organs for up to 20 years post-treatment. The copper transporter 1 (CTR1) receptor might play an important role in platinum drug influx. Since GI function is controlled by enteric neurons embedded into the GI wall, we hypothesized that oxaliplatin treatment would induce platinum accumulation within and damage mitochondrial function of the longitudinal muscle-myenteric plexus (LMMP). The aims of this study were to investigate: 1) nuclear and mitochondrial platinum and copper concentrations; 2) expression of CTR1; and 3) mitochondrial function of LMMP preparations from oxaliplatin- and sham-treated mice.

Methods: Animal experimentation was approved by the Victoria University Animal Ethics Experimentation Committee and performed in accordance with the Australian Code of Practice for the Care and use of Animal for Scientific Purposes. Balb/c mice received intraperitoneal injections of oxaliplatin (3mg/kg/d) or sterile water tri-weekly for 2 weeks. Mice were culled *via* overdose with sodium pentabarbitone followed by intracardiac perfusion. The LMMP was harvested, dissociated, and the nuclear and mitochondrial fractions were isolated to determine platinum and copper concentrations by atomic absorption spectrophotometry. The expression of CTR1 in LMMP preparations was investigated immunohistochemically. For mitochondrial function studies, the LMMP was harvested, dissociated and cultured for one day prior to assessment of mitochondrial and anaerobic function *via* extracellular flux analysis (Seahorse Bioscience, USA).

Results: Total platinum concentration was significantly greater in the LMMP preparations (11.26ppm ± 0.20, $P<0.0001$, mean ± SEM) from the oxaliplatin-treated compared to the sham-treated cohort (0.0ppm). A significant amount of platinum was found in both the nuclear (4.56ppm ± 0.01, $P<0.0001$) and mitochondrial fractions of the LMMP (2.95ppm ± 0.01, $P<0.0001$) in the oxaliplatin-treated group, but not in the sham-treated cohort (0.0ppm for both). No significant difference in copper concentrations within the total, nuclear or mitochondrial fraction of the LMMP preparations was observed following oxaliplatin treatment. However, oxaliplatin treatment induced a significant reduction in CTR1 in the LMMP preparations (1.30a.u ± 0.34 *vs* sham: 3.05a.u ± 0.31, $P<0.01$). Assessment of mitochondrial function demonstrated a significant depression of basal respiration and metabolic flexibility (both mitochondrial and glycolytic) as indicated by reduced oxidative and anaerobic metabolic potential (all $p<0.05$) in the oxaliplatin-treated cohort when compared to sham-treated controls.

Conclusions: Treatment with oxaliplatin results in platinum accumulation in the LMMP as well as a reduction in CTR1 receptor expression. The CTR1 degrades upon oxaliplatin-binding and the reduction is suggestive of direct toxicity of the LMMP. Despite reduced CTR1 availability to copper transport, the LMMP copper pool was demonstrably normal and therefore, not the cause of mitochondrial and glycolytic potential depression (normal mitochondrial function - specifically cytochrome oxidase (COX; Complex IV) - is dependent on copper homeostasis). Our data suggest, however, that oxaliplatin-induced mitochondrial dysfunction in longitudinal myocytes and myenteric neurons may underlie the chronic GI side-effects associated with this drug.