Meeting Report: Inaugural Chemotherapy-Induced Peripheral Neuropathy

Symposium - Santa Barbara, CA, February 2015

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Abstract

Chemotherapy-induced peripheral neuropathy is a common, dose-limiting side effect of cancer treatment. This conference was the first of its kind to bring together a wide range of clinicians, researchers, and industry professionals to address the potential causes, prevented, and treatments for this drug toxicity. Intraepidermal nerve fiber loss, axonal degeneration, immune cell infiltration, alterations in tubulin protein expression and microtubule stability, axonal transport, and mitochondrial dysfunction were addressed as possible mechanisms. Problems with animal models of the disease were discussed, as well as the potential of patient-derived induced sensory neurons to serve as a novel \textit{in vitro} model.
Introduction

The Chemotherapy-Induced Peripheral Neuropathy Symposium was held in Santa Barbara, California to address the current research and the many challenges involved in understanding and treating peripheral neuropathy caused by cancer chemotherapeutic drugs. Chemotherapy-induced peripheral neuropathy (CIPN), a common side effect of cancer therapy characterized by numbness and pain in the feet and hands, is often dose-limiting and difficult to control without resorting to dose reduction or cessation of treatment.\(^1\) This one-day conference was the first of its kind to focus entirely on this off-target toxicity, and included seminars by a wide range of experts, from the medical field to academic research and industry. The main goal of this meeting was to bring together members of a dispersed field of clinicians and researchers studying this topic to create a forum for dialogue. The meeting was sponsored by the American Society for Cell Biology (ASCB) through the ASCB local meetings program.

Clinical Aspects of Peripheral Neuropathy

To emphasize the translational focus of this meeting, the conference opened with a seminar by oncologist Daniel Greenwald (Cancer Center of Santa Barbara, Santa Barbara, CA) discussing the current state of CIPN symptoms and diagnosis, as well as the risk of neuropathy with various cancer drugs. Peripheral neuropathy is becoming more of a concern as cancer therapy has improved. Currently, there is an overall 67% 5-year survival rate for all cancers, and approximately 40% of cancer patients develop peripheral neuropathy as a result of their treatment, which can be chronic and life-long, profoundly affecting patient quality of life. Dr. Greenwald described current methods used to manage
symptoms of peripheral neuropathy, one of the most successful being serotonin/norepinephrine reuptake inhibitors, such as duloxetine. Thus far, no clinical trials have demonstrated any successful prevention treatments in the development of peripheral neuropathy in cancer patients.

**Clinical Studies in CIPN Detection and Susceptibility**

The first keynote seminar of the day was presented by Patrick Dougherty (University of Texas Health Science Center at Houston, Houston, TX), where he described clinical methods for the detection of peripheral neuropathy in cancer patients. Dr. Dougherty discussed the surprisingly common symptom of cold allogynia, a burning pain sensation from normally non-painful cold stimuli. Aβ-nerve fiber inactivity with normal C-fiber function can result in this effect, suggesting Aβ-nerve fiber dysfunction in this patient population. Intraepidermal nerve fiber loss and regrowth is also a potential cause of neuropathic pain, due to the continual discharge that is a normal occurrence in growing nerve endings. In addition, skin biopsies of patients with CIPN showed not only loss of intraepidermal nerve termini, but a distinct loss of Meissner’s corpuscle mechanoreceptors. Clinical trials are underway to develop a non-invasive method of visualizing and counting the Meissner’s corpuscle density in the fingertips of patients before and after chemotherapeutic treatment, which may prove to be a useful method to identify patients with the highest risk of developing CIPN. Surprisingly, chemotherapy-naïve cancer patients often show low Meissner’s corpuscle densities before the onset of treatment. The cause of this apparent neuronal damage is not currently known, however chemotherapy-naïve colon cancer patients with a baseline sub-clinical, but measureable
Peripheral neuropathy were twice as likely to develop CIPN over the course of their treatment.\(^3\) The involvement of toll like receptor (TLR) signaling pathways in an \textit{in vivo} rat model of CIPN suggests a potential mechanism for neuronal dysfunction via an immune response and macrophage infiltration, which was seen in rat dorsal root ganglia in response to paclitaxel.\(^4, 5\) Colocalization of TLR4 with the nociceptive ion channel TRPV1 in both human and rat dorsal root ganglia further supports the involvement of the TLR pathway in the development of pain.

**Mitochondrial Toxicity**

Neurons require an enormous energy supply to maintain a polarized state, generated by the constant activity of Na\(^+\)/K\(^+\) pumps. In addition, intraepidermal nerve fibers are in a constant state of remodeling and outgrowth to compensate for the daily turnover of skin cells. These two energy requirements necessitate proper mitochondrial function in peripheral neurons. Gary Bennett (McGill University, Montreal, Québec) highlighted this fact during his keynote seminar on his theory of mitotoxicity as the primary mechanism of chemotherapy-induced peripheral neuropathy.\(^6\) Dr. Bennett discussed the minimal nerve degeneration in a rat model of CIPN with low doses of three chemotherapeutic drugs: paclitaxel, bortezomib, and oxaliplatin. These low drug doses caused neuropathic pain and numbness in animals, displaying similar symptoms to human patients.\(^7\) Mitochondria in the axons of sensory neurons of treated animals were often swollen and atypical in both large, myelinated A-fibers and smaller C-fibers, however Schwann cell mitochondria appeared normal.\(^8\) Oxygen consumption in sensory nerve fibers from chemotherapy-treated animals was decreased, showing a
distinct defect within the electron transport chain. This defect persisted even weeks after chemotherapy was discontinued. ATP production was also reduced in the sensory nerves of chemotherapy-treated animals, which again persisted weeks after treatment cessation. Interestingly, unlike sensory neurons in the dorsal root, the ATP production of motor neurons in the ventral root was not affected and mitochondria appeared normal. Mitochondrial toxins enhanced the pain symptoms in rats treated with paclitaxel or oxaliplatin, while antioxidant compounds, which support mitochondrial function, reduced pain. Spontaneous, irregular discharge from nerve fibers in chemotherapeutic-treated animals increased in A- and C-fibers, which was also sensitive to mitochondrial toxins. Likewise, intraepidermal nerve fiber loss was increased when mitochondrial toxins were administered concurrently with chemotherapeutics. Subepidermal nerve bundles appeared normal, indicating the initial degenerative effects occur at the level of the epidermis in the sensory terminal arbor.

**Axonal Effects**

Two seminars addressed the effects of microtubule-targeting agents *in vivo* using mice treated with maximum tolerated doses of paclitaxel and eribulin mesylate. Mohamed Farah (Johns Hopkins School of Medicine, Baltimore, MD) described the research performed by his laboratory and the laboratory of his collaborator at Johns Hopkins University, Barbara Slusher. Eribulin is a novel microtubule inhibitor approved for metastatic breast cancer after prior treatment with a taxane and an anthracycline. To examine the potential of eribulin to exacerbate paclitaxel-induced peripheral neuropathy, sequential dosing of the two drugs was given to animals, and peripheral nerve activity
and morphology were examined. There was a significant reduction in caudal nerve conduction velocity after paclitaxel treatment, which was not enhanced with subsequent eribulin treatment.\(^{(12)}\) Nerve conduction amplitude was strongly decreased after paclitaxel treatment, and this was further reduced after secondary treatment with eribulin, however the reduction was lesser than subsequent paclitaxel treatment. Two weeks after cessation of paclitaxel treatment, nerve conduction velocity returned to control levels, but amplitude was not recoverable, indicating axonal degeneration in the nerve. Axons in the sciatic nerve displayed a distal to proximal degeneration, and swollen unmyelinated fibers and macrophages were present. In addition to peripheral nerves, axons in the spinal cord also showed degeneration after paclitaxel treatment, however cell bodies in the dorsal root ganglia displayed minimal damage.

Dr. Farah also detailed the results of an axonal transport study, which documented a reduction in anterograde transport in mouse sciatic nerves after paclitaxel treatment at the maximum tolerated dose, while eribulin induced a lesser effect on transport. Axonal transport is thought to be involved in CIPN, due to the fact that neurons with the longest axons are affected first and most strongly, namely those of the feet and hands. Eribulin, which binds to microtubules only at the ends, potentially has a weaker inhibitory effect on kinesin motility compared to paclitaxel, which decorates the entire length of the microtubule. The effects on microtubules and tubulin by microtubule-targeting agents was further discussed in the following seminar, given by doctoral candidate Brett Cook (University of California, Santa Barbara, Santa Barbara, CA), wherein he examined the biochemical changes in tubulin protein expression in the axons of sciatic nerves of mice treated with the maximum tolerated dose of paclitaxel or eribulin. After chemotherapeutic
drug treatment, axons were sectioned and immunostained for tubulin, the microtubule end-binding protein, EB1, and tubulin acetylation, a marker of microtubule stability. Paclitaxel and eribulin both increased tubulin protein expression and tubulin acetylation, however eribulin, a microtubule destabilizing compound, had a profound increase which was significantly greater than that of paclitaxel. Eribulin also unexpectedly produced an increase in EB1 expression, a protein involved in increased microtubule polymerization. An increase in microtubule polymerization and stability, which could lead to greater axonal stability, coupled with a lesser inhibition of axonal transport may begin to explain why eribulin has a lower clinical risk of CIPN compared to paclitaxel.

Patient-Derived Stem Cell Model

Future research using patient-derived induced pluripotent stem (iPS) cells yields the promise of testing cancer patients for genetic markers of susceptibility to peripheral neuropathy. Postdoctoral fellow, Amandine Rovini (Mayo Clinic, Rochester, MN) described a novel method of reprogramming cancer patient fibroblasts cells to iPS cells, and subsequent differentiation into sensory neurons. These induced sensory neurons hold the potential of patient-specific investigation of the neurotoxic response to chemotherapeutic drugs in human sensory neurons. Genetic expression data can also be examined in these cells, and mechanisms of neuropathy may be elucidated based on specific changes in protein and mRNA levels. Potential neuroprotective agents could also be tested with this model using a high throughput analysis.

Pharmaceutical Perspectives
To conclude the seminar sessions, Nicola Stagg (Genentech, Inc., South San Francisco, CA) described the difficulties pharmaceutical agencies face in predicting the side effects of novel chemotherapeutics in preclinical models. The development of antibody-drug conjugates was based on the expectation that targeting cytotoxic compounds directly to tumor cells using antibodies specific for extracellular proteins expressed by tumor cells would reduce toxicities of the drug and increase the drug’s therapeutic window.(14) The microtubule inhibitor, monomethyl auristatin E (MMAE), conjugated to various targeted antibodies showed a low level of peripheral neuropathy in a non-human primate preclinical model, however high neutropenia was dose-limiting and required the maximum tolerated dose to be lowered. When these antibody-MMAE conjugates were administered in clinical trials, a high incidence of peripheral neuropathy was seen in patients. Multiple factors appeared to contribute to this discrepancy, including a patient population that was predisposed to neuropathy due to prior chemotherapy, and the uptake of the antibody-drug conjugate into sensory neurons, despite neurons not expressing the extracellular target proteins.

Closing Discussion Panel

A discussion panel addressing the day’s seminar topics followed the seminar sessions. Dr. Greenwald answered questions regarding patient self-treatment of peripheral neuropathy with natural compounds, and offered his opinion that although natural treatments did not appear to help, neither did they appear to harm treatment outcome. The discussion session closed with a lively debate on the potential of the patient-derived iPS cell model to accurately predict the likelihood of an individual
patient’s peripheral neuropathy susceptibility, with attendee Dr. Eileen Dolan of the University of Chicago strongly supporting the model.

**Summary**

Cancer therapy has improved greatly over the past 50 years, and survival rates have steadily increased. The number of cancer survivors living with long-term, chronic peripheral neuropathy has likewise increased, necessitating more attention being dedicated towards preventing and treating this side effect that can reduce quality of life. The 2015 CIPN Symposium was the first national conference dedicated entirely to this topic. The conference highlighted the disparate effects of chemotherapeutic drugs on peripheral neurons, and the difficulty in parsing which toxicities are most causal in the development of neuropathy. While intraepidermal nerve fiber loss is most certainly a major, if not the root cause of numbness and neuropathic pain with CIPN, it remains unclear whether mitochondrial dysfunction, immune cell-driven inflammatory response, or microtubule inhibition and reduced axonal transport may be the primary mechanism for this degeneration. Additionally, the influence of axonal degeneration is uncertain, with definite effects on axonal nerve conductance and nerve morphology at higher drug doses; the question arises what relevant intracellular concentrations are involved in human neuropathy, and could axonal damage be the cause of the severe neuropathy seen in only a subset of patients. Lastly, what models are best used for the study of this toxicity? Animal models have been widely used, however despite similarities in disease symptoms, human clinical trials have not been able to reproduce the protective effects of multiple compounds in preventing neuropathy with chemotherapeutic drugs in rodents.
Perhaps patient-derived induced sensory neurons may yield a new look at not only the mechanisms of neurotoxicity, but potentially identify compounds that may protect human nerves from the various negative effects of these life-saving, but toxic, drugs.

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References


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