Fighting to eradicate chemotherapy-induced peripheral neuropathy

Reactive oxygen species are implicated in cell function, stress response, and have been linked to a staggeringly diverse range of diseases including nerve inflammation. They are also being increasingly employed as markers in the development of novel analytical, diagnostic and therapeutic tools. **Dr Mikhail Berezin's** current research at the Washington University School of Medicine is looking at novel ways of harnessing the power of imaging techniques in the diagnostics and potentially treatment of chemotherapy-induced peripheral neuropathy; a devastating, painful side effect of cancer treatment that occurs in almost 40% of patients.

A t the Washington University School of Medicine, USA, Dr Mikhail Berezin leads a lab investigating optical phenomena and contrast agents for medical diagnostics, clinical treatment and environmental monitoring. His lab also has a long research history exploring and imaging inflammation within cells and tissues, having had earlier success in harnessing the power of optically active molecular and nanoprobes to identify tissue damage caused by ischemia, lung inflammation and nerve injury.

OPTICAL WIZARDRY

Dr Berezin's labs' work extends to the development of spectroscopic instruments to detect novel optical probes and the discovery of novel advanced near infra-red (NIR) materials. They are also currently working on hyperspectral imaging systems from UV to infrared for a variety of life science applications.

CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

Recently, collaborating with the nerve expert Dr Matthew Wood (Washington University School of Medicine) and imaging scientist Dr Walter Akers (previously at Washington University School of Medicine, now at St Jude Children's Research Hospital), with the support of the National Cancer Institute at NIH, the teams' work has focused on investigating inflammation in peripheral nerves.

Millions of cancer patients suffer from chemotherapy–induced peripheral neuropathy (CIPN) as a common side effect of chemotherapy. The effects of this can be disabling, with symptoms primarily sensory in nature, and acute symptoms often occurring within hours to days of treatment. These generally include hypersensitivity to painful stimuli and cold, muscle cramps, as well as difficulty swallowing. In severe cases, chronic neuropathy can develop, causing pain to persist after the course of ▶

> **So far, no trials for chemotherapy–induced peripheral neuropathy preventatives or treatments have demonstrated positive results for patients**

chemotherapy has ended. This side effect is one of the most common reasons patients choose to discontinue their treatment, so it's imperative a solution is found to alleviate against it. Unfortunately, so far no trials for CIPN preventatives have demonstrated any positive results for patients.

Finding an effective treatment for CIPN therefore remains a major challenge. Dr Berezin and his team are leading the way in tackling this, with the identification of novel approaches capable of preventing and/or treating CIPN being one of their project's primary long term objectives. However, before this can be achieved, the researchers needed to find a way of identifying the neurotoxicity that underpins CIPN, in order to understand and intervene in its development.

MISSING MECHANISMS
This is a difficult task, not least because there are currently no biomarkers available to measure the degree of CIPN a patient suffers from, or even to help investigate CIPN in animal models. Research has been further hindered by the struggle to elucidate the causative biological mechanism responsible for the effects of CIPN.

Fortunately, a key mechanism has now been identified that may play a major role in the initiation of acute CIPN: oxidative stress within nerves caused by reactive oxygen species (ROS) – also suspected to drive its progression. ROS are a group of small aggressive molecules that contain oxygen and possess high chemical reactivities (potent mediators of cellular stress response). Although cells have endogenous mechanisms that normally repair oxidative damage, these systems can become overwhelmed when an excess of ROS accumulates, resulting in oxidative damage. Dr Berezin and his colleagues hypothesised that bursts of ROS in nerve tissue directly after the drug has been administered sparks a neuroinflammatory cascade of reactions, which ultimately leads to the neuropathy that patients experience.
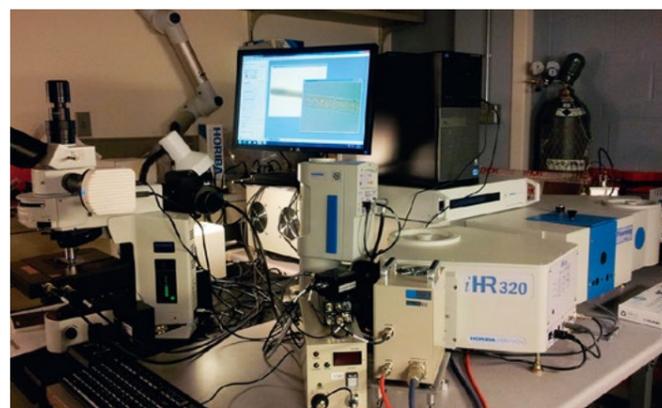

Normal nerve

After acute stress

Strong fluorescence indicates the presence of radicals in the sciatic nerve of the rat after acute stress

The Berezin Research Laboratory investigates the application of molecular excited states for medical imaging. The team develops novel imaging approaches to understand peripheral neuropathies *in vivo*.

ROS IN NERVE CELLS
Based on this, Dr Berezin and his team are developing a method to assess the extent of CIPN non-invasively *in vivo*, using fluorescent molecules specific to ROS. These fluorescent probes can be delivered to peripheral nerves which, when they encounter ROS, become activated – highlighting the presence of ROS. Traditional visible fluorescent probes might be less suitable for use within tissues due to low levels of photon penetration, as well as interference from endogenous autofluorescence. Dr Berezin and his team figured out a way to overcome this problem by using NIR fluorescent molecules instead.

Visualising the fluorescent probes and associated ROS also required imaging technology able to do so accurately. Therefore, Dr Berezin and his team developed a powerful approach to enable

the detection of chemotherapy-induced ROS. They have since successfully tested the efficacy of the novel molecular probes and detection system *in vivo*.

More specifically, they directly administered the NIR fluorescence molecular probes via intraneural injection into the nerves of rodents. Due to the sensitivity of the fluorescent probe molecules to hydroxyl radicals, they became activated after encountering ROS. This causes the molecules to emit fluorescence that can be subsequently detected and imaged. The results provided live, *in vivo* imaging of the response of the tissue to the molecular probe, which could then be correlated with nerve injury and neural stress response. When tested within the rat nerves, the probes exhibited rapid activation in both injured and stressed nerves. Overall, Dr Berezin's novel imaging approach appears more sensitive for detecting stress-induced changes within nerves than any existing techniques for evaluating molecular changes.

CURING THE PAIN OF CIPN
For the first time, this provides an opportunity to identify and monitor the neuroinflammation that occurs during early acute stages of CIPN as it happens. In collaboration with oncologist Dr Nina

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Q&A

What led you to focus your research on chemotherapy-induced peripheral neuropathy in particular?
First, observing the members of my family suffering from acute CIPN after each round of chemotherapy treatment and at the end developing the chronic form of CIPN. The fear and anxiety before every round of the chemotherapy and the pain they experienced strongly motivated my decision to solve the problem.

Talking to cancer patients, survivors and oncologists, I was shocked how common and widespread CIPN was and how little is known about this debilitating condition.

Out of the research your laboratory has carried out, what have been some of your most exciting discoveries and developments?
Being able to access the degree of neurodegeneration in the animal models of CIPN quantitatively and correlate the results with progression of CIPN.

Do you think your novel imaging method could be adapted for use in other medical applications beyond assessing nerve stress and injury?
Oxidative stress is quite a common response to inflammation caused by a stress or an injury and the imaging methods could be used to visualise these effects *in vivo*.

What have been the most significant challenges you have faced in conducting this research so far?
One of the strongest challenges in our research is the reliance on correlating nerve damage between the rodent models and humans. This is primarily because taking a nerve biopsy from humans is a highly invasive procedure and surgical removal of a piece of nerve causes chronic pain. We therefore do not have much histological data from human patients suffering from CIPN, and have mostly relied on the significant amount of histological evidence of CIPN in mice and rats that we have accumulated.

How do you see your project advancing over the coming years?
In the initial stage of this project we will be focusing on the mechanism of chronic CIPN using a strategy of combined imaging, histological, biochemical and behavioural methods to identify the molecular roots of chronic pain in living animals. We then plan to extend our studies to several common chemotherapy drugs that act with different cytotoxic mechanisms but show similar CIPN behaviour. Finally, we will test several pharmacological intervention strategies to minimize ROS and chronic CIPN in cancer animal models without affecting the efficacy of the chemotherapy treatment itself.

Wagner-Johnston, MD (Johns Hopkins), the team is currently applying this approach to measure the level of radicals after chemotherapy drug administration. It is suspected that the ROS-induced neuroinflammation and resulting neurological symptoms could be minimised if the spikes in ROS that occur during chemotherapy can be suppressed. Dr Berezin is therefore working to correlate the dosing of oxaliplatin, the expression of immunohistochemical markers of oxidative stress and the patient outcomes, by measuring the signal intensity of the fluorescent probe in nerve tissue.

The researchers are expecting to discover a positive correlation between dosage and fluorescence triggered by the presence of ROS as well as other markers of inflammation. Once this has been ascertained, the team will apply their novel imaging method to investigate several potential therapeutic interventions aimed at reducing early ROS response, thus minimising the degree of CIPN that occurs. They can then assess the neurotoxicity of both conventional and novel chemotherapeutic drugs and work on discovering new formulations with reduced CIPN side effects.

Detail

BIO
Dr Berezin has a background in medicinal chemistry and optical imaging. After being a research scientist at Pfizer, he joined Washington University in St Louis where he has an appointment at the Department of Radiology and the Department of Chemistry. He has authored more than 70 papers, patents, and book chapters.

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