Title: Platinum-based chemotherapy: gastrointestinal immunomodulation and enteric nervous system toxicity

Abbreviated Title: Platinum-based chemotherapy: immunomodulation and neurotoxicity

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Abstract: The efficacy of chemotherapeutic treatment of colorectal cancer is challenged by severe gastrointestinal side-effects which include nausea, vomiting, constipation and diarrhoea. These symptoms can persist long after the treatment has been ceased. An emerging concept is the ability of platinum-based drugs to stimulate immunity, which is in contrast to conventional chemotherapeutic agents that are immunosuppressive. Here we review the immunomodulatory aspects of platinum-based anti-cancer chemotherapeutics and their impact on gastrointestinal innervation. Given the bi-directional communication between the enteric nervous system and gastrointestinal immune system; exploring the consequences of platinum-induced immunogenicity will facilitate better understanding of gut dysfunction caused by chemotherapeutic agents. We propose that the development of future successful chemotherapeutics should rely on targeting the mechanisms underlying long-term gastrointestinal side-effects.

Keywords: Platinum, chemotherapy, gastrointestinal side-effects, enteric nervous system, immune response, neuro-immune interactions
Chemotherapeutic treatment of colorectal cancer: current challenges

Colorectal cancer (CRC) is one of the leading causes of cancer-related death worldwide (31). Surgical resection is an effective treatment strategy for CRC diagnosed at stages I and II. However, given the asymptomatic nature of this disease, patients are often diagnosed at stages III and IV when metastasis to secondary organs such as the mesenteric lymph nodes, spleen and liver occur. These patients benefit more significantly from chemotherapeutic treatment (32, 65). Chemotherapy and/or radiotherapy can be used prior to and subsequent to surgery, improving the efficacy of anti-cancer treatment. Platinum-based chemotherapeutic agents (cisplatin, carboplatin and oxaliplatin) have shown significant anti-tumour efficacy and oxaliplatin is now used as the first-line treatment for CRC (51). Oxaliplatin is given in combination with 5-fluorouracil (anti-metabolite agent) and leucovorin (folinic acid), making up the FOLFOX regimen (79). In some cases, patients may receive a combination of all three cytotoxic drugs which includes oxaliplatin, 5-fluorouracil and irinotecan (topoisomerase I inhibitor), also known as the FOLFOXIRI regimen (20, 79). All three agents induce DNA damage; however, their mechanisms of action and the type of cell death they induce differ. There is emerging evidence that some anti-cancer chemotherapeutic agents have shown the capacity to modulate immune responses which could be harnessed to enhance anti-tumour immunity by causing immunogenic cell death (40, 43, 84). This concept of immunogenic cell death challenges the notion that anti-cancer chemotherapeutic agents are immunosuppressive or do not induce innate or adaptive immune responses upon cytotoxic events.

Although anti-cancer chemotherapeutic agents are effective, their use is associated with unfavourable side-effects which is a major hurdle that compromises their efficacy. The
side-effects associated with platinum-based drugs include central and peripheral neurotoxicity, cardiotoxicity, nephrotoxicity as well as severe gastrointestinal (GI) complications such as nausea, vomiting, constipation and diarrhoea, which are debilitating to patients and account for dose-limitations and/or cessation of treatment (2, 15, 16, 52, 81). Chemotherapy-induced mucosal damage plays a significant role in the acute form of the GI side-effects such as constipation and diarrhea, however, these symptoms can persist up to 10 years after the treatment has been ceased (14). Retention of reactive platinum compounds which are still capable of inducing DNA adducts can be found in the body up to 20 years post-treatment with platinum-based agents (26).

A new emerging concept implicates that persistent symptoms of GI dysmotility are due to the damage to the enteric nervous system caused by platinum-based agents. The enteric nervous system functions to regulate GI motility, secretion, vascular tone and the absorption of nutrients (21). Recent studies have demonstrated that platinum-based agents have the capacity to induce morphological and functional changes in enteric neurons (74, 78). However, the exact mechanism for chemotherapy-induced enteric neuropathy remains unknown, and further investigation is required to determine whether it is a direct toxic effect of anti-cancer chemotherapy, or whether it is induced by indirect mechanisms. Current treatments to ameliorate the side-effects of anti-cancer chemotherapy are not always effective, and can cause adverse reactions. Therefore, there is the crucial need to explore potential gateways for enteric neuropathy to discover novel treatment strategies for the improvement of anti-cancer therapy.
Chemotherapy-induced immunogenic cell death

Until recent years chemotherapy-induced cell death was deemed an immunologically ‘silent’ or ‘tolerogenic’ event. However, it is becoming more evident that platinum-based chemotherapeutic agents can in fact prompt a fatal immune response to stressed or injured cancer cells, which is known as immunogenic cell death. Cellular stress, particularly endoplasmic reticulum and oxidative stress caused by some anti-cancer agents used for the treatment of CRC can induce the translocation of intracellular proteins to the plasma membrane, and can also result in the release of molecules which act as ‘eat me’ signals for the recognition by immune cells. These signals are known as damage-associated molecular patterns (DAMPs) and their presentation is critical for triggering or enhancing an anti-tumour response (24, 38). DAMPs vital for eliciting immunogenic cell death include calreticulin, high-mobility group protein B1 (HMGB1), ATP as well as some heat shock proteins (HSPs) (24, 48). Under normal conditions calreticulin is an endoplasmic reticulum chaperone protein; it functions to regulate calcium homeostasis, and is involved in the assembly of Major Histocompatibility Complex (MHC) class I molecules. Upon stressful stimuli, calreticulin translocates to the cell surface and acts as a potent ‘eat me’ signal recognised by phagocytes and dendritic cells (24, 38). The nuclear protein HMGB1, which functions to regulate DNA and chromatin transcription, can act as a chemoattractant when released from stressed or dying cells (38). HMGB1 also induces dendritic cell activation and maturation via toll-like receptor 4 (TLR4) which is crucial for T cell priming and activation (38). ATP is involved in many cellular functions such as differentiation, proliferation, adhesion and death. The release of ATP by cells undergoing apoptosis acts as a ‘find me’ signal which is recognised by monocytes (38). ATP also activates the purigenic receptor P$_{2X}$ on dendritic cells, leading to the secretion of IL-1β and polarization of IFN-γ producing
cytotoxic CD8+ T cells (18, 25). HSPs are chaperones involved in the folding of newly synthesized proteins. In circumstances of cellular stress, HSPs such as HSP70 and HSP90 can translocate to the cell surface and interact with a number of receptors belonging to antigen presenting cells (APCs), as well as activating natural killer (NK) cells and cross presenting antigens to CD8+ T cells (24).

The type of DAMPs which are presented or released, as well as their recognition by immune cells, depends on the type of stimulus (anti-cancer drug) and the resulting cellular stress induced (Table 1). Although the GI tract comprises the largest portion of immune cells within the body, little is known about the effects of anti-cancer chemotherapeutic drugs on the induction of DAMPs as well as their effects on resident immune cells within the intestines. There are only a few studies which have examined the effects of anti-cancer chemotherapeutic agents on GI immunity and CRC cell lines. The majority of studies have used human peripheral blood and various tumor cell lines exclusively or co-cultured with immune cells including, macrophages, dendritic cells, natural killer (NK) cells, CD4+ and CD8+ T cells (Table 1). Future studies investigating the effects of anti-cancer chemotherapy directly on the resident GI immune cells and populations within the Peyer’s patches and mesenteric lymph nodes is warranted. Employment of conventional fluorescence-activated cell sorting, immunohistochemistry, cytochemistry, cytokine analysis and molecular methods can be used to investigate the effects of chemotherapeutic agents on immune cells.

**Platinum-based chemotherapeutic agents: induction of DAMPs and immune responses**

Cisplatin (cis-diaminedichloridoplatinum(II)) is the first platinum-based anti-cancer
chemotherapeutic drug and is the predecessor to carboplatin (cis-diammine(1,1-cyclobutanedicarboxylato)platinum(II) (54) and to the third-generation agent oxaliplatin [(1R,2R)-cyclohexane-1,2-diamine](ethanedioato-O,O')platinum(II) (55). Although structurally different, these platinum analogues exert their anti-cancer effects via the formation of similar DNA platinum adducts or intrastrand and interstrand cross-links (51). At the site of bound platinum adducts, DNA denatures, which leads to strand breaks (1) (Figure 1). Ultimately, there is DNA synthesis arrest, inhibition of RNA synthesis and transcription, followed by the activation of apoptotic pathways and essentially reduction of tumour cell replication (1). Research on the effects of platinum-based drugs on GI cancers and immunity remains quite limited; however, there are a few studies which highlight the ability of cisplatin, carboplatin and oxaliplatin in inducing beneficial immune responses against tumors.

**Cisplatin**

Eliciting immunogenic cell death requires endoplasmic reticulum and/or oxidative stress, as well as the presentation of DAMPs. Cisplatin has the ability to induce tumour cell release of HMGB1, but fails to prompt the translocation of calreticulin to the cell surface, given its inability to cause severe endoplasmic reticulum stress (48, 72). The inability to prompt such translocation consequently renders the apoptotic process as non-immunogenic. Analysis of NK cell functions in peripheral blood of patients with GI cancer revealed that a low-dose of cisplatin and 5-fluorouracil can prevent the suppression of NK cells, enhancing innate anti-cancer immunity (30). Cisplatin and carboplatin can enhance the phagocytic activity of peritoneal macrophages by increasing the number of lysosomes formed; essential for the lysis of tumor cells (63). Furthermore, cisplatin and carboplatin induce the dephosphorylation of STAT6 in
dendritic cells derived from melanoma and CRC patients as well as tumour cells (41).

STAT6 is important for the regulation of the T cell inhibitory molecules known as programmed cell death ligand 1 (PD-L1) and 2 (PD-L2) expressed on dendritic cells. A decrease in dephosphorylated STAT6 is associated with the downregulation of the inhibitory molecule PD-L1, but more so PD-L2, therefore enhancing the activation of T cells by dendritic cells (41). A recent study investigating the effect of the anti-cancer chemotherapeutic agents cisplatin in combination with a taxane drug paclitaxel (mitotic inhibitor) on ovarian anti-tumour immunity revealed that this combination prompted the recruitment of macrophages and CD8+ T cell responses which were tumour specific in the peritoneal cavity of the abdomen (10).

Carboplatin
There is little evidence in regards to carboplatin’s ability in eliciting DAMPs and inducing immunogenic cell death. However, like its predecessor cisplatin, carboplatin can also exhibit a positive anti-tumour immune response. Exposure to antigens from ovarian cancer cells treated with carboplatin and paclitaxel led to the induction of dendritic cell phagocytic and antigen recognition activity (83). Apoptotic bodies from cancer cells can drive dendritic cell maturation as they are internalized and processed for antigen presentation, increasing CD80, CD83 and CD86 expression essential for T cell priming and activation.

Oxaliplatin
Oxaliplatin is regarded as a potent stimulator in inducing the presentation of DAMPs and immunogenic cell death (40, 72). Oxaliplatin prompts the translocation of calreticulin and HSP70 to the surface of dying tumour cells, the release of HMGB1 and the secretion of ATP, thereby instigating their recognition by APCs for eventual
presentation to effector T cells (Figure 1) (38, 72). The above mentioned DAMPs are vital for triggering an immune response, and the failure to elicit one or more of these danger signals can abolish the immunogenic apoptosis pathway. HMGB1 function has been investigated in Balb/c TLR4−/− mice bearing CT26-induced colon cancer (72). The TLR4 serves an important role as it is a key receptor in which dendritic cells use to recognise HMGB1. This is taken up by dendritic cells and expressed on MHC-I (also known as cross presentation). Dendritic cells will then travel to the lymph nodes to activate CD8+ T cells; resulting in cytotoxic T cell induction and ultimate killing of cells bearing HMGB1 and calreticulin peptide; MHC-I complexes. However, the above functions are impeded in the absence of TLR4 on dendritic cells (72). Investigation of the effects of oxaliplatin on cultured dendritic cells derived from the blood of healthy donors demonstrated an increase in T cell activation as marked by the heightened production and secretion of cytokines IL-2 and IFN-γ (43). The activation of CD8+ T cells could be hindered if sufficient numbers of anti-inflammatory myeloid-derived suppressor cells (MDSCs), tumour-associated macrophages (TAMs) and regulatory T cells (Tregs) are found in the microenvironment, thus reducing the effectiveness of oxaliplatin. There is only one study to date which has assessed the impact of oxaliplatin on CD4+ T cell subsets (46). This study had demonstrated that oxaliplatin is effective at reducing Treg numbers when combined with other chemotherapeutic agents in CRC patients. Together with MDSCs and TAMs, these cells secrete the antagonistic Th2 cytokine IL-10, ultimately causing suppression of Th1 responses (22). Thus chemotherapeutic agents that target these IL-10 producing cells will give the immune system an opportunity to surmount Th2 responses thus clearing the way for Th1 anti-tumour responses. This may justify further studies on immune cell functions in response to oxaliplatin treatment.
To date, platinum-based agents are amongst the most effective in killing cancer, however, they are also cytotoxic against a range of healthy cells leading to many side-effects.

**Side-effects of platinum-based anti-cancer chemotherapy**

Anti-cancer chemotherapeutic agents can induce side-effects to the nervous, cardiovascular, renal and GI systems (2, 16, 52). The incidence of central nervous system side-effects have been concomitant with the use of cisplatin, which accounts for acute encephalopathy presented as alterations in consciousness, seizures, cerebral infarctions, paralysis, ototoxicity (7, 75). Peripheral sensory neuropathy generally surface as distal paraesthesia (tingling or numbness), cold-induced dysesthesia (burning sensations), pain and loss of sensations (81). Approximately 90% of patients undergoing oxaliplatin treatment show symptoms of acute peripheral sensory neuropathy within the first 24-48 hours of chemotherapeutic infusion which can be transient due to neuron hypersensitivity (37), and/or cumulative and persistent up to 29 months following chemotherapy due to chronic neuropathy (64). Platinum-based drugs have also been implicated with cardiac toxicity, causing diastolic dysfunctions, hypertension and myocardial ischemia (2, 26). Cisplatin and high-dose carboplatin treatments cause acute kidney injury and renal insufficiency in 20-30% of patients leading to progressive and permanent nephrotoxicity (52). These side-effects along with prominent GI side-effects, discussed below in more detail, are major reasons for dose limitations, compromising optimal anti-cancer chemotherapeutic treatment and overall patient quality of life (50, 70).
Gastrointestinal side-effects of platinum-based chemotherapy: current treatments and their limitations

Diarrhoea, constipation, oral mucositis, nausea and vomiting are common GI side-effects of chemotherapeutic medications including platinum-based agents (50, 53, 70). As a result of these side-effects, patients develop malnutrition and dehydration which lead to rapid weight loss (cachexia) (27, 53). In some cases, the addition of platinum-based drugs to combination therapies causes severe intestinal inflammation, bowel wall thickening and ulceration (39). The incidence of chronic post-treatment diarrhoea amongst cancer survivors varies from 14% to 49% and episodes of diarrhoea can persist for more than 10 years (14, 50). Various antiemetic drugs are available in clinical practice: corticosteroids and drugs acting on various neurotransmitter receptors including dopaminergic, histaminic, muscarinic and serotoninergic (57, 68). However, all these agents have side-effects including central nervous system (headache, insomnia, dizziness, nervousness, anxiety, fever, tremor or twitching, ataxia), cardiovascular (arrhythmia, heart failure), GI (constipation, diarrhoea), hepatic and renal disorders (19, 68). A new class of antiemetics, a selective NK1 receptor antagonist, aprepitant, inhibits cytochrome P450 isoenzyme 3A4 and can lead to significant drug interactions, resulting in need for dose modification of concomitant therapy (56, 68). Moreover, delayed nausea and vomiting remain a significant clinical problem occurring frequently after treatment, but the pathophysiology of delayed emesis is not well understood (69).

Despite the number of clinical trials evaluating therapeutic or prophylactic measures in chemotherapy-induced diarrhoea, the most common current treatment is a µ-opioid receptor agonist loperamide which causes abdominal pain, bloating, nausea, vomiting, constipation, paralytic ileus, dizziness, rashes and anaphylaxis (50, 66, 70). GI side-effects associated with anti-cancer chemotherapy are traditionally thought to be due to
mucosal damage. Although mucosal insult plays a significant role in the acute symptoms associated with chemotherapy (33), the persistence of GI symptoms long after the treatment suggests that anti-cancer drugs may induce damage to intestinal innervation.

Taking into account that platinum-based chemotherapeutic agents have the ability to accumulate and enhance immune responses, changes in neuro-immune interactions could possibly impact the GI innervation, and consequently, cause long-term gut dysfunctions which are experienced by cancer patients.

**Neuro-immune interactions in the gastrointestinal tract**

The enteric nervous system (ENS) is a complex orchestration of neurons innervating the GI tract and controlling its functions (21). Several different classes of neurons reside in the ENS and differ in terms of cell body morphology, electrophysiological properties, neurotransmitter synthesis and release, and types of synaptic inputs received (21). Functional types of neurons within the ENS include interneurons, intrinsic primary afferent, muscle motor, secretomotor and vasomotor neurons. Neurons are arranged into ganglia forming two major plexuses: myenteric located between the circular and longitudinal muscles, regulating the movement of the contents along the gut (motility) and submucosal located between the circular muscle layer and submucosa, regulating secretion, fluid and electrolyte balance as well as vascular tone (21). In addition to this, it is becoming more evident that the ENS may play a role in GI immunity, and vice versa. Interactions between enteric neurons and immune cells have been shown in both normal and pathological conditions (34). These interactions may be via direct cell-cell contact or by the production and release of neuronal and immune soluble mediators. Direct anatomical and functional communication occurs between enteric nerve fibres
and lymphoid tissues embedded in the intestines such as the Peyer's patches (45, 77) as well as immune cells located in the lamina propria and mucosa (59). Enteric glial cells also play an important role in neuro-immune communication via cytokine receptors and the ability to produce both cytokines and neurotransmitters (58). Enteric neurons express receptors for soluble immune mediators such as cytokines and chemokines, and immune cells attain receptors on their surface for neuropeptides (12, 36). Several neuropeptides in the ENS can be recognised by immune cells including neuropeptide Y (NPY), vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP) and substance P. NPY can inhibit the production and release of pro-inflammatory cytokines such as IFN-γ by Th1 cells and promote the secretion of anti-inflammatory mediators such as IL-4 by Th2 cells (12). VIP functions similarly to NPY in that it also induces a shift in the production of anti-inflammatory cytokines in contrast to the pro-inflammatory type and inhibits leukocyte migration (4). Substance P is primarily involved in the activation of myeloid cells for the induction of inflammatory responses (12). CGRP and substance P induce secretion of pro-inflammatory cytokines and leukocyte migration (12). Moreover, enteric neurons are capable of producing pro-inflammatory cytokines such as IL-8 (73) and immune cells are capable of producing neuropeptides. VIP is produced by T cells, B cells, mast cells and eosinophils (13); substance P is secreted by macrophages, eosinophils, lymphocytes, and dendritic cells (35, 62). One of the major neurotransmitters in the GI tract, acetylcholine, is released by both preganglionic vagal efferents and enteric neurons. Non-neuronal cells such as mononuclear leukocytes, bone marrow-derived dendritic cells and skin mast cells can also synthesize acetylcholine as determined by immunohistochemical and high-performance liquid chromatography methods (82). The enzyme choline acetyltransferase which is essential for acetylcholine production is found in immune
cells such as macrophages, dendritic cells and lymphocytes (12, 82). In addition, these cells express other markers of the cholinergic system, including nicotinic and muscarinic acetylcholine receptors, and the enzyme acetylcholinesterase, thus forming a non-neuronal cholinergic system (3). Interaction between efferent vagus nerve signalling and nicotinic acetylcholine receptors expressed on macrophages and other non-neuronal cytokine producing cells residing in the GI tract plays an important anti-inflammatory role (23). Stimulation of vagal efferent nerve terminals inhibits the release of pro-inflammatory cytokines (47, 80) and production of the pro-inflammatory mediator HMGB1 (29). This “cholinergic anti-inflammatory pathway” has been recognized as a physiological mechanism by which the nervous system interacts with the innate immune system to restrain inflammatory responses (12). As each system has the potential to regulate the functions of the other, aberrant immune responses and neuro-immune interactions may therefore cause detrimental effects on neurally-controlled GI functions such as motility and secretion. It has been shown that cytokines such as IL-1β and IL-6 have the ability to influence neuronal electrophysiological activity and modulate neurotransmission, supporting the notion that inflammation or local changes of cytokine levels can impact on GI motility and secretion (49). In inflammatory conditions, it has been shown that hypertrophy of neurons and degeneration of axons and ganglia becomes apparent leading to a decrease in the number of neuronal synapses, whilst there is an increase in the amount of lysosomes within the soma (36). The increase in lysosomes in inflammatory states is thought to be one of the mechanisms for inducing neurodegeneration.

Given that chemotherapeutic agents have the capacity to kill not only cancer, but other off-target cells, they can impact enteric neurons leading to alteration in neurally-
controlled GI functions, which could underlie side-effects experienced by patients undergoing anti-cancer chemotherapeutic treatment.

**Effects of platinum-based chemotherapy on the enteric nervous system**

Damage to the enteric neurons and their subsequent death underlie the symptoms of persistent pain, disorders of motility and secretion in the intestine, including diarrhoea, constipation and slow transit disorders (34). The correlation between ENS damage and the long-term changes in GI functions have been shown in previous studies on diabetes (9) and GI inflammation (42, 44, 61). Despite mounting support for the possibility of chemotherapy-induced enteric neuropathy, research in this area is scarce. To date, the effects of platinum-based anti-cancer chemotherapeutic agents on enteric neurons and the changes in GI functions caused by cisplatin and oxaliplatin have been shown by two research groups (74, 78). Both studies in animal models provided strong evidence that platinum-based treatment causes death of enteric neurons, morphological alterations and increases in the proportion of nitric oxide synthase (NOS)-immunoreactive inhibitory muscle motor neurons. These changes in the ENS are correlated with impairment of colonic motility and GI symptoms (diarrhoea and constipation).

Treatment of patients with combination chemotherapy including oxaliplatin and 5-fluouracil causes the translocation of Hu protein (Figure 2). Hu proteins are important for the regulation of mRNA in the nucleus and cytoplasm. The loss of cytoplasmic Hu protein contributes to mRNA degradation, which is indicative of neuronal stress and damage (28). Immunohistochemical labelling of the myenteric plexus derived from CRC patients treated with 5-fluorouracil also show some degree of Hu translocation in the
absence of oxaliplatin (unpublished data). However, there is greater Hu translocation in
the FOLFOX-treated group. Damage to the ENS might be caused directly by platinum-
based chemotherapeutics due to their accumulation in the enteric ganglia and formation
of DNA adducts in the enteric neurons (Figure 3A). This hypothesis is supported by the
fact that long-term retention of platinum in the plasma and tissues has been observed
8–75 months after treatment with cisplatin and oxaliplatin (6). Accumulation of
platinum compounds and neuronal apoptosis has been found to occur in dorsal root
ganglia (71). In our studies using the atomic absorption spectrophotometry we were
able to detect significant amount of platinum in dissociated enteric neurons in mice
following repeated in vivo injections of oxaliplatin (unpublished data). This suggests a
direct enteric neuronal toxicity caused by oxaliplatin. Whether damage/death induced
by oxaliplatin is specific to certain enteric neuronal subtypes should be further
investigated as this may be correlated with gut dysfunctions. Other methods for the
detection of platinum include inductively coupled plasma mass spectrometry,
synchrotron-based scanning transmission X-ray spectromicroscopy, transmission
electron microscopy and use of fluorophore-conjugated platinum drugs (5, 17, 67).
Another possible mechanism for enteric neuropathy associated with anti-cancer
chemotherapy may be indirect effects via immune responses to platinum-based agents.
The chemotherapeutic influence on immune activity may elicit changes in neuro-
immune interactions. Thus it can be speculated that the recruitment of leukocytes
and/or stimulation of the production and release of soluble mediators such as cytokines
evoked by chemotherapy can inadvertently induce changes in the ENS structure and
functions, or even induce neuronal damage and death (Figure 3B). Further studies
should be conducted to investigate the GI immune response to platinum-based agents
and correlated with the morphological and functional changes in the ENS.
Concluding remarks and future perspectives

Platinum-based chemotherapeutic agents have demonstrated significant anti-tumour efficacy, and have shown the ability to modulate immune responses which could potentially be exploited for immunotherapy against cancers. However, platinum-based anti-cancer chemotherapeutic agents are also associated with neurotoxicity, along with a range of side-effects. The GI symptoms in particular are generally thought to arise as a consequence of chemotherapy-induced mucositis. However, the persistence of the GI side-effects suggests that enteric neuropathy is induced by the treatment. Investigations into the two possible mechanisms for enteric neuropathy: platinum accumulation within enteric neurons and/or chemotherapy-induced immunomodulation, which could lead to aberrant neuro-immune interactions/collateral damage to neurons, affecting GI functions controlled by the ENS is warranted.

Current knowledge on chemotherapy-induced immune responses within the GI tract, particularly in response to platinum-based chemotherapeutic agents, remains fairly limited and requires further investigation. Understanding the fate of the metabolites from these platinum-based drugs as well as the immune response and neuro-immune interactions could lead to novel therapeutic strategies to prevent neuropathy, ameliorate the GI side-effects and ultimately improve the treatment outcome and patient quality of life.

Acknowledgements

The authors are grateful to Professor Dale Godfrey (Department of Microbiology and Immunology, The University of Melbourne) for reviewing the manuscript.

Table 1. Summary of the immunogenic potential of anti-cancer chemotherapeutic agents used for the treatment of CRC.
<table>
<thead>
<tr>
<th>Chemotherapeutic agent</th>
<th>Mechanism of action</th>
<th>Immunogenicity</th>
<th>Organ</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cisplatin</strong></td>
<td>DNA platinum-adduct formation</td>
<td>Activates immune response by inducing the release of HMGB1 (but does not cause immunogenic cell death) Improves the number of NK cells Induces an increase in lysosome formation by macrophages (tumor lysis mechanism) Induces macrophage recruitment and CD8+ T cell responses Decreases STAT6 resulting in the downregulation of PD-L1 and PD-L2, enhancing T cell</td>
<td>CT26 colon cancer cell line</td>
<td>(48, 72)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Human peripheral blood</td>
<td>(30)</td>
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<td></td>
<td></td>
<td></td>
<td>Murine macrophage culture (derived from peritoneum)</td>
<td>(63)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Human peripheral blood</td>
<td>(10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dendritic cell culture (cells derived from myeloma and colorectal cancer patients)</td>
<td>(41)</td>
</tr>
<tr>
<td><strong>Carboplatin</strong></td>
<td>DNA platinum-</td>
<td>Induces dendritic cell phagocytic and</td>
<td>OVCAR-3 ovarian cancer</td>
<td>(83)</td>
</tr>
<tr>
<td>Drug</td>
<td>Adduct formation</td>
<td>Antigen recognition, increases the expression of CD80, CD83 and CD68, improved CD8+ T cell numbers and the secretion of IFN-γ</td>
<td>Cell line and dendritic cell co-cultures, human peripheral blood</td>
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<td>---------------------------------------------------------------</td>
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<tr>
<td><strong>Oxaliplatin</strong></td>
<td>DNA platinum-adduct formation</td>
<td>Activated immunity by inducing immunogenic cell death via the presentation and secretion of DAMPs (calreticulin, HMGB1, ATP and HSP70)</td>
<td>CT26 colon cancer cell line (38, 72)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Induces dendritic cell antigen presentation and T cell activation resulting in the marked increase in the production and secretion of IL-2 and IFN-γ</td>
<td>Peripheral blood, A549 lung cancer cell line (43)</td>
<td></td>
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<tr>
<td><strong>5-fluourouracil</strong></td>
<td>DNA metabolite incorporation and thymidylate synthase inhibiton</td>
<td>Can enhance anti-tumor immunity by selectively depleting myeloid derived suppressor cells in spleen and tumor bed, however cell</td>
<td>Isolated myeloid derived suppressor cells and EL4 lymphoma cancer cell line (76)</td>
<td></td>
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<tr>
<td><strong>Irinotecan</strong></td>
<td>Topoisomerase I inhibition leading to DNA strand breaks</td>
<td>Induces severe myelosuppression (neutropenia, leukopenia, anemia and thrombocytopenia)</td>
<td>Peripheral blood</td>
<td>(8, 20)</td>
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Death is not considered immunogenic. Causes the release of HMGB1 from colon carcinoma cells but does not induce immunogenic cell death due to the inability to cause the translocation and surface expression of calreticulin, a critical DAMP. Can cause myelotoxicity by oxidative stress. 5-FU induces the induction of haem oxygenase-1 and a decrease in glutathione content in bone marrow cells.

MC38 colon cancer cell line and peritoneal fluid

Murine bone marrow

(11)

(60)
Single nucleotide polymorphism in ABCG2 gene which codes for proteins involved in detoxification and transport of irinotecan metabolite SN-38 can alter transport activity of drug metabolite and elevate systemic circulation, leading to severe myelosuppression.
Table 2. Outstanding questions on chemotherapy-induced immunogenic cell death and damage to the ENS.

<table>
<thead>
<tr>
<th>Question</th>
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<tbody>
<tr>
<td><strong>How do platinum drugs modulate the effects of all types of immune cells?</strong></td>
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<tr>
<td>o Do the changes in immunity in response to chemotherapeutic agents contribute to enteric neuropathy?</td>
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<tr>
<td>o Which immune cells are involved in neuronal damage and/or death? What are the mechanisms?</td>
</tr>
<tr>
<td>o Which specific cytokines are involved in the changes in neuronal functions?</td>
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<tr>
<td><strong>Do metabolites from platinum-based drugs accumulate within the ENS?</strong></td>
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<td>o Is the platinum accumulation specific to enteric neurons only or affects glial cells in the ENS as well?</td>
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<tr>
<td><strong>Do the platinum-based drugs affect all neurons in the ENS, or is there a particular type of neuron more susceptible to damage and/or death?</strong>*</td>
</tr>
<tr>
<td><strong>Which neuroprotective treatments are the most effective for reducing/preventing enteric neuropathy associated with platinum-based chemotherapy</strong></td>
</tr>
<tr>
<td><strong>Would the neuroprotective treatments affect the anti-cancer efficacy of these platinum drugs?</strong></td>
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</table>
**Figure Legends**

**Figure 1. Immunogenic apoptosis of the tumour cell induced by oxaliplatin**

A. Oxaliplatin is metabolised into monochloro, dichloro and diaquo complexes, which form DNA platinum-adducts (B) causing denaturation of DNA. C. In an oxaliplatin-induced apoptotic event, there is translocation of intracellular proteins such as calreticulin and HSP70 to the cell surface and the release of HMGB1 and ATP. Dendritic cells can recognise these proteins in addition to antigens. D. The release of soluble tumour antigens into the extracellular environment may initiate innate mechanisms of tumour immunity. Antigen recognition and presentation can induce a form of receptor-mediated endocytosis and subsequent phagocytosis by antigen presenting cells (APCs) such as macrophages and dendritic cells. E. Dendritic cells may also uptake tumour antigens which are then processed and presented on Major Histocompatibility Complex (MHC) class II molecules. They can also capture antigens from the surface of tumour cells (not shown in figure) and process them on MHC class I molecules (this is known as cross presentation). F. Cytotoxic T cells then have the ability to kill tumour cells expressing MHC class I tumour antigen complexes. This interaction leads to the release of cytolytic proteins (perforin) and cytotoxic granules (granzyme B). H. Opsonisation of membrane-bound tumour antigens (including translocated proteins such as calreticulin) by IgG antibodies is another mechanism which can be used to induct the direct killing of tumour cells by natural killer cells, in a process called antibody dependent cell-mediated cytotoxicity (ADCC). Like cytotoxic T cells, natural killer cells can also release perforin and granzyme B, inducing tumour cell death.
Figure 2. Effect of treatment with FOLFOX on human myenteric neurons. Confocal images of the colon wholemount preparations labelled with an antibody to the pan-neuronal marker human neuronal protein (Hu). A. Hu-immunoreactive myenteric neurons in the colon tissue resected from colorectal cancer patient untreated with chemotherapy. B. Translocation of Hu protein to the nuclei of myenteric neurons in the colon tissue from a colorectal cancer patient treated with FOLFOX prior to surgery.

Figure 3. Proposed mechanisms underlying oxaliplatin-induced enteric neuropathy

A. Oxaliplatin metabolites entering the GI tract via arterial circulation, can accumulate within the myenteric and submucosal ganglia. These platinum metabolites form adducts on enteric neuronal DNA, leading to DNA denaturation and eventual cell death. B. Oxaliplatin metabolites can form platinum adducts in the DNA of mucosal epithelial cells inducing damage/death. Under these conditions, epithelial cells release various chemotactic cytokines, leading to the infiltration of enterotoxins from the lumen into the lamina propria. This can prompt activation of resident leukocytes which can release a variety of cytokines and neurotoxins, inducing damage to the neuronal processes projecting to the mucosa. Alongside this, leukocytes may also invade the submucosal and myenteric ganglia, where they release cytokines and neurotoxins which can lead to neuronal damage/death. In both instances, damage to the enteric neurons will induce altered gut functions (motility and secretion) and cause severe GI symptoms such as diarrhoea and constipation, which can persist long after chemotherapeutic treatment.
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<td>APCs</td>
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randomized trial of bolus 5-fluorouracil/leucovorin plus or minus oxaliplatin: a prospective analysis.


58. Neunlist M, Van Landeghem L, Mahe MM, Derkinderen P, des Varannes SB, and Rolli-


Oxaliplatin is metabolized into monochloro-, dichloro- and diaquo-diaminocyclohexane complexes

Oxaliplatin metabolites form DNA platinum adducts, causing DNA strand breaks, cell cycle arrest and death

Oxaliplatin induces translocation of calreticulin (C) and HSP70 to the cell surface, and release of HMGB1 and ATP recognized by dendritic cells for antigen presentation

Membrane-bound tumor antigens are opsonized by IgG antibodies for induction of direct killing by natural killer cells via antibody-dependent cell-mediated cytotoxicity (ADCC)

Colorectal cancer cells expressing MHC class I tumor antigen complexes are killed by cytotoxic T cells via the release of cytolytic proteins (perforin) and cytotoxic granules (granzyme B)

Dendritic cells uptake soluble tumor antigens for processing and presentation in lymph nodes via MHC class II molecules for CD4+ T cell polarization, and MHC class I for CD8+ T cells cross presentation (not shown)

Antigen binding to innate receptors induces receptor-mediated endocytosis and subsequent phagocytosis by antigen-presenting cells (macrophages and dendritic cells)
A Platinum accumulation

1. Platinum metabolites enter gastrointestinal tract via arterial blood flow

2. Platinum metabolite accumulation in submucosal and myenteric ganglia leading to neuronal dysfunction and death

3. Neuronal DNA platinum-adduct formation

B Inflammatory neuropathy

1. Intestinal epithelial cell damage/death and release of cytokines

2. Infiltration of enterotoxins

3. Activation of the innate immune system

4. Activation of the adaptive immune system

5. Leukocyte secretion of cytokines and neurotoxins

6. Axonal damage and neuronal dysfunction and death

Legend:
- Platinum metabolite
- Erythrocyte
- Neutrophil
- Macrophage
- Dendritic cell
- Cytokines
- Eosinophil-derived neurotoxins
- Nerve fibre
- Damaged nerve fibre