SIGNED ..... DATE ...23/05/2024.....

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Targeting ion channels to combat pathophysiology of chemotherapy-induced peripheral neuropathy (CIPN)

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Of the many different types of clinical pain, pain caused by the medicine taken to combat an existing disease is particularly devastating; it typically worsens the existing condition and leads to poorer clinical outcomes. A prevalent example is pain caused by the strong drugs used to treat cancer. Many common anti-cancer drugs can cause nerve damage, often felt as tingling, numbness or burning-shooting pain. Due to such serious symptoms, it is often necessary to reduce or even stop drug treatment. Additionally, there is no preventative or treatment for the nerve damage pain caused by anti-cancer drugs and the use of currently available analgesic drugs is often associated to serious side effects.

Nerve cells, including those that signal pain, can talk to each other via proteins on the cell surface called 'ion channels'. Aim of this project is to focus on ion channels that regulate the flow of calcium into nerve cells; in particular, the 'T-type' calcium channel. Calcium channel function is further controlled by other proteins called auxiliary subunits. One of these auxiliary subunits is the target for 'gabapentinoid' pain drugs. We have recently identified a new protein, called CACHD1, that has similar properties to the subunit targeted by gabapentinoid drugs. This protein is found in high levels in pain pathways. Thus, we have proposed a potential new drug target.

We will investigate if T-type calcium channels and the CACHD1 protein represent new drug targets to prevent pain caused by anti-cancer drugs. We will use a preclinical animal model of pain induced by anti-cancer drug to study these effects and determine if there are any differences between male and female animals.

It is hoped that this fundamental research will eventually lead to much-needed new treatments for pain caused by anti-cancer drugs.

We have 3 main aims for this project:

AIM 1: To fully characterise the mouse model of chemotherapy-induced neuropathic pain (CIPN) in male and female mice.

AIM 2: To examine the role of Ca_V3 T-type calcium channels and CACHD1 subunits in CIPN using pharmacology, genetic manipulation and patch clamp electrophysiology methodologies

AIM 3: To deduce whether central and peripheral nervous system changes to autophagy contribute to the initiation and/or maintenance of CIPN and potential involvement of Ca_V3 T-type calcium channels and CACHD1 subunits.

RESULTS

AIM 1: To fully characterise the mouse model of chemotherapy-induced neuropathic pain (CIPN) in male and female mice.

We are using a mouse model of CIPN induced by repeated injection of a commonly used anticancer drug, paclitaxel. We have already developed a CIPN model in adult mice and measured mechanical sensitivity

using von Frey filaments. Our data shows that repeated injections of paclitaxel cause an increased sensitivity to mechanical stimulation in comparison to saline injected mice (Fig 1).

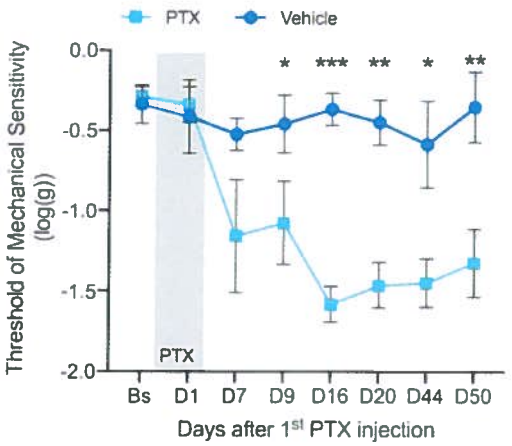

Fig 1: Repeated paclitaxel injections induce increased sensitivity in male mice. N=5/5. Data shown as Mean±SEM. *P<0.05; **P<0.01; ***P<0.001.

AIM 2: To examine the role of Ca_v3 T-type calcium channels and CACHD1 subunits in CIPN using pharmacology, genetic manipulation and patch clamp electrophysiology methodologies

We have previously investigated the contribution of high-voltage-activated Ca_v1 and Ca_v2 family voltage-gated calcium channels to whole-cell current in isolated nociceptive dorsal root ganglion (DRG) cells in the mouse partial sciatic nerve ligation model of neuropathy. In this study, we will use *in vitro* patch clamp electrophysiology to investigate the effects of pharmacologically blocking Ca_v3 channels using latest, more Ca_v3 selective agents such as TTA-P2(2).
Voltage- gated patch clamp technique is applied to study the spontaneous post synaptic excitatory current changes across the membrane of pyramidal cells in the cortical layer 5/6 of mice brain slices. This allows to understand the changes in the membrane current due to the opening and closing of ion channels across the cell membrane of a neuron, hence providing an inference on the properties of the ion channels.
The principle of voltage gated patch clamp technique works based on measuring the change in current across the membrane after a seal is formed between the electrode pipette and the cell membrane, by maintaining the membrane potential at a given stable potential (here -70mV or resting membrane potential). We have started to collect data to measure spontaneous post synaptic currents. This technique will be used to investigate the role of Cav3 T-type calcium channel in a model of pain induced by anticancer drugs.

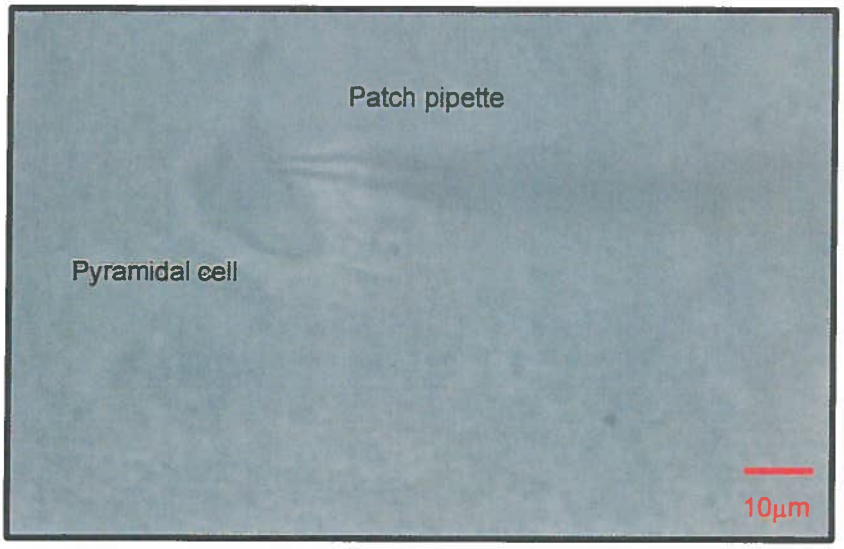

Fig 2: Image of whole cell path of pyramidal neuron (60x magnification)

We are confident that our project will substantiate a link between CIPN and CaV3 T-type calcium channels, opening the way for the development of more effective treatments.